SECTION 15

The patient with genetic renal disease

CHAPTER 301

Ethical aspects of genetic testing

Frances Flinter

The impact of inherited renal disease

Inherited renal diseases cause 50% of paediatric chronic renal failure (CRF) and 20% of adult CRF (Ong and Devuyst, 2011). Genetic causes of CRF include around 60 rare single-gene disorders, a variety of chromosomal abnormalities, and many multifactorial disorders (Flinter et al., 2003). An underlying genetic diagnosis may be suspected because of a known family history (e.g. of adult polycystic kidney disease) or the simultaneous occurrence of other medical problems (e.g. cardiac rhabdomyomas or cerebral tubers in tuberous sclerosis), but in many cases the presentation is unexpected. A detailed family history going back over at least three generations may provide a clue, and consanguinity is important to note as it increases the chance of an autosomal recessive disorder.

The important of making a genetic diagnosis

It is important to make an accurate diagnosis in order to optimize health and life expectancy; to identify other manifestation of a syndrome for which a patient should be screened; to establish the underlying pattern of inheritance so that recurrence risks can be determined and at-risk relatives offered screening; to enable a discussion of all possible reproductive options, if indicated; and to facilitate recruitment into treatment trials. Failure to make a genetic diagnosis may have adverse implications, not only for the proband, but also for relatives who are not aware that they are at risk of being affected or of passing the condition on to their children. Clinicians have a duty to be vigilant and consider the possibility of a genetic diagnosis, particularly if the use of a donated kidney from a living relative is being considered.

Tests to diagnose genetic causes of renal disease

A genetic diagnosis does not always require a molecular or cytogenetic test. The majority of cases of adult (autosomal dominant) polycystic renal disease are diagnosed following an ultrasound scan of the kidneys and many cases of Alport syndrome are diagnosed after tissue from a renal biopsy is examined under the electron microscope. Whether or not testing of genetic material is involved, clinicians must be aware of the responsibilities that are associated with making a genetic diagnosis, not only for the proband but also for relatives who may be affected or at risk. Primary diagnosis of renal disease will often be the role of the nephrologist, whilst counselling and testing of the extended family can be undertaken by clinical geneticists/genetic counsellors; nevertheless, nephrologists must discuss the implications of considering a genetic diagnosis in a renal patient before undertaking diagnostic tests so that the patient has an opportunity to consider the implications for family members and to discuss these with them.

DNA tests to diagnose single-gene disorders

Genetic testing involving the identification of mutations in single genes is constantly evolving and a suspected clinical diagnosis of a single-gene disorder can sometimes be confirmed at a molecular level following mutation screening within a single gene or a panel of relevant genes. The turnaround times for mutation screening and the costs involved are falling all the time, and in some cases molecular testing can replace invasive procedures such as renal biopsy, for example, in the diagnosis of Alport syndrome. It is important that nephrologists know that not all mutations within a gene are identifiable (some may be deeply hidden within an intron, for example), so a negative results does not exclude the diagnosis. Clinicians requesting genetic tests also need to be aware of the possibility of generating unexpected or un-interpretable information such as molecular variants of unknown significance or non-paternity. Properly accredited laboratories will do their best to provide an interpretation of results, and will be aided in reaching useful conclusions if they are provided with sufficient clinical information; but help from clinical geneticists may also be required. In some cases it may be necessary to test other affected relatives to see if they carry the same genetic variant before the significance of a novel DNA variant can be established.

Storing DNA samples

New genes are identified every week, but there is a still a number of inherited renal disorders for which the underlying gene (or genes) remains elusive. It is important to store DNA from patients affected with these conditions (especially if their life expectancy is likely to be limited) so that, as and when genetic testing becomes possible in the future, their samples can be analysed and the results provided to clinicians looking after relatives for whom this information may be useful. Clinical genetics departments usually provide a DNA storage service.

Predictive testing versus diagnostic testing

Diagnostic tests may confirm a suspected clinical diagnosis, for example, a patient presenting with symptoms and signs of renal failure may have a biopsy, ultrasound scan, or molecular test which provides a specific diagnosis. Predictive tests are different—they are performed on healthy, asymptomatic people who are aware of a

family history which may have implications for them. For example, they may have a parent with von Hippel–Lindau syndrome, an autosomal dominant condition that is associated with renal tumours, cerebral haemangiomas, and phaeochromocytomas and may elect to have their DNA tested to see if they have inherited a mutation from their parent, which is possible if the mutation in the family has already been identified. This then enables them to undergo regular surveillance to screen for manifestations of the condition that would benefit from early intervention. Patients in whom a mutation has been identified, whether they are symptomatic or not, can be offered prenatal diagnosis or pre-implantation genetic diagnosis, if appropriate. Predictive testing is normally offered in conjunction with genetic counselling so that the medical implications and also practical implications (for future employment and insurance purposes, for example) can be discussed.

Genetic testing in childhood

For conditions that are expected to manifest in childhood, or for which early intervention (pre-symptomatically) is indicated, it may be appropriate to perform genetic testing so that health surveillance or other specific management plans can be made. If an inherited condition is not expected to manifest before adult life, then genetic testing in childhood is not indicated and there are clear guidelines in many countries about the importance of protecting the child's autonomy, or right not to know their genetic status (American Medical Association, 1996; British Society for Human Genetics, 2010).

Young people in this position should be offered genetic counselling at an appropriate age (typically in their mid teens) so that they can explore the implications of their positive family history and make an informed decision about whether or not to undergo predictive testing. Some countries have legislation to protect people who have an inherited condition from discrimination. The Genetic Information Nondiscrimination Act (GINA) of 2008 is an Act of Congress in the United States designed to prohibit the use of genetic information in health insurance and employment, but in many other countries there is no such protection.

Genetic testing and patient pathways

It is important that genetic tests are performed on the right person at the right time, with a clear understanding of the sort of information that may be expected and knowledge about how to interpret it. There are a number of useful resources available describing specific genetic tests and the criteria that patients should meet before genetic testing is indicated. (See the websites of the UK Genetic Testing Network (<http://www.ukgtn.nhs.uk>), Eurogentest (<http://www.eurogentest.org>), and Gene Tests (<http://www.ncbi.nlm.nih.gov/sites/GeneTests>), all publicly funded resources that provide detailed information about tests available, testing criteria and laboratories that offer these services.)

Over-the-counter genetic testing

An increasing number of private companies now offer genetic testing services that are marketed direct-to-consumer. These generally bypass a medical intermediary and are often advertised on the Internet, avoiding the regulations of individual jurisdictions. The UK Human Genetics Commission developed a framework of principles that could be used in any country to define good practice, comprising a voluntary set of guidelines designed to enable the development of relevant laws and regulation around the world. (Human Genetics Commission, 2010) but the development of legislation is patchy (Borry et al., 2012).

Whole-genome sequencing

At present, exome capture and whole-genome sequencing techniques are often used on a research basis for discovering new candidate genes. However, the first clinical applications of so-called Next-Gen sequencing techniques are happening now. Ethical frameworks to deal with the new issues this will raise are not fully developed even in a research setting (Green et al., 2013). Wright et al. (2013) describe the issues in an African setting, but the questions are broadly the same anywhere in the world. May et al. describe some of the clinical issues in a developed world, paediatric context, which is where the implications are likely to impact on clinical practice very soon (May et al., 2013). Some examples of the questions raised include the following:

- Whether to report incidentally discovered abnormalities of definite significance for health or life and for which there is an effective therapy.
- And what if there is not a preventive treatment, but possible significance for relatives?
- Whether to report incidentally discovered abnormalities of uncertain significance.
- Whether to pass on information to other family members without the consent of the index patient.
- As knowledge expands in the years and decades after initial testing, how/whether to keep patients informed.
- What to do with information that may be significant for other family members after a patient's death.

Conclusion

Genetic testing used to be the preserve of clinical geneticists, but is now increasingly used by other clinicians, a phenomenon referred to as 'mainstreaming' (Burton, 2011). This trend will continue and non-geneticists need to remain up to date with the range of tests available and understand clinically appropriate ways of using them, in order to exploit their clinical utility and enhance patient care. In conjunction with this lie responsibilities for understanding both the potential power and also the limitations of these tests, and the ethical implications of genetic testing for both the proband and their relatives.

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CHAPTER 302

Antenatal diagnosis and pre-implantation genetic testing

Frances Flinter

Antenatal screening

In many countries, screening is offered antenatally to all women, whether or not their pregnancy is known to be at increased risk of abnormality. This may include non-invasive screening for structural abnormalities by ultrasound scanning and blood tests to look for markers suggestive of an underlying chromosome abnormality. The detection of an abnormality or marker of increased risk may lead subsequently to an invasive test that enables a sample of fetal tissue to be obtained for analysis.

Antenatal ultrasound scanning

See also Chapter 361 on ante- and post-natal imaging.

Garrett and colleagues reported the first antenatal diagnosis of a renal abnormality by ultrasound scanning (USS) in 1970 (Garrett et al., 1970).The quality of images obtained by antenatal USS has improved considerably, but it is still dependent on the presence of a reasonable amount of amniotic fluid, which may be absent/reduced with underlying bilateral renal disease. It is also difficult to obtain good images if the pregnant woman is significantly overweight or if the baby is not in an optimal position for visualization. Charts of standard fetal size are available for comparison (Chitty and Altman, 2003). Normal kidneys become visible at around 9 weeks and by 12/13 weeks good images can be obtained in the majority of cases. Serial scans reveal the progress of renal development and in some cases, such as antenatally detected hydronephrosis, the problem may improve or even resolve altogether. A thorough USS will include the assessment of the fetal kidneys, bladder, and liquor volume.

Renal tract anomalies comprise around 15% of all prenatally detected renal anomalies and > 70% are associated with other anomalies. Renal tract anomalies are found in 250–300 syndromes and in around 35% of all chromosome anomalies (Firth and Hurst, 2005); however, the common abnormality, hydronephrosis, with renal pelvic dilatation, is often isolated. The role of renal pelvic dilatation as a soft marker for chromosomal abnormalities became less significant after the introduction of combined screening test in 2007 in the United Kingdom.

Renal agenesis

Unilateral renal agenesis occurs in 0.15/1000 neonates and bilateral renal agenesis affects 0.12/1000 neonates. The birth incidence has been decreasing over the last 10 years due to an increase in antenatal detection of bilateral renal agenesis (Loane et al., 2011). It is more common in males than females (2.45:1) and may be associated with an underlying genetic syndrome such as Fraser syndrome, cerebro-oculo-facial syndrome, acro-renal-mandibular syndrome, and branchio-oto-renal syndrome. The absence of a kidney should prompt a search in ectopic locations, especially the fetal pelvis as antenatally suspected unilateral renal agenesis is confirmed in only 47% of cases postnatally (Chow et al., 2005). If there is no pelvic kidney then unilateral renal agenesis is the most likely diagnosis; but another possibility is crossed renal ectopia, in which the contralateral kidney is large and unusually shaped. If both kidneys are absent, this is usually accompanied by severe oligohydramnios and Potter sequence (pulmonary hypoplasia, micrognathia, and talipes). Renal agenesis is found in VATER (vertebral defects, anal atresia, tracheo-oesophageal fistula, oesophageal atresia, renal anomalies), VACTERL (vertebral defects, anal atresia, cardiac anomalies, tracheo-oesophageal fistula, oesophageal atresia, limb and renal anomalies), and MURCS (Mullerian duct anomalies, renal aplasia, cervicothoracic somite dysplasia), all three of which generally occur as sporadic events with a low recurrence risk in subsequent pregnancies.

Multicystic dysplastic kidney disease

Multicystic dysplastic kidney disease (MCDK) is often an incidental finding on prenatal USS. Unilateral MCDK occurs in 1/3000–5000 births and liquor volume in usually normal. Bilateral MCDK occurs in 1/10,000 births and there is oligohydramnios (Firth and Hurst, 2005). Karyotyping is indicated if there is bilateral renal involvement or an additional extra renal abnormality on USS; if there is an associated cardiac abnormality then the possibility of an underlying deletion on the long arm of chromosome 22 (Di George syndrome) should be considered.

Polycystic kidneys

Renal cystic disease *in utero* (see also Chapter 305, approach to the child with renal cysts) may occur as a feature of a number of mostly rare syndromes (see Box 302.1); as the predominant feature in infantile autosomal recessive polycystic kidney disease, or occasionally as an early presentation of adult autosomal dominant polycystic kidney disease; or it may be secondary to obstructive anomalies *in utero* such as congenital urethral obstruction, which may lead to the development of prune-belly syndrome. It can also be a rare feature of other syndromic conditions including Beckwith-Wiedemann, Fryns and Zellweger syndromes.

Box 302.1 Genetic disorders associated with renal cystic disease (see also Chapter 305)

Chromosomal

- Trisomy 13 (Edward syndrome)
- Trisomy 18 (Patau syndrome)
- Turner syndrome (X0).

Autosomal dominant

- Adult polycystic kidney disease (types 1 and 2) (Chapter 306)
- Tuberous sclerosis (Chapter 330)
- Von Hippel–Lindau (Chapter 332)

Autosomal recessive

- Infantile polycystic kidney disease (Chpater 313)
- Meckel syndrome (Chapter 314)
- Bardet–Biedl syndrome (Chapter 314)
- Jeune syndrome (Chapter 314).

X-linked

- Type 1 orofacial digital syndrome (Chapter 319).

If the possibility of an underlying genetic diagnosis arises, then it is important to ask about consanguinity and to obtain a three-generation family history. It may also be appropriate to examine the parents and consider a renal USS of them in order to look for further clues. The value of storing DNA extracted from fetal tissue either obtained prenatally, or during post-mortem examination, cannot be over-emphasized. It may enable molecular confirmation of a suspected underlying genetic diagnosis, without which definitive early prenatal diagnosis or preimplantation genetic diagnosis may not be possible in future pregnancies.

Finally, the potential role of teratogens such as maternal alcohol ingestion, maternal diabetes, rubella, and the use of angiotensin-converting enzyme inhibitors should be considered in any pregnancy with renal tract abnormalities.

Prenatal diagnosis

Some pregnant women know that their pregnancy is at increased risk of abnormality, either because they or their partner have a genetic diagnosis, because they have had previously had an affected pregnancy/child, or because of their family history. The availability of accurate prenatal diagnosis will depend on knowledge of the precise diagnosis and in some cases this will require specific molecular diagnostic tests on DNA taken from a previously affected pregnancy/relative in order to identify the precise mutation for which testing can be offered.

Counselling

All women considering prenatal screening or testing should be routinely counselled beforehand about the possible outcomes and implications of these tests. If a genetic diagnosis is considered, referral to a clinical genetics department may be indicated to allow genetic counselling, discussion about possible genetic tests, and consideration of the implications of making a genetic diagnosis for other members of the family too. Pregnant women in whom a significant renal abnormality is identified may suddenly find themselves being asked whether or not they wish to consider terminating the pregnancy and it is important that it is known in advance that this is where screening/testing may lead (as they may prefer not to know if they would not chose to intervene). If they do go ahead with screening/testing, they should be well supported throughout the process, with the provision of accurate information that facilitates decision-making in a non-directive manner (Abramsky and Chapple, 2003).

Non-invasive prenatal diagnosis (maternal blood sampling)

A few conditions can be diagnosed by taking a sample of maternal blood. Finnish nephrotic syndrome or bladder exstrophy may be suspected if the alpha fetoprotein in the maternal blood stream is high, especially if a previous pregnancy has been affected; however, with routine screening this opportunity does not arise very often as the quadruple test, in which alpha fetoprotein is one of the biochemical components measured, is offered only to a minority of women who missed their first trimester screening test. Abnormalities of a combination of markers used to screen for Down syndrome and other chromosomal abnormalities may raise the suspicions of an abnormality, but subsequent invasive prenatal diagnosis will usually be required to make a more specific diagnosis.

It is now known that a small amount of fetal DNA escapes into the maternal bloodstream and current research is focusing on the identification of non-maternal DNA markers that may enable specific prenatal diagnosis without the need for an invasive procedure. This is already being done successfully with testing for male DNA in order to sex a pregnancy during the first trimester (indicating whether or not invasive prenatal diagnosis is indicated in a pregnancy at risk of inheriting an X-linked disorder) and current work is attempting to identify paternal markers that may be inherited by a fetus at risk, for example, in a pregnancy at risk of inheriting an autosomal dominant condition from the father.

Invasive prenatal diagnosis

Prenatal sampling of fetal tissue enables detailed chromosome analysis or molecular analysis to test for specific mutations. If either/both parents carries a known mutation and they wish to know if the pregnancy is at risk, they may request prenatal diagnosis. The choice of tests will depend on the gestation of the pregnancy and the sample required.

Fetal tissue for analysis can be obtained by chorion villus biopsy (performed at around 11–14 weeks' gestation), amniocentesis (performed in the second trimester), and fetal blood sampling (second or third trimester). The procedure-related miscarriage risk following amniocentesis and chorionic villus sampling (CVS) is 1%. The overall risk of miscarriage following CVS is usually higher, 1–2%, as it is done earlier in pregnancy when spontaneous miscarriage is more likely; however, earlier testing generates a result more quickly, which may be important if termination of pregnancy is an option. If the pregnant woman is Rhesus negative, then she should receive anti-Rh-D antibody at the time of the procedure to prevent Rhesus sensitization.

If detailed chromosome analysis by karyotyping is required, the cells are cultured and then stained before examination by microscopy. Increasingly, however, chromosome abnormalities are detected using targeted molecular techniques such as qf-PCR (quantitative fluorescent polymerase chain reaction) or aCGH (array comparative genomic hybridization).

Fetal DNA can also be extracted and used to test for specific mutations in single genes if the mutation(s) is already known in the family. However, because the mutations in most single-gene disorders are novel and unique to an individual pedigree, it is necessary to identify the specific mutation(s) involved in the family before it is possible to offer prenatal diagnosis. Once the results are available, the pregnant woman will need to make a decision about whether or not to consider terminating the pregnancy, and the need for supportive genetic counselling during this period should always be considered. The likely renal prognosis will often not be the only consideration, especially in the context of a complex syndromic diagnosis in which other factors may have an even greater effect on the long-term prognosis.

Prenatal intervention

Although antenatal intervention is unusual, there are a few situations where fetal medicine specialists may consider an invasive procedure to try and optimize the outcome of a pregnancy in which a renal abnormality has been identified. For example, drainage of distended bladder by percutaneous bladder shunt is thought to reduce the back pressure on the kidney and increase the volume of amniotic fluid, thereby improving the development of the fetal lungs. Such procedures are technically challenging and should only be undertaken in specialist centres and in research settings (Pluto Collaborative Study Group et al., 2007).

Pre-implantation genetic diagnosis

For some couples known to be at risk of conceiving a pregnancy with an underlying genetic abnormality, the option of prenatal diagnosis and termination of an affected pregnancy is unacceptable, but they may be keen to maximize the chances of having a healthy child. During genetic counselling a number of alternatives will be discussed, including the use of donor gametes: donor egg if the woman carries an X-linked condition such as Alport syndrome, or donor sperm to avoid an autosomal dominant condition carried by the male partner or an autosomal recessive condition. Other options include avoiding pregnancy altogether, or adoption. For couples who want to have a child that is genetically related to both of them, but with minimal risk of inheriting the genetic condition that they carry, then pre-implantation genetic diagnosis (PGD) may be possible (Braude and Flinter, 2007).

PGD involves the use of *in vitro* fertilization to obtain early embryos in the laboratory, which are biopsied on day 3–5 and then tested for the genetic condition that the couple carry. Super-ovulation is achieved by hormonal stimulation so that a sufficient number of eggs can be fertilized *in vitro* before they are tested (Fig. 302.1). If an unaffected embryo is identified, it is transferred into the uterus in the hope that implantation will take place. Up to 50% of embryos will implant, so single-embryo transfer is preferred in order to minimize the chance of a multiple pregnancy. Spare unaffected embryos can be frozen and then used in subsequent cycles, if requested.

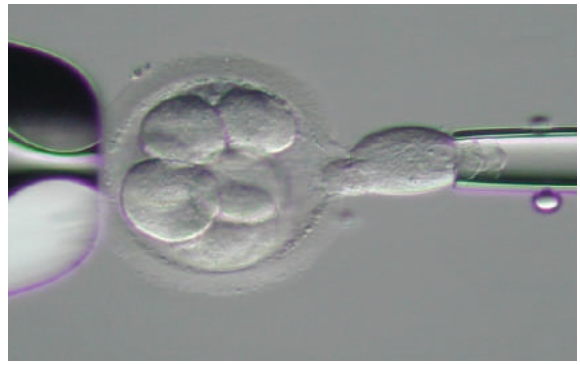

Fig. 302.1 Biopsy of eight-cell embryo (day 3) for pre-implantation genetic diagnosis.

Some people object to PGD on ethical grounds, because the procedure involves embryo biopsy with those affected being discarded, but for many couples this is preferable to prenatal diagnosis and subsequent termination of pregnancy. PGD is only available in a few centres as the technology involved is highly specialized and the success rate depends on experience. PGD can only be offered to couples at significant genetic risk in whom the cytogenetic/molecular basis of the condition they carry has been identified. Obviously it is only an option for couples who present before they become pregnant, so those who are at increased risk of conceiving a pregnancy with a genetic condition must be referred for genetic counselling prior to conception (Lashwood, 2009; Ross, 2010).

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CHAPTER 303

The molecular basis of ciliopathies and cyst formation

Wolfgang Kühn and Gerd Walz

The discovery that cystic diseases are 'ciliopathies'

In autosomal dominant polycystic kidney disease (ADPKD), cysts are slowly expanding spherical structures, filled with fluid, and surrounded by enlarged epithelial cells that disconnect from the tubular system at later stages. Early research suggested that cultured cystic epithelial cells were incompletely differentiated, continue to proliferate, and eventually form cysts; hence the hypothesis that cysts behave like 'neoplasia in disguise' (Grantham, 1990). Tubular epithelial cells typically absorb fluid. However, abnormal localization of membrane proteins and ion channels including CFTR, instead promote fluid secretion and cyst expansion. Mechanical compression of the surrounding tissue and reactive changes of the interstitial matrix compromise the normal nephrons, leading to progressive renal failure.

The observation that the epithelial cells lining the cysts in patients with ADPKD secrete fluid led to the discovery of several components within the cyst fluid, including antidiuretic hormone (ADH) that promotes ion transport through generation of cAMP. Cloning of the two genes responsible for ADPKD, *PKD1* and *PKD2*, not only provided the opportunity to study the function of the corresponding gene products polycystin-1 and polycystin-2/TRPP2, but also led to the two-hit theory of cyst formation (Watnick and Germino, 1999) based on the observation that tubular epithelial cells involved in cyst formation lose their second *PKD1* or *PKD2* allele due to a somatic mutation.

The finding that gene products mutated in cystic kidney disease localize to primary cilia heralded a paradigm change, now linking the formation of kidney cysts to a dysfunction of the cilium (reviewed in Simons and Walz, 2006). The first hint that cilia might be involved in the pathogenesis of polycystic kidney disease (PKD) came from the *orpk* mouse, which carries a hypomorphic mutation of the *Tg737* gene, and is characterized by dilation of the proximal tubules and cystic lesions in the collecting ducts (Yoder et al., 1995). Mutant strains of the flagellated green algae *Chlamydomonas rheinhardtii* with defective *IFT88*, an intraflagellar transport protein (IFT) and homologue of *Tg737*, fail to assemble flagella (Pazour et al., 2000). Similarly, mice with mutated *Tg737* lack cilia on the ventral node and on tubular epithelial cells. These findings suggested that IFT is important for both flagellar biogenesis and assembly of primary cilia in mammalian cells, and prompted the ciliary hypothesis of PKD.

Studies in the worm *Caenorhabditis elegans* provided further support for his hypothesis. The *C. elegans* homologues of *PKD1* and *PKD2, lov-1* and *pkd-2*, were the first cystoproteins to be identified in cilia and cell bodies of male-specific sensory neurons, where they participate in a shared signalling pathway (Barr et al., 2001).

The mammalian polycystin-1, polycystin-2, polaris/IFT88, and cystin were subsequently identified in the primary cilium of tubular epithelial cells (Yoder et al., 2002). Other PKD proteins followed, and the list of cilia-associated cystoproteins continues to grow. Finally, the kidney-specific inactivation of the ciliary kinesin motor protein KIF3A in mice resulted in cystic kidneys with absent cilia and provided a convincing link between defective ciliogenesis and cystic kidney disease (Lin et al., 2003).

The proteins mutated in cystic kidney diseases are not confined exclusively to the ciliary compartment, and participate in cellular functions outside of the cilium. Furthermore, it is difficult to differentiate between primary ciliary defects and secondary perturbations caused by the expansion and other indirect effects of cyst formation. Therefore, the loss of a gene product that is causative for the development of cystic kidney disease may trigger signalling pathways not necessarily connected to the underlying function of the diseased protein. Hence, the identification of abnormal signalling pathways, especially those that are shared across different gene defects, might provide the most efficient way to treat PKD.

Primary cilia

Biology of cilia

Cilia formed approximately 700 million years ago, when single-cell organisms started assembling into larger cell clusters and multicellular tissues (Ainsworth, 2007). Cilia are microtubular organelles derived from the centrosome. The centrosome consists of two centrioles, the mother and daughter centriole, which form the microtubule-organizing centre in interphase cells. Almost all body cells form cilia (Singla and Reiter, 2006). In polarized epithelial cells, the two centrioles migrate towards the apical membrane. The mother centriole then converts into the basal body, which consists of a characteristic cartwheel-like arrangement of nine microtubule triplets and nucleates the ciliary axoneme. The axoneme of non-motile, primary cilia consists of nine microtubular doublets; motile cilia in addition contain a central doublet, inner and outer dynein arms, as well as radial spokes (Fig. 303.1A, B).

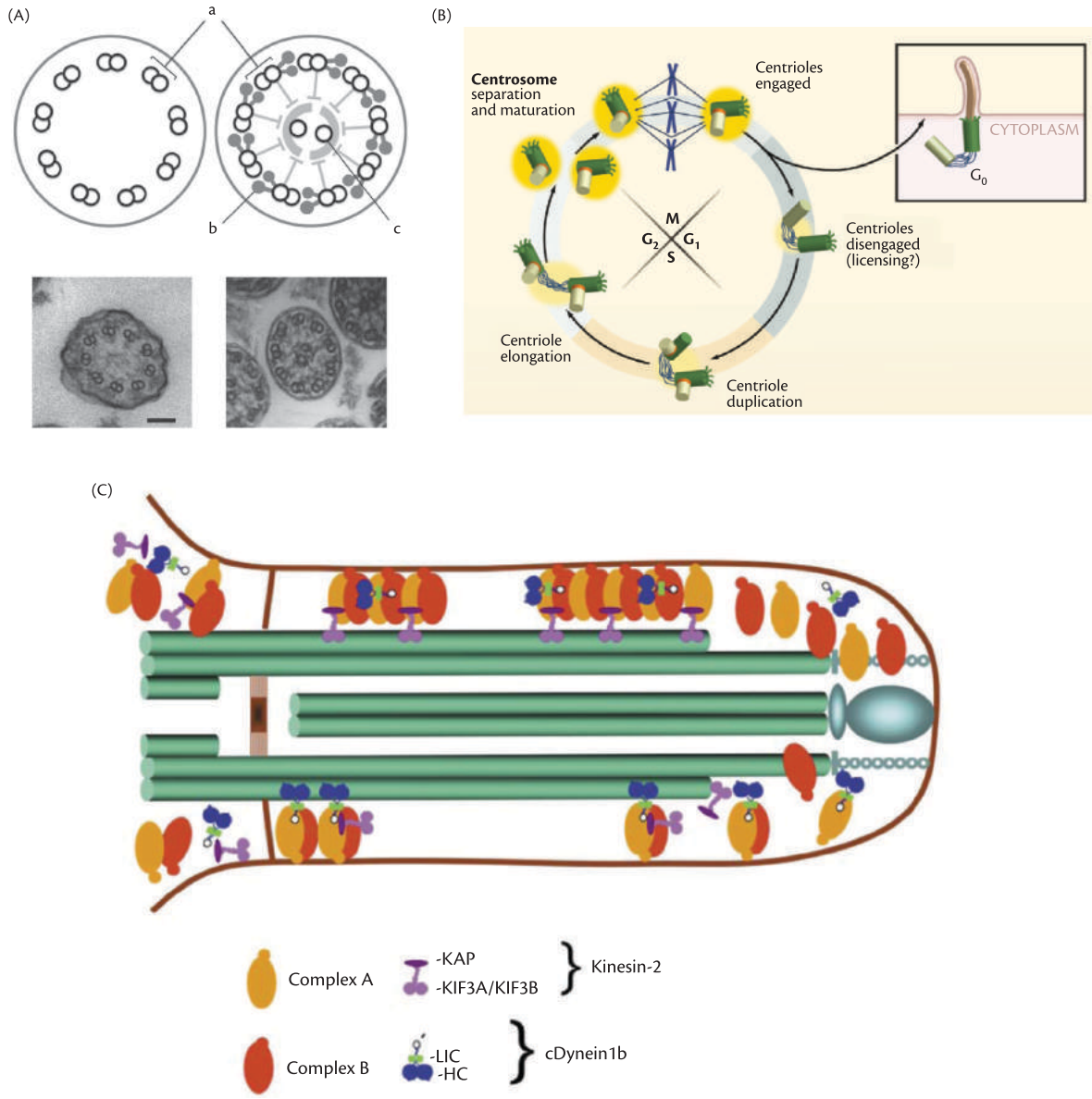

Fig. 303.1 Cilia are microtubule-containing organelles originating from the mother centriole. (A) A 9 + 0 primary cilium from the embryonic node shows nine peripheral microtubule doublets in a circular arrangement (axoneme) (a). Motile cilia, as shown here from trachea, have an additional central microtubule pair (c) and inner and outer dynein arms (b). (B) Cilia are resorbed upon entry into the cell cycle (G1). During S phase the centrioles duplicate and elongate in G2. After mitosis the centrioles migrate to the cell periphery and the mother centriole docks at the plasma membrane to serve as basal body from which the cilium emerges. (C) Cilia contain an intraflagellar transport (IFT) system to shuttle ciliary cargo with the help of specific multi-protein complexes (complex A and complex B) along the axoneme. Kinesin-2 mediates anterograde transport to the ciliary tip, retrograde transport occurs by cytoplasmic dynein. HC = heavy chain, LIC = light intermediate chain. Modified versions reproduced from Takeda and Narita. *Differentiation*, 2012; 83(2): S4–11 (A), Nigg and Raff. *Cell*, 2009; 139(4): 663–78 (B) and Pedersen et al. *Curr Biol*, 2006; 16(5): 450–9 (C).

Assembly of the cilium and subsequent maintenance of the ciliary axoneme requires a bi-directional intraflagellar transport (IFT) system that is powered by two types of motor proteins: the anterograde transport towards the tip is driven by kinesin-2, the retrograde transport towards the base of the cilium is mediated by cytoplasmic dynein (Rosenbaum and Witman, 2002) (Fig. 303.1C). Both motor proteins interact with IFT protein complexes known as IFTA and IFTB particles. Disruption of either kinesin-2 or IFTB particles prevents ciliogenesis, while disruption of the dynein motor or IFTA results in shortened, bulged cilia (Goetz and Anderson, 2010). Cilia are anchored to the actin and microtubule cytoskeleton (Werner et al., 2011), and disruption of the local environment prevents normal ciliogenesis.

Ciliary trafficking

The ciliary proteome is estimated to encompass approximately 1200–2500 proteins (Liu et al., 2007; Oh and Katsanis, 2012). Access to this organelle is tightly orchestrated and restricted by three physical barriers: the transition zone, the septin ring, and the peri-ciliary membrane domain (Nachury et al., 2010; Garcia-Gonzalo and Reiter, 2012; Reiter et al., 2012).

Transition fibres radiate from the basal body to the plasma membrane. Immediately distal, a transition zone exists consisting of microtubular rods that are linked to axonemal microtubule doublets and anchor to the membrane by bifurcated structures called Y-links (Reiter et al., 2012) (Fig. 303.2A). This gate-like organization

represents functional pores that prevent diffusion of larger molecules into this compartment. Only small molecules (< 10 kDa in most cell types) can pass through the transition zone and reach the ciliary compartment by diffusion. This means that most proteins undergo active transport to reach the ciliary compartment. A large number of ciliopathy proteins localize at the ciliary gate and play important roles in protein import and export. These include proteins of the NPHP family, mutated in nephronophthisis (see Chapter 317), as well as the MKS and JBTS family, mutated in Meckel–Gruber and Joubert syndromes (Reiter et al., 2012) (see Chapter 314). Recent observations suggest that the cilium also co-opted the nuclear import machinery to recruit cargo proteins to the ciliary axoneme (Gruss, 2010; Huang and Tsao, 2010; Fan and Margolis, 2011; Obado and Rout, 2012). Proteins of the nucleoporin complex are located at the transition zone (Kee et al., 2012). The ciliary motor protein KIF17 interacts with the nuclear import receptor importin-β2A via a short carboxy-terminal stretch of several basic amino acids similar to a nuclear localization signal and releases its cargo via RanGTP which similar to the nucleus is present in the cilium (Dishinger et al., 2010) (Fig. 303.2B).

The transition zone seems to be the site of another diffusion barrier: a septin ring. Septins are small GTPases that interact with plasma membrane lipids to restrict free diffusion across membranes in yeast, sperm, and dendrites. They have been found as rings at the ciliary base of cultured renal epithelial cells and the motile cilia on *Xenopus* skin (Fig. 303.2C) (Hu et al., 2010; Kim et al., 2010b). Loss of septins disturbs cilia-based Hedgehog (Hh) signalling and interferes with ciliogenesis in *Xenopus*. Interestingly, septins functionally interact with Fritz, a planar cell polarity (PCP) protein that has been linked to Meckel–Gruber and Joubert syndromes (Kim et al., 2010b). A second peri-ciliary diffusion barrier has been described with a diameter of approximately 2–3 μm, consisting of a condensed lipid zone that excludes GPI-linked apical membrane markers (Vieira et al., 2006; Nachury et al., 2010; Francis et al., 2011) (Fig. 303.2D). This diffusion barrier precedes cilia formation and seems to prevent diffusion of actin anchored proteins within the apical membrane towards the ciliary base. The determinants of the peri-ciliary domain are not yet known.

The function of cilia as platforms for sensing the environment requires the regulated recruitment of transmembrane molecules into the ciliary membrane. As outlined above, free diffusion is inhibited by membrane barriers suggesting a need for indirect delivery routes of proteins to cilia. Indeed vesicular transport seems to be the main route of targeting receptor molecules to the cilium (Nachury et al., 2010). After leaving the Golgi, ciliary cargo vesicles likely travel through intermediate compartments, and acquire different adaptor and coat proteins en route to the cilium. No universal pathway for ciliary protein delivery exists. Some cargo destined for the cilium including rhodopsin appears to be transported by vesicles to the base of the cilium (Papermaster et al., 1985). Other transmembrane proteins appear to reach the cilium by lateral diffusion, such as agglutinins in *Chlamydomonas* or the Hh protein Smoothened (Smo) (Hunnicutt et al., 1990; Milenkovic et al., 2009). However, at least some plasma membrane proteins are recycled through the recycling endosome and delivered to the cilium (Boehlke et al., 2010a; Garcia-Gonzalo and Reiter, 2012)(Fig. 303.2E). Vesicle trafficking to the peri-ciliary membrane involves Rab GTPases, small GTP-binding proteins that have been implicated in almost every aspect of vesicle trafficking, including vesicle budding, transport, and tethering, particularly rab8 and rab11 (Moritz et al., 2001; Yoshimura et al., 2007; Kaplan et al., 2010; Knodler et al., 2010). Other Rab GTPases are likely to be involved and include Rab23 which has been implicated in the ciliary recycling of Smo (Boehlke et al., 2010a). Peri-ciliary protein transport involves IFT molecules (particularly IFT20, 52, 57, and 88) and a complex of Bardet–Biedl syndrome (BBS) proteins that functions as a coat for ciliary cargo and is termed the 'BBSome' (Follit et al., 2006; Nachury et al., 2007; Nachury et al., 2010; Sedmak and Wolfrum, 2010) (Fig. 303.2F). In many instances, protein entry to the cilium requires short amino acid sequences, so-called ciliary targeting signals, for instance, the C-terminal octapeptide in rhodopsin or the N-terminal RVxP motif in TRPP2 (Deretic et al., 1998; Tam et al., 2000; Geng et al., 2006). Ciliary targeting sequences often contain myristoylation and/or palmitoylation sequences, implying that association with lipid microdomains, also called lipid rafts, is part of cilia-directed protein delivery (Tao et al., 2009; Follit et al., 2010). The altered lipid composition at the ciliary base may in addition play an important role in vesicle fusion. The role of the cytoskeleton in these delivery pathways is not explored. Actin seems to inhibit ciliogenesis and to impede protein transport to the cilium (Kim et al., 2010a). An important issue only just beginning to be understood are the factors determining recycling and exit from the cilium as a means to determine the protein composition of this organelle (Lechtreck et al., 2009).

Cilia and the cell cycle

Since increased cell proliferation has been suspected for many years as a major underlying cause for cyst formation and expansion, it is relevant to discuss the link between cilia and the cell cycle (reviewed in Kim and Tsiokas, 2011). Cilia form in interphase cells during the G_1 or G_0 phase, and are disassembled as cells re-enter the cell cycle. A link between ciliary disassembly and cell cycle is suggested by members of the NIMA-related kinases, a family of serine/threonine kinases implicated in mitosis (reviewed in O'Regan et al., 2007). Depletion of Nek8 in zebrafish results in longer cilia (Liu et al., 2002) and targeting Nek1 and Nek8 is associated with cystic kidney disease. Several ciliary (e.g. TRPP2, fibrocystin/polyductin) (Rundle et al., 2004; Zhang et al., 2010) and IFT (e.g. IFT20, IFT52, and IFT88) (Deane et al., 2001; Follit et al., 2006, 2008) proteins localize to the mitotic spindle, suggesting a direct involvement in cell cycle progression and cytokinesis. IFT88 forms a complex with the microtubule-dependent, minus-end directed motor protein dynein-1 to transport microtubule-nucleating proteins such as γ-tubulin and EB1 to spindle poles to ensure formation of normal mitotic spindles (Delaval et al., 2011). In addition, loss of IFT88 has been shown to increase proliferation (Robert et al., 2007). The presence of integral membrane proteins such as the ion channel TRPP2 and fibrocystin as components of the mitotic spindle apparatus is poorly understood. Loss of polycystin-1 or transgenic overexpression of TRPP2 causes centrosomal and chromosomal instabilities (Battini et al., 2008; Burtey et al., 2008), suggesting a close functional relationship between the cilia/centrosome complex and control of mitosis.

Cilia and polarity

The earliest role for cilia in polarity was recognized at the embryonic node, a ciliated structure that defines the left–right asymmetry of the body during embryonic development (reviewed in Hirokawa

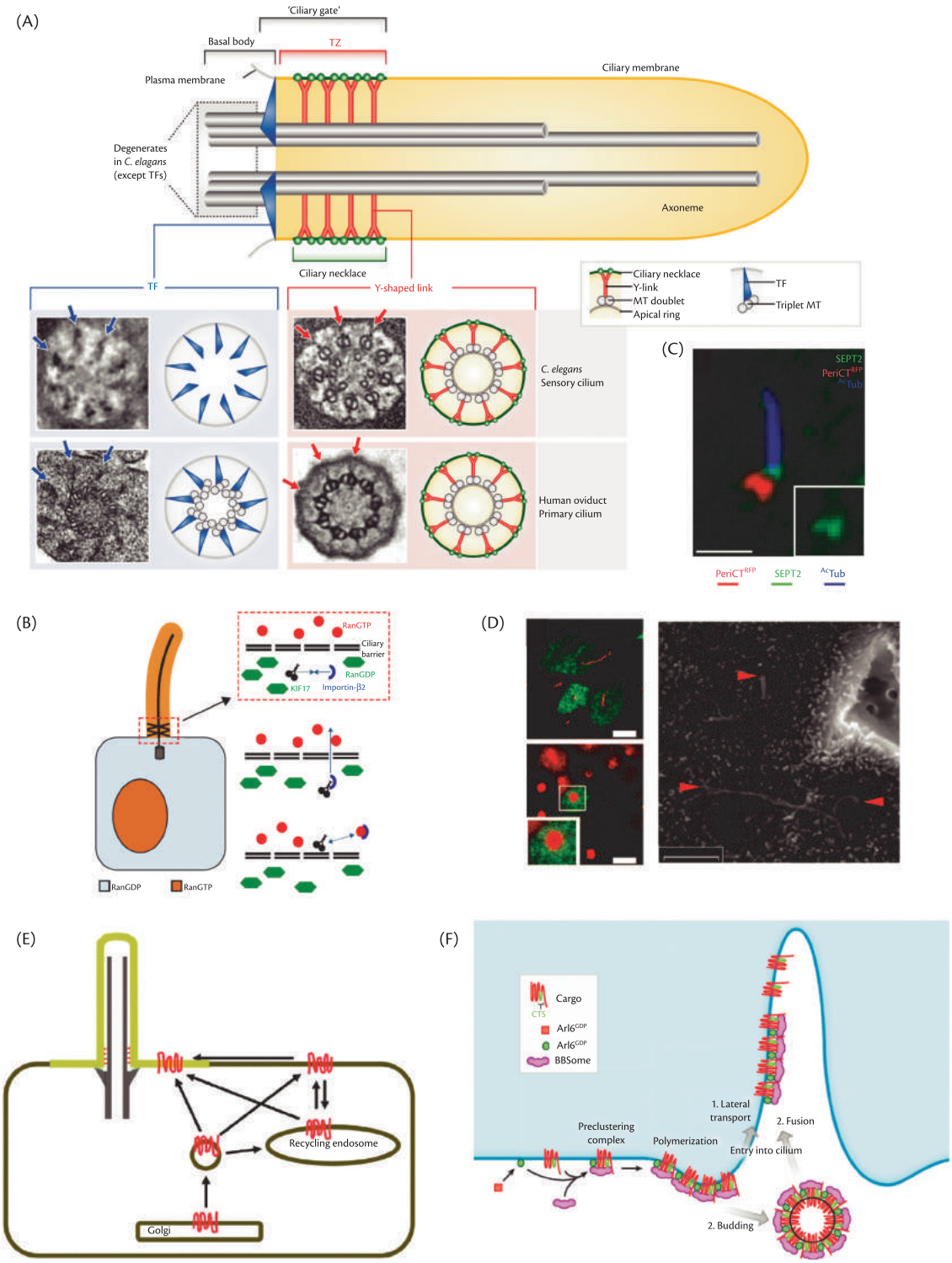

Fig. 303.2 Multiple pathways of protein transport to the cilium exist and access to the ciliary compartment is restricted by physical and molecular barriers. (A) Schematic view of the ciliary gate: transition fibres radiate from the microtubular triplets of the basal body to the plasma membrane and mark the transition to the microtubule doublet structure of the ciliary axoneme. Immediately distal lies the transition zone which contains Y-links that anchor the microtubules to the plasma membrane constituting the ciliary necklace. (B) The base of the cilium contains importins and uses an entry mechanism similar to the nucleus involving RanGTP/GDP to shuttle the monomeric KIF17 motor to the cilium. (C) A septin ring (green) forms a membrane diffusion barrier at the base of the cilium. (D) The cilium outgrowth site is surrounded by a circular membrane domain (CMD) devoid of microvilli (EM). It contains a different membrane composition as shown by exclusion of GPI anchored YFP-GL (green) and accumulation of galectin-3 (red). (E) Transport of transmembrane molecules to the ciliary compartment is not uniform and involves different rab GTPase directed vesicular transport routes. (F) A multi protein complex of BBS proteins ('BBSome') acts as a coat complex in shuttling proteins to the cilium.

Reproduced from Reiter et al. *EMBO Rep*, 2012; 13(7): 608–18 (A), Dishinger at al. *Nat Cell Biol*, 2010; 12(7): 703–10 (B), Hu et al. *Science*, 2010; 329(5990): 436–9 (C), Vieira et al. *Proc Natl Acad Sci U S A*, 2006; 103(49): 18556–61 (D left), Francis et al. *J Cell Biol*, 2011; 193: 219–233 (D right), Garcia-Gonzalo and Reiter. *J Cell Biol*, 2012; 197(6): 697–709 (E) and Nachury et al. *Annu Rev Cell Dev Biol*, 2010; 26: 59–87.

et al., 2009). Motile cilia are polarized to assume a coordinated beating pattern and move fluid or particles in a certain direction. The tilted monocilia which are localized at the posterior end of the node cells create a leftward flow that is sensed by cilia in the periphery involving TRPP2 and guides unilateral gene expression and subsequent left–right determination (Fig. 303.3A). In addition, cilia and polarity are connected in more complex ways. Three kinds of polarization characterize motile cilia (Wallingford, 2010): *rotational polarity*, that is, the orientation of the basal body determined by appendages such as the basal foot; *tissue-level polarity*, that is, the coordination of beating patters between different multi-ciliated cells; and *translational polarity*, that is, the position of the cilium within the two dimensions of the apical surface. The underlying programmes may have extensive implications for cystic kidney disease. Studies of the skin of the *Xenopus laevis* embryos, for instance, revealed that the polarization process is mediated by a positive feedback mechanism (Mitchell et al., 2007). Mechanosensory properties help motile cilia to sense the direction of the fluid flow, and to reorient their beating pattern accordingly (Fig. 303.3B). At a molecular level, this involves Dishevelled, an adaptor protein that plays a crucial role in non-canonical Wnt signalling (see below) and PCP. In *Drosophila*, PCP signalling is characterized by an asymmetric distribution of the PCP core proteins with Frizzled, Dishvelled, and Diego in the cell (Strutt and Strutt, 2009). Although first characterized in *Drosophila*, it is now clear that this evolutionary conserved signalling programme controls the morphogenesis of tissues and organs in vertebrates as well as mammals. Several studies have elucidated the relationship between cilia and PCP signalling. Knockdown of Dishevelled as well as the PCP effector proteins Inturned and Fuzzy prevent normal ciliogenesis (Park et al., 2006; Park et al., 2008; Gray et al., 2009). Conversely, the requirement for cilia to establish polarized structures has been shown for the inner hair cells of the organ of Corti: positioning of the centrosome requires expression of ciliary motor and IFT proteins and loss of these essential cilia proteins results in disordered stereocilia bundles (Jones et al., 2008).

Cilia serve as secluded organelles hosting several canonical signalling cascades implicated in complex cellular programmes, including cell proliferation, collective cell migration, and planar cell polarity. The prototypical ciliary signalling pathway in vertebrates is Hh signalling (Goetz et al., 2009; Wong and Reiter, 2008). The Hh signalling pathway orchestrates tissue patterning and organ development. Defective Hh signalling causes multiple congenital abnormalities, including neural closure defects, holoprosencephaly, and polydactyly, while inappropriate activation leads to cancer such as medulloblastoma or basal cell carcinoma. Vertebrate cells need intact cilia to respond to Hh. The first link between the cilium and the Hh pathway resulted from a forward genetic screen, which identified several IFT mutations associated with embryonic defects typical for abnormal Hh signalling (Huangfu et al., 2003; Huangfu and Anderson, 2005). The identification of Hh core components in the cilium (Corbit et al., 2005; Haycraft et al., 2005) has led to the current concept of Hh signalling in mammalian cells (Goetz and Anderson, 2010). In the absence of Hh, Ptch1 is present in the cilium and blocks accumulation of Smo in the cilium. Binding of Hh removes Ptch1 from the primary cilium (Rohatgi et al., 2007), allowing Smo to enter the cilium (Fig. 303.3C). At the tip of the cilium this promotes activation of the transcriptional activator Gli2 and its transport out of the cilium.

Wnt signalling is another pathway associated with cilia, albeit in a more complex manner (Wallingford and Mitchell, 2011). Wnt signalling is delineated by two branches, canonical beta catenin dependent and non-canonical Wnt signalling. In canonical Wnt signalling, soluble Wnt molecules bind to Frizzled receptors to recruit LRP co-receptors (Benzing et al., 2007; Lancaster and Gleeson, 2010). The Frizzled/LRP receptor sequesters Dishevelled and components of the β-catenin degradation machinery to initiate TCF-dependent gene transcription. While canonical Wnt signalling controls cell proliferation and cell fate, the non-canonical Wnt signalling pathway shapes tissues and maintains their function by controlling cell migration and orientation (Simons and Mlodzik, 2008). Excessive canonical Wnt signalling causes cyst formation. Transgenic mice expressing a degradation-resistant β-catenin develop severe cystic kidney disease (Saadi-Kheddouci et al., 2001). The ability of cilia to modulate Wnt signalling (Gerdes et al., 2007; Corbit et al., 2008) is supported by observations that knockdown of ciliary proteins associated with renal cyst formation (e.g. KIF3A, IFT88, PKD1, PKD2, and OFD1) causes abnormal Wnt signalling and upregulation of ß-catenin activity (Lin et al., 2003; Patel et al., 2008; Happé et al., 2009; Kim et al., 2009; Macca and Franco, 2009). However, recent reports showed that cilia per se are not required for canonical Wnt signalling (Huang and Schier, 2009; Ocbina et al., 2009); rather, cilia may serve to seclude Wnt components. For example, Jouberin shunts β-catenin to the cilium, dampening the response to Wnt3a (Lancaster et al., 2011b). In the absence of cilia Wnt signalling is hyper-reactive in response to Wnt3a, as Jouberin facilitates transport of β-catenin to the nucleus.

Mutations of ciliary gene products in mouse models typically affect kidney development during the second half of embryogenesis. At this time point, early kidney organogenesis such as cell fate determination and tissue specification has been completed, resulting in immature glomeruli and tubules. During the second half of kidney development, the tubules differentiate into mature nephrons, acquiring the highly specialized functions of each segment. Collective cell migration and oriented cell division appear to represent essential components of the morphogenetic programmes that generate tubules with precise final programmes to orient cells within the plane of a tissue. This insight came from the observation that Inversin, a protein mutated in nephronophthisis type II, is required for convergent extension movements during *Xenopus* gastrulation (Simons et al., 2005; Simons and Walz, 2006). Subsequent work revealed that mice mutant for *BBS* genes display abnormalities associated with planar cell polarity defects, including open eyelids, neural tube defects, and disrupted cochlear stereociliary bundles (Ross et al., 2005).

Two PCP-like events occur during kidney development: convergent extension (CE) and oriented cell division (Fig. 303.3D, E). Oriented cell division appears to extend the tubules of the growing kidney in the immediate postnatal period (Fischer et al., 2006). This observation indicates that dividing tubular epithelial cells are endowed with a positional cue along the anterior–posterior axis of the developing nephron. Mice defective in HNF1B or *pck* rats with a mutation in the human *Pkhd1* homologue fail to align the mitotic angles perpendicular to the axis of the nephron and develop cysts (Fischer et al., 2006). Defective oriented cell division was observed in other animal models of cystic kidney disease, including the kidney-specific elimination of KIF3A, which interferes with ciliogenesis in tubular epithelial cells (Patel et al., 2008). However,

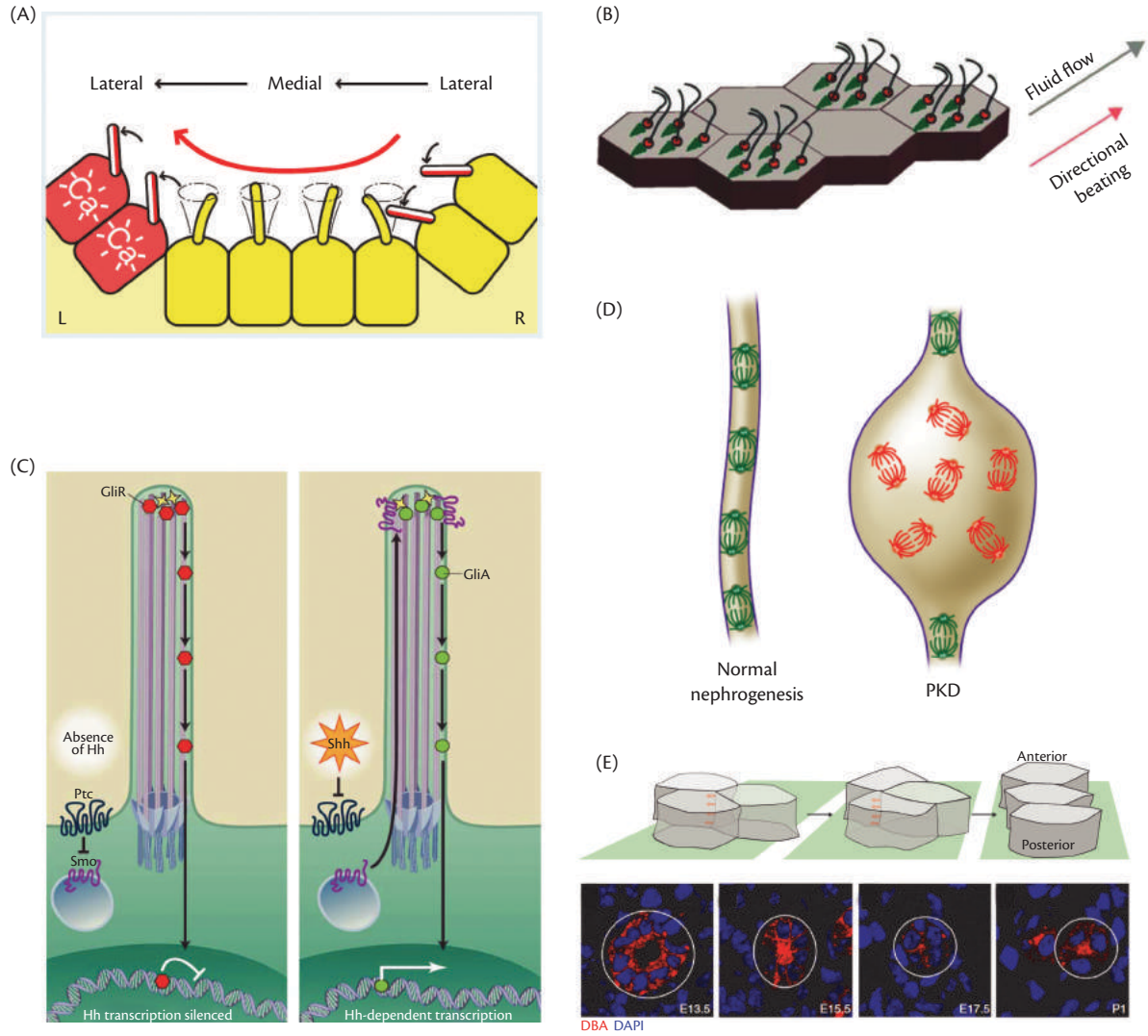

Fig. 303.3 Cilia are signalling platforms involved in polarity and patterning. (A) In the embryonic node central cilia create a leftward flow to transport fluid and particles. Flow is sensed at the left margin involving PKD2 and is required for left–right patterning of the embryo. (B) In the skin of the *Xenopus* embryo beating cilia generate fluid flow which orients the basal bodies (red) and basal feet (green) in a feedback loop. (C) The cilium is a signal transduction compartment for hedgehog (Hh) signalling. In the absence of secreted Hh protein patched retains the transmembrane receptor Smoothened (Smo) in the cell. Ciliary Gli repressor (red circles) silences transcription of target DNAs. Upon binding of Hh to Patched, Smo enters the cilium to activate Gli (green circles) and enable Gli-dependent transcription. (D) In different animal models of PKD, including cilia mutants, cysts are associated with misalignment of the mitotic spindles. (E) Cell intercalation is a mechanism in gastrulation which elongates the embryo (convergent extension—top panel). In tubule development a similar mechanism leads to successive narrowing of the tubules between E13.5 and P1. Note the decreasing number of nuclei per tubule diameter.

Reproduced from Marshall and Nonaka. *Curr Biol*, 2006; 16(15): R604–14 (A), Wallingford. *Curr Opin Cell Biol*, 2010; 22(5): 597–604 (B), Singla and Reiter. *Science*, 2006; 313(5787): 629–33 (C and D), Green and Davidson. *Nat Cell Biol*, 2007; 9(9): 1010–5 (E, top panel) and Karner et al. *Nat Genet*, 2009; 41(7): 793–9 (E, lower panel).

random orientation of the spindle axis during mitosis alone is not sufficient to cause kidney cysts. KIF3A mutant mice develop cysts only after injury repair. The *Pkhd1* (–/–) mouse model does not develop cysts despite randomized cell division, whereas *Pkd1* and *Pkd2* mutant mice develop randomization of mitotic spindles only after the onset of cyst formation (Nishio et al., 2010).

The intercalation of tubular cells by CE during tubular development leads to narrowing of the lumen (Fig. 303.3E). This process requires Wnt9b and when disturbed results in cyst formation (Karner et al., 2009). It is interesting to note that Wnt9b acts cell non-autonomously to regulate tubule diameter: Wnt9b depletion causes cyst formation predominantly in the proximal nephron segments; however, these segments do not normally express Wnt9b, raising the possibility that cyst formation ensues if complex morphogenetic programmes involving the entire nephron are disturbed. For example, this could encompass collective cell migration, which arises in distal nephron segments, but helps to shape the proximal nephron. In mice, the proliferative index remains high within the first 10 days after birth, consistent with the observation that differentiation and maturation of the rodent kidney continues postnatal (Piontek et al., 2007); this may be different in the human kidney. Whether CE requires cilia has not been determined, yet the role of cilia in Wnt signalling (see above) makes this an interesting possibility.

Principles of cyst formation

For many years, the attempt to find one unifying molecular pathogenesis dominated the research in PKD. Insight into the molecular functions of gene products mutated in cystic kidney disease (Hildebrandt et al., 2011) has changed this view, and suggests that kidney cysts represent a 'final common structural abnormality', which can arise from multiple different molecular defects.

Several pathways have been identified that are abnormally regulated in cell culture and animal models, or in tissues of ADPKD patients. Abnormally regulated signalling pathways identified in cystic kidneys do not necessarily establish a causal relationship between the signalling pathway and the molecular function of the mutated gene product. Another consideration is that multiple factors may contribute to cyst formation, and the aberrantly activated signalling pathways may change over time. To find effective therapies, it will be particularly important to identify 'canonical' signalling pathways that uniformly contribute to cyst growth and disease progression. As discussed below, two of these pathways, mammalian target of rapamycin (mTOR) and cyclic adenosine monophosphate (cAMP), have emerged as potential targets of pharmacological intervention to slow the progression of cystic kidney disease in human patients. However, inhibiting one pathway may allow cysts to divert to alternative growth pathways, resembling the drug resistance observed in cancer treatments. Thus, the initial hypothesis that cysts are 'benign tumours in disguise' may continue to be relevant and herald the need for multimodal drug therapies.

Genetic considerations

The two-hit hypothesis

Analysis of the cells lining the cysts in patients with ADPKD caused by mutations of *PKD1* revealed that these cells lost the second normal *PKD1* allele, in addition to the germline *PKD1* mutation, inherited from the affected parent. The two-hit-hypothesis in ADPKD suggests therefore that the disease, although inherited in an autosomal dominant fashion, is at the cellular level a recessive disease caused by tubular epithelial cells that lost both alleles of *PKD1* (Qian et al., 1996; Germino, 1997; Badenas, 2000) (Fig. 303.4A, B). This hypothesis was extended to ADPKD caused by mutations of *PKD2* (Wu et al., 1998), and confirmed by the mouse models generated with a targeted disruption of the mouse homologues for *PKD1* or *PKD2*. While mice with a homozygous deletion of *PKD1* or *PKD2* die during embryonic development or shortly after birth (Lu et al., 1997), heterozygote mice are largely unaffected and do not develop a significant number of kidney cysts. To explain the increased frequency of second, somatic PKD mutations, several theories including chromosomal instability have been invoked; yet mutational analysis of *PKD1* in many patients with ADPKD has not uncovered a particular hot spot that would have been the logical consequence. However, damage that forces tubular epithelial cells to regenerate accelerates cyst formation, suggesting that repetitive damage and repair may facilitate the loss of the second *PKD1* allele and provoke cyst formation (Weimbs, 2006, 2007; Happé et al., 2009, 2011a; Prasad et al., 2009), or cause cyst formation as a result of haplotype insufficiency (Prasad et al., 2009). Activation of mTOR in the context of repair mechanisms with compensatory hypertrophy of the kidney structures has recently been considered as the third hit in cystogenesis (Takakura et al., 2009; Bell et al., 2011; Weimbs, 2011).

The mutational load in polycystic kidney disease

Several observations are inconsistent with a two-hit-theory in ADPKD, and require modification of this hypothesis. Mouse models with a timed inactivation of *Pkd1* have demonstrated that PC1 is not essential for maintaining the adult kidney (Piontek et al., 2007). In humans, genotype–phenotype correlations have, with few exceptions, not revealed a close relationship between the causative mutation and the resulting clinical manifestations, indicating that other confounding factors determine the disease severity in PKD. Altering the levels of polycystin-1 or TRPP2 can cause cystic kidney disease in mouse and other animal models (Pritchard et al., 2000; Qian et al., 2003; Lantinga-van Leeuwen et al., 2004; Thivierge et al., 2006; Fu et al., 2008; Park et al., 2009), suggesting that haplotype insufficiency and/or unbalanced PKD protein levels contributes to the penetrance and manifestation of ADPKD (Rossetti et al., 2009). *TSC2*, a gene immediately adjacent to *PKD1*, has been identified early on as a confounding factor in ADPKD. Large deletions that disrupt both *TSC2* and *PKD1* result in severe ADPKD typically associated with early-onset end-stage renal disease (Brook-Carter et al., 1994); however, early ESRD has not been observed in all cases (Smulders et al., 2003), suggesting that even in this setting additional factors determine the clinical course of the disease. Although most renal cysts in ADPKD are characterized by a homozygous loss of either *PKD1* or *PKD2*, trans-heterozygote cysts with a germline mutation in one and a somatic mutation in the other gene have been found in both ADPKD1 (Koptides et al., 2000) and ADPKD2 (Watnick et al., 2000). While heterozygote mice are unaffected, compound heterozygote *Pkd1*(+/−) /*Pkd2*(+/−) mice develop renal cysts (Wu et al., 2002), supporting the clinical finding that germline mutations in either *PKD1* or *PKD2* can be aggravated by additional mutations in the corresponding gene. Bi-lineal disease with germline mutations in *PKD1* and *PKD2* (Pei et al., 2001) as well as compound heterozygote *PKD1* combined with homozygote *PKD2* mutations have been reported (Dedoussis et al., 2008). Additional genetic interactions with autosomal recessive disease genes are predicted from compound mouse models of cystic kidney disease. Deletion of one *Pkd1* allele aggravates the development of cystic kidney disease in *Pkdh1* (del3-4/del3-4) mice (Garcia-Gonzalez et al., 2007). In a complex genetic analysis using conditional knockout mice for *Pkd1, Pkd2, Pkhd1, Sec63*, and *Prkcsh, Pkd1* was identified as the rate-limiting gene product in cyst formation (Fedeles et al., 2011). In recessive diseases of the BBS/MKS spectrum compound heterozygote mutations have been shown to affect the severity of disease manifestations (Leitch et al., 2008). Based on these findings, it is very likely that next-generation sequencing will identify disease-aggravating mutations in other cystic kidney disease and ciliopathy genes. Indeed, compound mutations in addition to *PKD* have been found in patients with severe disease manifestations, for instance in HNF1β (Bergmann et al., 2011). A cumulative mutational load in PKD-related genes may also explain the accelerated course of disease in patients with ADPKD: the onset of end-stage renal disease appears to occur earlier in every following generation—an observation also known as anticipation (Fick et al., 1994; Reed et al., 2008); however, the underlying mechanism has remained elusive so far. It is conceivable that genetic factors, that is, the 'cumulative mutational load' in kidney disease-relevant genes, are the most important predictive factors for disease progression and onset of end-stage renal disease, that is, patients seem to accumulate more and more disease-facilitating mutations in other genes

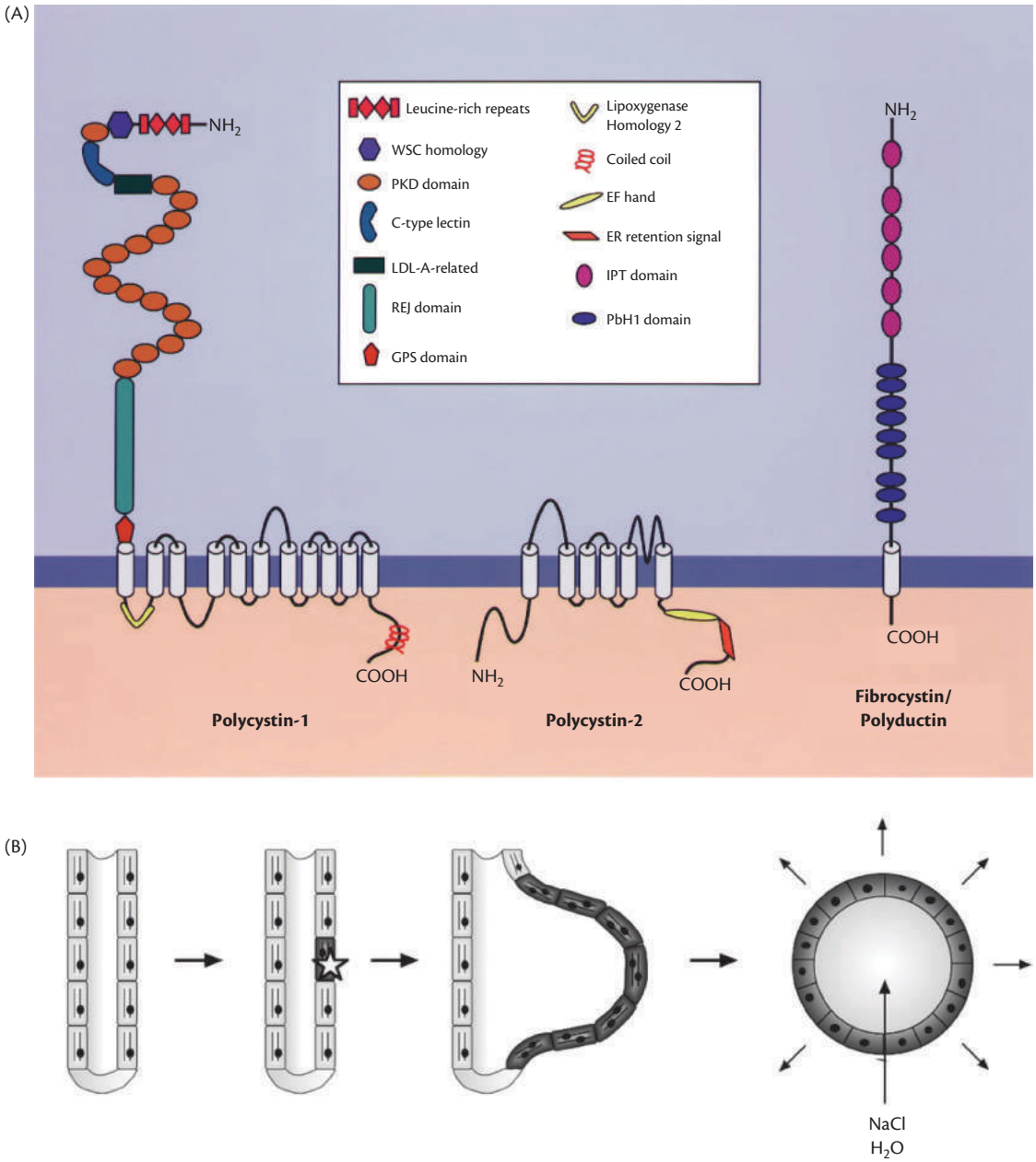

Fig. 303.4 (A) Domain structure of the proteins encoded by *PKD1*, *PKD2*, and *PKHD1*. (B) A cell in a heterozygote state receives a somatic mutation on the second locus (second hit). This triggers changes which after secondary events trigger cyst formation.
Reproduced from Igarashi and Somlo. *JASN*, 2002; 13(9): 2384–98 (A) and Köttgen. *Biochim Biophys Acta*. 2007; 1772(8): 836–50 (B).

that ultimately determine the onset of renal failure. Given the high prevalence of mutations in *BBS* genes despite the rare incidence of overt BBS, this appears to be a plausible hypothesis for accelerated disease in ADPKD families.

Ciliopathy proteins mutated in cystic kidney disease

Autosomal dominant polycystic kidney disease

PKD1 encodes for polycystin-1, a > 450 kD integral membrane protein with 11 transmembrane domains, while *PKD2* encodes for polycystin-2/TRPP2, a member of the TRP family of calcium-permeable ion channels (Fig. 303.4A) (The International Polycystic Kidney Disease Consortium, 1995; Mochizuki et al., 1996; Chapin and Caplan, 2010). Both gene products localize to the cilium, and have been implicated in flow- dependent calcium transients (Nauli et al., 2003; Sharif-Naeini et al., 2009; Boehlke et al., 2010b). PC1 undergoes N-terminal cleavage at a G-protein-coupled receptor proteolytic cleavage site located immediately before the first transmembrane domain. Cleavage within the C-terminus liberates 15- and 35-kD fragments that have been shown to translocate to the nucleus with components of the Wnt and Stat6 pathways (Chauvet et al., 2004; Zatti et al., 2005; Low et al., 2006; Lal et al., 2008; Merrick et al., 2012). In most cells, TRPP2 is retained in the endoplasmic reticulum due to a retention signal that is phosphorylated by casein kinase II, and subsequently recognized by PACS

adaptor molecules (Cai et al., 1999; Kottgen et al., 2005). TRPP2 retained in the ER or TGN has been suggested to interact in *trans* with membrane-associated polycystin-1; the close proximity of the TGN to the ciliary base would make such an interaction feasible; however, there is currently no experimental evidence that ER- or TGN-based TRPP2 interacts directly with ciliary or other transmembrane proteins. Nevertheless, ER-based TRPP2 controls cytoplasmic calcium levels and apoptotic thresholds (Wegierski et al., 2009), and appears to modulate cilia-dependent functions (Fu et al., 2008). The PC1/TRPP2 complex seems to control several pathways (Fig. 303.5) (Torres and Harris, 2009; Chapin and Caplan, 2010); however, it is difficult to discern whether this control originates in the cilium. PC1 curtails mTOR activity by stabilizing the TSC1/TSC2 complex (Distefano et al., 2009;Dere et al., 2010), and activates STAT1/STAT3, elevating p21, a Cdk2 and cell cycle antagonist (Bhunia et al., 2002; Talbot et al., 2011). PC1/TRPP2 may also be involved in the ciliary control of canonical and non-canonical Wnt signalling, although TCF/β-catenin reporter transgenes have not revealed increased activity in *Pkd1* or *Pkd2*-deficient mice during embryogenesis and cyst formation (Miller et al., 2011), and the role of *PKD1* and *PKD2* in regulation of canonical Wnt signalling and its relevance for cyst formation remains controversial (Wuebken and Schmidt-Ott, 2011). An involvement of the PC1/TRPP2 complex in non-canonical Wnt/PCP signalling is equally doubtful. Defective oriented cell division, a down-stream event controlled by PCP signalling, occurs in *Pkd1*- and *Pkd2*-deficient mice after the onset of cyst formation (Nishio et al., 2010). Furthermore, defective mitotic spindle alignment is also observed in heterozygote *Pkd1* (+/−) mouse kidneys in the absence of cyst formation, suggesting that disoriented cell division alone does not account for cystic kidney disease (Bonnet et al., 2009). The hypothesis that cyst formation and expansion is caused by persistent proliferation of cystic epithelial cells has strongly biased the interpretation of experimental findings, emphasizing the anti-proliferative or pro-apoptotic properties of PC1 or TRPP2. However, the morphogenetic programmes controlled by the PC1/TRPP2 complex to prevent cyst formation still remain largely elusive.

Autosomal recessive polycystic kidney disease

The gene product encoded by *PKHD1*, fibrocystin, is a large integral membrane protein with 4074 amino acids (Fig. 303.4A) (Onuchic et al., 2002; Ward et al., 2002), which associates with the basal body and the cilium (Menezes et al., 2004; Zhang et al., 2004). The overall domain structure of the protein suggests a functional involvement in cell–cell interaction similar to polycystin-1. However, as for polycystin-1, a definitive ligand for fibrocystin has not been identified. Transcription of *PKHD1* is regulated by hepatocyte nuclear factor-1B (HNF1B); hence, renal cysts observed in patients with maturity-onset diabetes of the young type 5 (MODY5) due to HNF1B mutations may result from defective *PKHD1* expression (Hiesberger et al., 2004). Deletion of different exons of the mouse homologue, *Pkhd1* is associated with severe biliary and

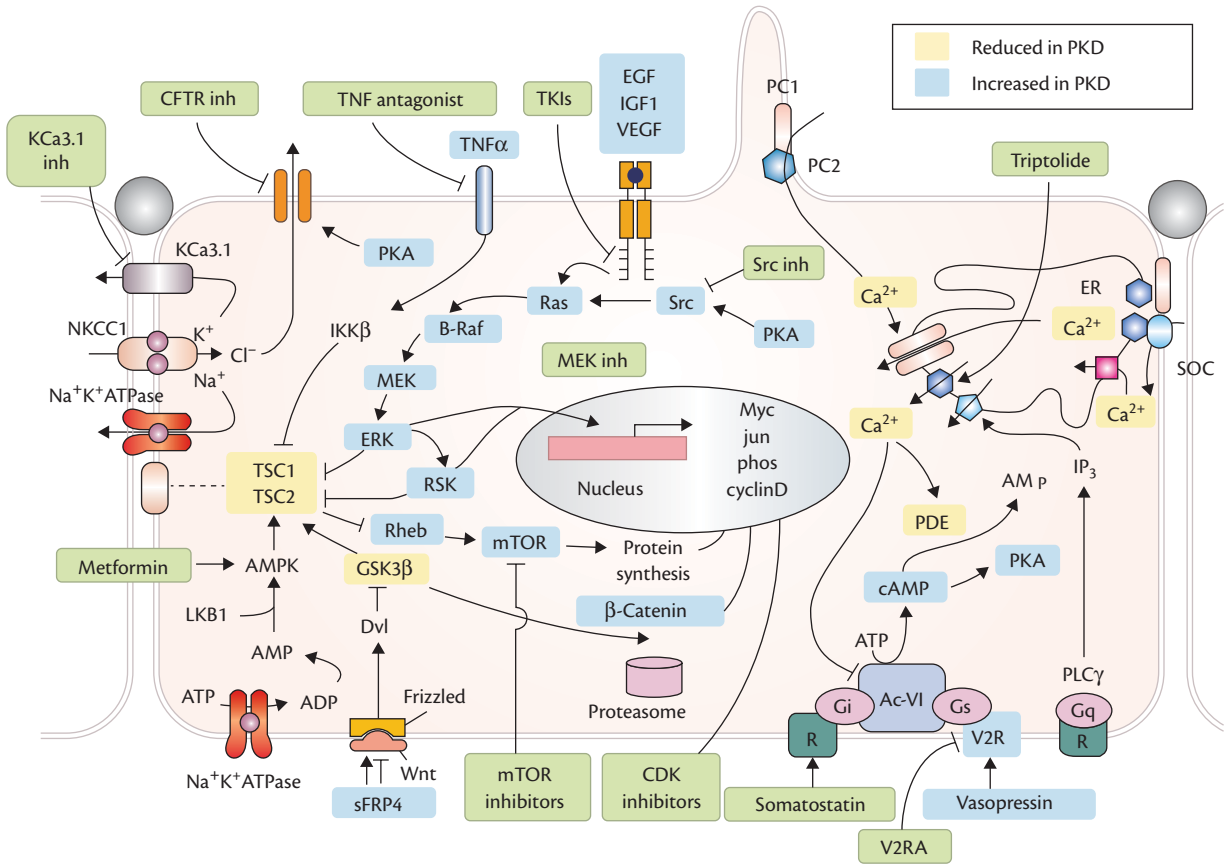

Fig. 303.5 Multiple signalling pathways are altered in PKD.
Reproduced from Torres and Harris. *Kidney Int*, 2009; 76(2): 149–68.

pancreatic dysgenesis, but with no or only mild renal abnormalities (Moser et al., 2005; Woollard et al., 2007; Gallagher et al., 2008). Fibrocystin interacts with TRPP2, and appears to regulate calcium transients mediated by TRPP2 (Wang et al., 2007). Mutation of *Pkhd1* in the rat (*pck*) rat and targeted knockout of *Pkhd1* in the mouse cause misoriented cell division; however, this is associated with cyst formation only in the rat model, while the *Pkhd1* knockout mouse does not develop overt renal cysts. It has been postulated that the misoriented cell division in the *Pkhd1*-deficient mouse is compensated by increased cell intercalation (Nishio et al., 2010). This hypothesis, although currently unproven, would suggest an exciting novel approach to prevent cyst formation by stimulating increased cell intercalation.

The NPHP/JBTS/BBS/MKS complex

Nephronophthisis (NPHP), the Joubert syndrome (JBTS), the Bardet–Biedl syndrome (BBS), and the Meckel–Gruber syndrome (MKS) have been historically classified into different entities. However, genetic analysis increasingly reveals that mutations of the same gene can cause different syndromic manifestations (Tobin and Beales, 2009). Most NPHP/JBTS/BBS/MKS gene products contain protein–protein interaction domains, and form complex protein networks among each other (Sang et al., 2011), supporting the idea that these proteins participate in overlapping signalling pathways to exert their molecular functions. Mutations in one component likely affect the composition of these protein complexes, resulting in syndrome-specific manifestation. This overview will focus on shared functions rather than highlighting individual family members.

Nephronophthisis

More than a dozen gene products have been identified that cause NPHP, characterized by kidney cysts and progressive renal failure in combination with specific extrarenal manifestations such as retinitis pigmentosa and cerebellar defects (see Chapter 317) (Benzing and Schermer, 2012; Shiba and Yokoyama, 2012). Some of these manifestations can be directly linked to ciliary defects. For example, the outer segments of photoreceptors are specialized cilia that are attached to the cell body by a connecting cilium, a structure that corresponds to the transition zone of primary cilia (Fliegauf et al., 2006). NPHP proteins typically localize to the connecting cilium, and seem to facilitate or control the delivery of lipids and proteins such as opsin required for the photon-transduction process as well as photoreceptor survival. Defective NPHP proteins result in photoreceptor degeneration and blindness (termed retinitis pigmentosa). The networks formed by NPHP products may also act as gate keeper that control access of proteins to the ciliary compartment (Omran, 2010). Although the precise molecular mechanisms remain unknown, NPHP and MKS proteins link the axoneme microtubules to the surrounding ciliary membrane (Williams et al., 2011), and together with JBST gene products appear to participate in protein sorting at the transition zone (Shiba and Yokoyama, 2012). However, NPHP gene products are also found outside of the cilium. For example, NPHP1, -4, and -8 form a distinct module (Sang et al., 2011) that localizes to the plasma membrane, where it may be involved in cell–cell contacts and the organization of the actin cytoskeleton. During kidney development in either zebrafish or *Xenopus* embryos, NPHP proteins seem to engage in morphogenetic programmes that involve cell migration. For example, NPHP4 is required for directed cell migration of distal pronephric duct cells and the formation of the cloaca opening in zebrafish, a process that requires the targeted contact with ectodermal cells that subsequently undergo apoptosis (Slanchev et al., 2011). NPHP2/Inversin is needed for directional cell movements within the proximal *Xenopus* pronephros (Lienkamp et al., 2010). These morphogenetic programmes may involve a crosstalk with non-canonical Wnt, a β-catenin-independent signalling cascade that shapes the wing and orients appendages during *Drosophila* development (Maung and Jenny, 2011; Lienkamp et al., 2012). NPHP2/Inversin interacts with Dishevelled, a central regulator of Wnt signalling, and can target this protein for proteasomal degradation, antagonizing canonical Wnt signalling (Simons et al., 2005). However, NPHP2 also promotes the translocation of Dishevelled to the plasma membrane in response to non-canonical Frizzled signalling, thereby facilitating planar cell polarity signalling (Lienkamp et al., 2010). Although other NPHP family members seem to antagonize canonical Wnt signalling (Burckle et al., 2011), it remains an unresolved puzzle to understand how these activities are linked to cilia and the morphogenetic programmes that shape the developing kidney.

Bardet–Biedl syndrome

More than 15 BBS proteins have been identified so far that cause cystic kidney disease together with extrarenal manifestation typical for the BBS (see Chapter 314) (Waters and Beales, 2011). Seven BBS proteins (BBS1, -2, -4, -5, -7, -8, -9) form a coat-like protein complex termed the BBSome (Nachury et al., 2007, 2010). Recruited by Arl6/BBS3, the BBSome coat facilitates the transport of cargo vesicles from the proximity of the ciliary base to the ciliary axoneme, controls the assembly of the IFT transport particles, and helps to reverse the transport direction at the tip of the cilium (Fig. 303.2F) (Wei et al., 2012). Other BBS proteins (BBS6, -10, and -12) form a complex with chaperons to support the assembly of the BBSome (Seo et al., 2010), suggesting that the BBS is largely the result of a defective ciliary transport machinery. BBS proteins, similar to NPHP proteins, have been implicated in Wnt signalling (Ross et al., 2005; Gerdes et al., 2007; Wiens et al., 2010), and mutations of BBS15 (Fritz), a WD repeat protein, is also involved in PCP signalling (Kim et al., 2010b). Whether these proteins affect the Wnt signalling cascade solely through their role in ciliary transport, or exert additional properties, is currently unknown.

Joubert/Meckel–Gruber syndrome

There is substantial overlap between the NPHP, BBS, Joubert, and MKS syndromes. Extensive NPHP gene defects, resulting in complete loss of gene function, are associated with the more severe MKS. For example, mutations of CEP290/NPHP6 are typically associated with nephronophthisis, but extensive mutations have also been identified in patients with BBS-, Joubert- and MKS-like disease (Baala et al., 2007). Conversely hypomorphic Meckelin (MKS3/TMEM67) mutations have been identified in patients with nephronophthisis type 11 (NPHP11) (Otto et al., 2009). The Joubert–MKS complex appears to act as a sorting complex at the transition zone (Garcia-Gonzalo et al., 2011), while Tectonic1 and -2 link MKS to defective ciliogenesis and Hh signalling (Garcia-Gonzalo et al., 2011; Sang et al., 2011). Jouberin, a gene product encoded by Ahi1 and mutated in Joubert syndrome, has been implicated in control of canonical Wnt signalling through sequestration of β-catenin within the cilium (Lancaster et al., 2011b), and defective regulation of canonical Wnt signalling also

appears to cause abnormal cerebellar midline fusion observed in a mouse model of Joubert syndrome (Lancaster et al., 2011a). These observations suggest that cilia-associated molecules (CAMs) affect Wnt signalling in a highly tissue-dependent manner: while blocking canonical Wnt in some tissues during certain developmental stages, the same ore other CAMs facilitate can also facilitate canonical Wnt signalling. Since most CAMs localize to specific subcellular localizations, their effects may be highly restricted to distinct cellular domains, and opposing effects may occur in different parts of the cell simultaneously.

The role of cilia and flow-dependent signalling

Primary cilia in renal epithelial cells protrude from the apical membrane into the tubular lumen and are ideally situated to act as flow sensors (Kotsis et al., 2013). Praetorius and Spring examined cilia in a cell based system and found that bending of the cilium elicits a calcium increase from internal stores (Praetorius and Spring, 2001). The same response was observed when ciliated cells were superfused by fluid. The calcium signal mediated by the cilium occurred with some delay and thus differed from mechanosensory signals provoked by direct stimulation of the apical membrane. Flow-induced calcium signals were also observed in explanted tubules from mouse kidneys and were altered in tubules from ift88 mutant cystic mice (Liu et al., 2005). The flow-induced calcium signals require Pkd1 and Pkd2 as was shown in immortalized cells from kidney cysts or mutant animals or protein depletion by inducible siRNA in MDCK cells (Fig. 303.6A) (Nauli et al., 2003; Xu et al., 2007; Boehlke et al., 2010b). Localization of TRPP2 in the cilium seems to be a prerequisite for flow-induced calcium currents. Loss of TRPP2 in the cilium has been demonstrated in Pkd1 and ift88 mutant cells and may explain altered calcium signals in these cells (Siroky et al., 2006; Xu et al., 2007). Furthermore, expression of a ciliary trafficking mutant TRPP2 suppresses the flow response but not expression of full-length or truncated TRPP2 (Kotsis et al., 2007). Depletion of TRPV4, a mechanosensory TRP channel that interacts with TRPP2 in the cilium abolishes flow sensing and TRPV4 mutant animals have a defect in flow-dependent potassium secretion, which suggests that TRPP2 forms hetero-tetrameric cation channels in the cilium to translate flow into calcium-dependent signalling (Taniguchi et al., 2007; Kottgen et al., 2008). *In vivo* evidence for the role of PKD2 in flow sensing comes from the embryonic node where peripheral cilia sense leftward flow employing TRPP2 to trigger unilateral gene expression which is required for left–right patterning (Pennekamp et al., 2002; Yoshiba et al., 2012). Yet, disruption of the ciliary flow sensor alone cannot explain cyst formation, since TRPV4 mutant zebrafish fail to form pronephric cysts and trpv4 null mice do not display a cystic phenotype (Taniguchi et al., 2007; Kottgen et al., 2008). Similarly, adult mice with induced cilia loss through inactivation of Kif3a or IFT88 fail to form cystic kidneys, demonstrating that cilia are not required to maintain normal tubular geometry in the absence of injury or increased cell proliferation (Davenport et al., 2007; Patel et al., 2008).

Irrespectively, the study of flow-induced downstream events has retrieved a number of findings that may be relevant for cyst formation. A C-terminal fragment is cleaved from PC1 and accumulates in the nucleus in kidneys with disturbed flow, for example, after ureteral obstruction (Chauvet et al., 2004). This fragment interacts with stat6 and its co-activator p100 and both are localized in cilia under flow (Low et al., 2006). In the absence of flow, stat6 translocates to the nucleus where it could enhance pro-cystic gene expression (Fig. 303.6B). Indeed, inactivating stat6 in polycystic mice or treatment with stat6 antagonists ameliorates cyst formation, indicating the biological relevance of this finding (Olsan et al., 2011).

Another set of data implicate ciliary flow sensing in polarity: The ciliary molecule inversin, mutated in NPHP type 2, interacts with Dishevelled and antagonizes the canonical Wnt pathway in favour of planar polarity signalling, seemingly involving flow (Simons et al., 2005). No direct evidence has linked flow sensing with tubular polarity programmes such as convergent extension and oriented cell division. Nevertheless, cilia-dependent polarity has been found in the epidermis of the developing *Xenopus* embryo where coordinated ciliary beating and flow affects the orientation of the basal bodies in a feedback loop (Mitchell et al., 2007). Flow chamber experiments in a cell-based system have shown that flow biases centriole movements requiring TRPP2-dependent calcium signals (Kotsis et al., 2008, 2013). These findings implicate cilia, flow, and calcium in polarizing events within epithelial cells.

Cilia regulate mTOR signalling and vice versa. The bending of cilia by flow activates a negative regulator of mTORC1: the Peutz–Jeghers kinase Lkb1 is localized in the cilium and phosphorylates AMPK at the basal body under flow (Fig. 303.6C) (Boehlke et al., 2010b). Since mTOR signalling is a controller of cell size this results in smaller cells and would be expected to affect the tubular diameter and shear stress within tubules. Interestingly, this mechanism is independent of TRPP2 and calcium signals. Studies in zebrafish and *Chlamydomonas* have revealed that mTOR activity positively regulates cilia length in a biologically relevant manner (Yuan et al., 2012). Therefore, mTOR may be part of a physiological feedback loop regulating cilia length depending on the amount of flow and shear stress (Kotsis et al., 2013). It is noteworthy that metabolic activation of the cilium recapitulates negative mTOR regulation via Lkb1 and AMPK in the cilium/basal body compartment without involvement of fluid flow (Teperino et al., 2012). This implies that the ciliary flow response may involve chemosensing or that flow is one of many mechanisms to engage this organelle. Indeed, purinergic signalling has been shown to play a part in ciliary flow sensing, and chemosensation has been postulated to mediate flow sensing at the embryonic node (Tanaka et al., 2005; Praetorius and Leipziger, 2009).

Abnormal cell activation and signalling

Since an increased proliferative index was detected in epithelial cells isolated from human ADPKD kidneys, increased cell proliferation has been the main paradigm for cyst growth for many years. This growth hypothesis was supported by the finding that overexpression of oncogenes, for example, c-myc, associated with increased cell proliferation, causes massive cyst formation (Trudel et al., 1991). However, genetic deletion of the death repressor Bcl-2, resulting in extensive apoptosis in many tissues, also causes cyst formation (Veis et al., 1993). Subsequently, increased apoptosis rates have been observed both in animal models (Savill, 1994) and human ADPKD (Woo, 1995), suggesting that an imbalance between proliferation of cyst tissue and apoptosis of neighbouring normal tissue causes cyst formation (Grantham, 1995; Lanoix et al., 1996).

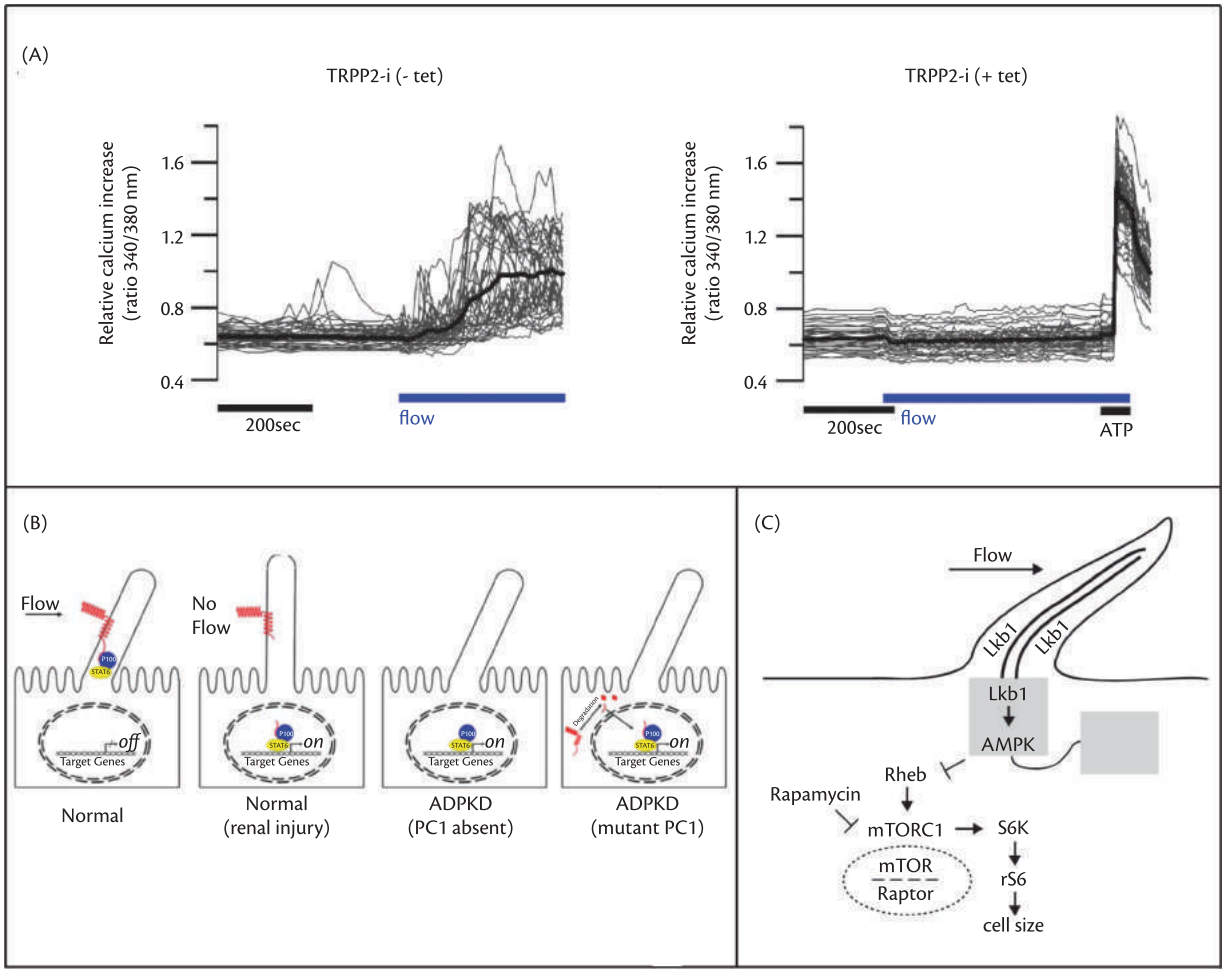

Fig. 303.6 Bending of the cilium by flow activates intracellular signalling. (A) Superfusion of ciliated cells in a flow chamber results in calcium release from internal stores as measured by fura-2 ratio imaging. After depletion of TRPP2 by tetracycline inducible siRNA no flow-induced calcium increases occur. (B) Model of Stat-dependent signal transduction under flow. The C-terminus of polycystin 1 (PC1) interacts with Stat6 and p100 in the cilium. Cessation of flow leads to cleavage of the PC1 C-terminus and translocation of the complex to the nucleus where it activates the transcription of target genes. In the absence or mutation of PC1 constitutive nuclear accumulation of the Stat complex occurs. (C) Bending of cilia by flow activates mTOR signalling. The kinase LKB1 accumulates in the cilium and upon engagement by flow phosphorylates AMPK at the basal body. AMPK inhibits the mTORC1 activator Rheb through the TSC complex (not shown. See Fig. 303.7).
(A) and (C) from Boehlke et al. *Nat Cell Biol*, 2010; 12(11): 1115–22). (B) reproduced from Low et al. *Dev Cell*, 2006; 10(1): 57–69.

STAT pathway

Polycystin-1 activates the JAK/STAT pathway, thereby upregulating p21WAF to arrest cells in G_1/G_0 (Bhunia et al., 2002). The C-terminal domain of polycystin-1 undergoes cleavage, can interact with STAT6 and P100, and has been shown to translocate to the nucleus (Low et al., 2006). Both membrane-associated and cleaved polycystin-1 activate STAT3 (Talbot et al., 2011). Sustained STAT3 upregulation is seen after ischaemia and in mouse models of cystic kidney disease, and in human ADPKD (Leonhard et al., 2011; Takakura et al., 2011). Both the non-selective mTOR and STAT inhibitor curcumin as well as the anti-parasitic compound and STAT inhibitor pyrimethamine ameliorate the progression of cystic kidney disease supporting a role of the STAT signalling pathway in cystogenesis (Leonhard et al., 2011; Takakura et al., 2011).

Activation of protein kinases and gene transcription

The signalling pathways controlled by polycystin-1 and TRPP2 have been carefully reviewed (Chapin and Caplan, 2010). The C-terminal cytoplasmic tail of polycystin-1 entails multiple properties that have been studied in cultured cells. Overexpression of the C-terminal domain of polycystin-1/TRPP1 induces activation of PKC and JNK, resulting in AP-1-dependent gene activation; this activation is blocked by dominant negative Rac1 or Cdc42 (Arnould et al., 1998). The C-terminus also contains a trimeric G protein activation domain, which appears to activate the NFAT pathway (Parnell et al., 1998; Parnell et al., 2002; Puri et al., 2004). However, these observations are confounded by endogenous levels of polycystin-1, resulting in either facilitating or dominant-negative responses elucidated by overexpression of the C-terminal polycystin-1 domain (Basavanna et al., 2007).

Control of cAMP levels

Abnormal cAMP concentrations have been long implicated in the pathogenesis of autosomal PKD and cyst formation (Friedlander and Amiel, 1987; Mangoo-Karim et al., 1989). Epithelial cells derived from ADPKD cysts contain elevated cAMP levels, and respond to cAMP agonists with fluid secretion and proliferation; furthermore, cyst fluid was found to contain a lipophilic substance

that promoted cAMP production and fluid secretion (Ye et al., 1992; Grantham et al., 1995a, 1995b; Yamaguchi et al., 2000; Belibi et al., 2004). Upregulation of cAMP seems to be a universal feature of cystogenesis and disease progression, since increased cAMP levels have been detected in several animal models of cystic kidney disease, including the *Pkhd1* rat model (*pck*), the *NPHP3* (*pcy*), and *Pkd2* mouse model (Torres, 2005). Inhibition of cAMP by vasopressin-2-receptor antagonists or genetic inactivation of ADH ameliorates disease progression in these models (Gattone et al., 2003; Torres et al., 2004; Wang et al., 2008), providing the preclinical basis for clinical trials in patients with ADPKD. Ciliary TRPP2 forms a complex with A-kinase anchoring protein 150 (AKAP150) and phosphodiesterase 4C (PDE4C) that controls the activity of adenylyl cyclase 5/6 and production of cAMP. Ciliary defects release the activity of AC5/6, resulting in increased cAMP levels (Choi et al., 2011).

mTOR activity

The mTOR signalling cascade has evolved as one of the key pathways involved in pathogenesis of disease progression and as a potential target for therapies designed to slow cyst growth (Huber et al., 2011). The mTOR protein kinase was originally discovered in yeast as the target of rapamycin, an immunosuppressive drug that slows cell growth in response to growth factors and nutrients (Heitman et al., 1991). In mammalian cells, mTOR consists of two kinase-containing complexes, mTORC1 and mTORC2, where rapamycin acts primarily on the first complex (Dazert and Hall, 2011). While nutrients and growth factor signalling activate mTORC1 to stimulate protein biosynthesis and cell growth, it is inhibited by cell stress via the kinase Lkb1 which is mutated in Peutz–Jeghers syndrome (Fig. 303.7). Inhibition of mTORC1 occurs through a complex of TSC1/TSC2, mutated in tuberous sclerosis, where positive as well as negative mTORC1 inputs are integrated.

The first indication that the mTOR kinase is involved in the progression of cystic kidney disease came from reports demonstrating that inhibition of mTOR by rapamycin ameliorates cyst formation and expansion in the Han:SRPD rat model of cystic kidney disease (Tao et al., 2005; Wahl et al., 2006). Subsequent work showed that the beneficial effect of rapamycin was not limited to the rat model, but reproducible in several mouse models of cystic kidney disease, including those relevant for human disease (Shillingford et al., 2006, 2010). Furthermore, signs of increased mTOR activity were detectable in the epithelial cells lining the cysts of ADPKD patients (Shillingford et al., 2006; Qian et al., 2008), and a limited analysis of ADPKD patients that had received rapamycin after kidney transplantation suggested that kidney cysts shrink in response to mTOR inhibition (Shillingford et al., 2006). These findings spurred a substantial interest in elucidating the role of mTOR in the pathogenesis and treatment of ADPKD. Depletion or lack of polycystic kidney disease proteins including polycystin-1 (Shillingford et al., 2010), TRPP2 (Spirli et al., 2010), OFD1 (Zullo et al., 2010) and fibrocystin (PKHD1) (Fischer et al., 2009; Zheng et al., 2009; Becker et al., 2010) results in upregulation of mTOR activity. However, how these proteins control mTOR activity is only partially understood. The C-terminus of polycystin-1 interacts with *TSC2* gene product Tuberin (Shillingford et al., 2006), and appears to protect *TSC2* against Akt-mediated phosphorylation and sequestration (Dere et al., 2010). Polycystin-1 also appears to inhibit the phosphorylation and inactivation of Tuberin on S664 by extracellular signal-regulated kinases (ERKs) (Distefano et al., 2009). Another report links the hyper-activation of mTOR to a defective ubiquitylation of c-Met and increased responsiveness to hepatic growth

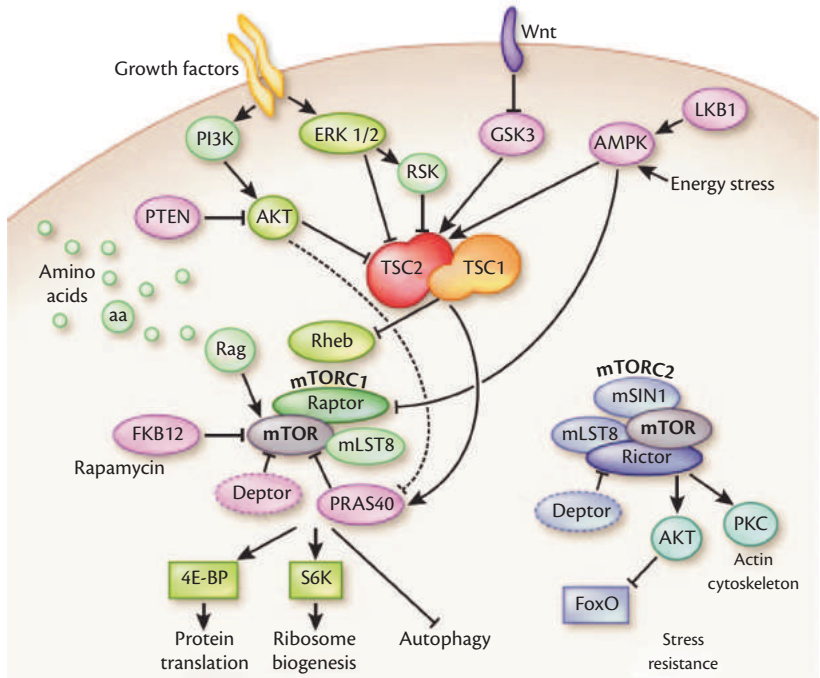

Fig. 303.7 The mTOR pathway. A multi-protein complex including mTOR (mTORC1) regulates cell mass through modification of protein biosynthesis and autophagy. The TSC complex acts as the central controller of mTORC1 activity and is regulated through various inputs including AMPK.
From Huber et al. *Kidney Int*, 2011 Mar;79(5):502–11.

factor (HGF), the ligand that stimulates the c-Met receptor, which leads to increased Akt activation (Qin et al., 2010).

Cilia control mTOR activity in a flow-dependent fashion (Boehlke et al., 2010b). Flow activates the kinase LKB1, which is present in the cilium (Fig. 303.6C). LKB1 phosphorylates and activates AMPK, resulting in inhibition of mTOR activity. Absent flow or defective cilia, for example, caused by knockdown of KIF3A, result in increased activity of mTOR associated with an increased cell size (Wrighton, 2010). Abnormal cell size regulation can also be observed *in vivo* in KIF3A-deficient kidney tubules, consistent with earlier findings reporting enlarged cells lining the cysts in kidneys of ADPKD patients (Grantham et al., 1987). If ciliary defects prevent flow-dependent AMPK activation by LKB1, pharmacological activation of AMPK should inhibit mTOR-dependent kidney cyst formation. Indeed, metformin, an activator of AMPK, can ameliorate cyst formation in animal models of PKD (Seo-Mayer et al., 2011; Takiar et al., 2011).

Hippo signalling

The Hippo pathway regulates the apical membrane and organ size through proliferation and apoptosis in response to polarity signalling and mechanical cues (Fig. 303.8). The Hippo cascade involves several protein kinases and adaptor molecules to control the activity of the transcriptional activator YAP1 (reviewed in Harvey and Hariharan, 2012). Active Hippo signalling results in sequestration of YAP1 in the cytoplasm and translocation of YAP to the nucleus when the pathway is switched off. Recently, the Hippo signalling pathway has been implicated in normal pronephros formation (Skouloudaki et al., 2009) and as a downstream target of the ciliary molecules nephrocystin-4 and -9 (Habbig et al., 2011, 2012). Interesting work in the ciliated unicellular protozoan *Tetrahymena* has shown that Mob1, a member of the Hippo cascade, has a role in ciliogenesis and is crucial for the establishment of cell polarity, although it is not clear that the two are related(Tavares et al., 2012). In PKD, nuclear accumulation of YAP has been found in cyst lining cells (Happé et al., 2011b).

Injury

Perhaps most informative was the generation of constitutive and conditional mouse knockout models. Homozygote *PKD1* and *PKD2* (−/−) mice develop severe cystic kidney disease that develops at around embryonal day 15–16 (E15–16). Cyst formation is accompanied by multiple additional organ manifestations, highlighting the broad role of these two molecules for the normal development of other tissues. Importantly, the inducible deletion of *Pkd1* uncovered a critical window for cyst formation in mice: deletion of *Pkd1* within the first 2 postnatal weeks causes rapidly progressive cyst formation, while a deletion past this time point results in mild disease with a slowly progressive cystic kidney phenotype (Lantinga-van Leeuwen et al., 2007; Piontek et al., 2007). The critical window for cyst formation coincided with a drop in cell proliferation, suggesting that PC1 function is required in rapidly developing tissues. Similar results were obtained after conditional elimination of cilia, using a KIF3A or IFT88 conditional mouse model (Davenport et al., 2007), confirming that cilia are not permanently required in adult tissues. However, ischaemic damage, forcing the kidney to undergo proliferation, facilitated cyst formation (Patel et al., 2008; Takakura et al., 2009), indicating that regenerating tubules require intact cilia. These findings reveal that cilia and cilia-dependent

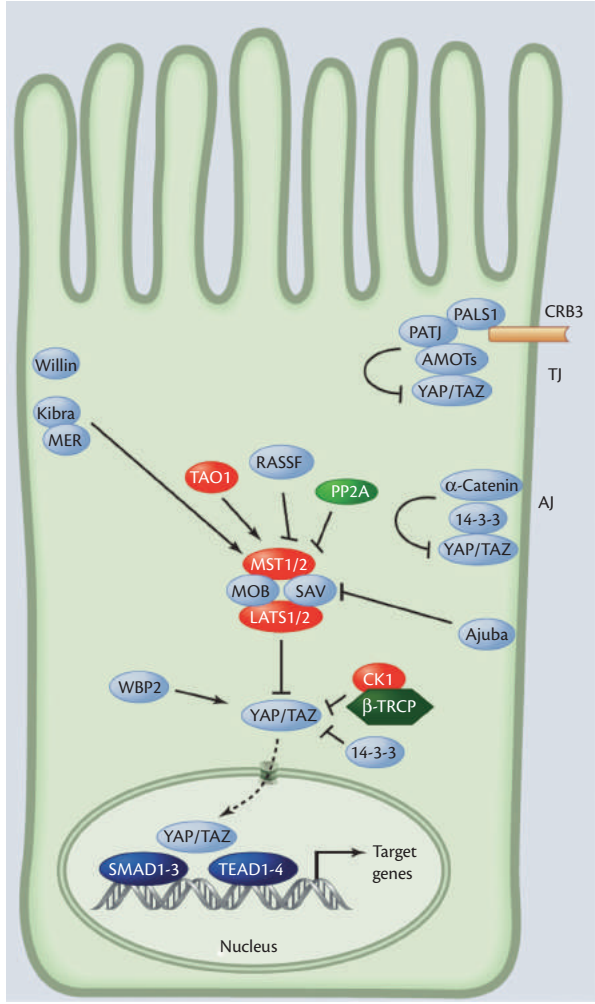

Fig. 303.8 The mammalian Hippo pathway, a regulator of organ size. Multiple inputs converge on a kinase complex including Hippo (MTS1 and 2 in mammals) and Warts (LATS1/2 in mammals) to phosphorylate and inhibit Yorkie (mammalian YAP/TAZ), a transcriptional co-activator. Active Yorkie increases transcription of positive cell growth regulators and inhibits apoptosis.
Reproduced from Harvey and Hariharan. *Cold Spring Harb Perspect Biol*, 2012; 4(8):a011288.

programmes are absolutely essential during development to ensure normal renal morphogenesis and tubular integrity. In mature kidney tubules, cilia are required for repair, supporting the hypothesis that renal regeneration recapitulates developmental programmes. If cilia are needed as mechano-, chemo-, or pressure-sensors, or in any other capacity remains unknown. Furthermore, cilia-independent functions are now increasingly recognized for IFT and other ciliary proteins (Finetti et al., 2009; Baldari and Rosenbaum, 2010; Finetti et al., 2011). The animal models cannot differentiate between ciliary and non-ciliary functions during embryogenesis and/or in tissue homeostasis.

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CHAPTER 304

The adult with renal cysts

Yves Pirson and Olivier Devuyst

Introduction

The vast majority of renal cysts derive from tubules. They are lined by an epithelial layer and filled with ultrafiltrate. Renal cysts of non-tubular origin (glomerular, lymphatic) are much rarer. Renal cysts found in the adult encompass a large number of conditions that may be categorized as hereditary, developmental, or acquired (Tables 304.1), knowing that some of the developmental category probably have a genetic basis. With improving sensitivity and increasing number of imaging procedures, a significant proportion of renal cysts are detected fortuitously. This chapter aims to guide the clinician investigating an adult subject found with renal cysts.

Keeping in mind the overall prevalence of the main renal cystic diseases (see Tables 304.1), the practitioner should first analyse the imaging findings (number and aspect of cysts, uni- or bilaterality, size of the kidney, existence of other renal or extrarenal abnormalities) as a preliminary clue to the differential diagnosis. The next step should be to integrate the information provided by the patient's investigations, family history, and any extrarenal feature. In many cases, the above information will be sufficient to make the diagnosis. When this is not the case, proper investigation of first-degree relatives may reveal it. In the category of hereditary disorders, a genetic analysis is then usually performed to establish a molecular diagnosis. Autosomal dominant polycystic kidney disease (ADPKD) is a noticeable exception, due to the complexity of genetic analysis.

With ultrasonography (US) and computed tomography (CT) or magnetic resonance imaging (MRI) of the kidney as a starting point, the following settings are successively considered:

- A single or a few cysts, uni- or bilaterally, in normal or small-sized kidneys
- Multiple cysts, unilaterally, in a normal or enlarged kidney
- Multiple cysts, bilaterally, in normal or small-sized kidneys
- Multiple cysts, bilaterally, in enlarged kidneys.

Only cystic diseases not covered in other chapters will be detailed here.

A comprehensive description of all cystic diseases of the kidney is outside the scope of this chapter and may be found elsewhere (Pirson et al., 2005; Torres and Grantham, 2008).

A single or a few cysts, uni- or bilaterally, in normal or small-sized kidneys

Simple renal cysts are common. Their prevalence depends on the resolution of the imaging technique and it increases with age. By US, at least one cyst in one kidney is found in 2% of normal people aged 30–49 years, in 11% of those aged 50–70 years, and in 22% of those aged > 70 years, and at least one cyst in each kidney in 1%, 4%, and 9% of these age groups, respectively (Ravine et al., 1993). By contrast-enhanced CT, at least one cyst ≥ 5 mm is found in 39% of potential kidney donors aged 19–49 years and in 63% of those aged 50–75 years (Rule et al., 2012). Simple cysts occur mainly in the cortex but may also be found in the medulla; they usually measure 0.5–10 mm but cysts up to 100 mm are not uncommon. Simple cysts increase in number and most often in size—at a mean growth rate of 4% per year (Terada et al., 2002)—with age. Cyst walls are thin and smooth and there is no contrast enhancement. Rarely, they are septated or contain calcium deposits, thus needing distinction from multilocular cysts or renal cell carcinoma, respectively (see below).

The presence of a few simple cysts was previously considered of little relevance in healthy adults. However, their recently described association with albuminuria, hypertension, and hyperfiltration (Grantham, 2012; Lee et al., 2012; Rule et al., 2012) suggests that simple cysts may in fact be a marker of early kidney injury, helping to identify patients at increased risk of developing chronic kidney disease. Interestingly, it has also been shown that some *PKD1* gene mutations are associated with mild cystic renal disease (Pei et al., 2012) which may be interpreted as simple, acquired cysts.

The characteristics of simple cysts are similar to those of uncomplicated cysts in early ADPKD. The presence of liver cysts or familial history may reveal the existence of ADPKD. The US criteria to distinguish simple cysts and ADPKD in at-risk families are detailed in Chapter 309.

Though simple cysts are usually asymptomatic, a large one may cause abdominal, flank, or lumbar discomfort or pain. Cyst infection and gross haematuria occur rarely; the latter should not distract from the investigation of another cause of haematuria.

A complicated simple cyst should be distinguished from a cystic renal cell carcinoma, which is a variety of clear cell carcinoma characterized by its cystic nature with < 25% of solid component (Suzigan et al., 2006). The Bosniak classification is a well-established, widely used method that uses CT findings to categorize complex cysts on the basis of imaging features associated with malignancy (Bosniak, 2012). In brief, Bosniak categories I and II lesions are simple and mildly complex cysts, respectively, requiring no further evaluation, though a subcategory IIF was subsequently introduced to define lesions needing follow-up. Category III refers to complex cysts with suspected malignant potential; they are most commonly managed with surgical excision. Category IV are lesions with almost certainly a malignant component (Smith et al., 2012).

Table 304.1 Hereditary renal cystic diseases in the adult

	Prevalence	Imaging findings	Extrarenal features
Autosomal-dominant			
Autosomal-dominant polycystic kidney disease (ADPKD)	1:1000	See Chapter 308	See Chapter 309
Tuberous sclerosis complex (TSC)	1:10,000	See Chapter 330	See Chapter 330
Von Hippel–Lindau disease (VHL)	1: 50,000	See Chapter 332	See Chapter 332
Hepatocyte nuclear factor 1B disease (HNF1B)	?	See Chapter 309	See Chapter 309
Glomerulocystic kidney disease (GCKD)	Very rare	See Chapter 305	No
Medullary cystic kidney disease (MCKD)	1:100,000	See Chapter 318	See Chapter 318
Hereditary angiopathy-nephropathy-aneurysms-muscle cramps syndrome (HANAC)	Very rare	See Chapter 325	See Chapter 325
Autosomal-recessive			
Autosomal-recessive polycystic kidney disease (ARPKD)	1:50,000	See Chapter 313	See Chapter 313
Nephronophthisis (NPHP)	1:100,000	See Chapter 317	See Chapter 317
X-linked			
Oral-facial-digital syndrome, type 1 (OFD1)	1:100,000	See Chapter 319	See Chapter 319

In other rarer conditions summarized below, imaging characteristics of the cystic process may point to a specific developmental or acquired condition (Tables 304.2 and 304.3) whereas certain associated renal or extrarenal features may orientate to a given inherited disorder (Table 304.1).

When cysts are confined to the medulla, medullary sponge kidney should be suspected. This is reinforced by the existence of stones/calcifications into the dilated tubules constituting the cysts (see Chapter 311). Of note, medullary sponge kidney may be superimposed to ADPKD and may be associated with multiple congenital abnormalities including hemihypertrophy (Rommel and Pirson, 2001) and Ehlers–Danlos or Marfan syndrome. The disease is associated with gross and microscopic haematuria as well as urinary tract infections.

When cysts are confined to the renal sinus, parapelvic cysts should be suspected. They usually appear as round or ovular cavities surrounding pelvis (Fig. 304.1). They may be multiple and bilateral. When they are large, their US appearance may mimic hydronephrosis. They are most frequently diagnosed after the age of 30 years. They are of lymphatic origin and lined by a layer of endothelial cells. They do not compromise kidney function.

When a cyst, usually unique, appear as saccular diverticulum from a calyx or from the pelvis—and thus filled by contrast dye—a

Table 304.2 Developmental renal cystic diseases in the adult

Disease	Prevalence	Imaging findings
Pyelocalyceal cyst	1: 1000	Usually unilateral, often unique diverticula arising from pyelon or calyx ± stone
Medullary sponge kidney	1: 5000	Cystic dilatation confined to the medulla, ± stones in pre-calyceal areas
Multicystic dysplasia	1: 5000	Unilateral non-functioning kidney, replaced by a multiloculated cystic mass
Unilateral renal cystic disease	Very rare	Multiple cysts (resembling ADPKD) in only one kidney, with normal excretory function
Multilocular cystic nephroma	Very rare	Multilocular (with septa) mass in one kidney

Table 304.3 Acquired renal cystic diseases in the adult

Disease	Prevalence	Imaging finding
Simple cysts	39% at age 50 63% at age 75 (by CT/MRI)	Simple or multiple, often cortical, thin and smooth wall
Renal cystic carcinoma	Rare	Uni- or multilocular cystic mass with a solid carcinoma component; see Bosniak classification
Cystic disease of the renal sinus (or parapelvic cysts)	1: 1000 in the elderly	Uni- or bilateral, often multiple; localized in the parapelvic area
Acquired cystic kidney disease	12% in ESRD 50% > 5 years of dialysis 100% > 10 years of dialysis	Multiple, varying size cysts in usually atrophic kidneys
Hydatic cyst	Rare	Large calcified cyst containing daughter cysts; most often similar liver cysts
Lithium-induced cysts	Rare	Multiple microcysts (rarely >3 mm) in normal sized kidneys
Hypokalaemia-related cysts	Rare	A few simple cysts, often medullary

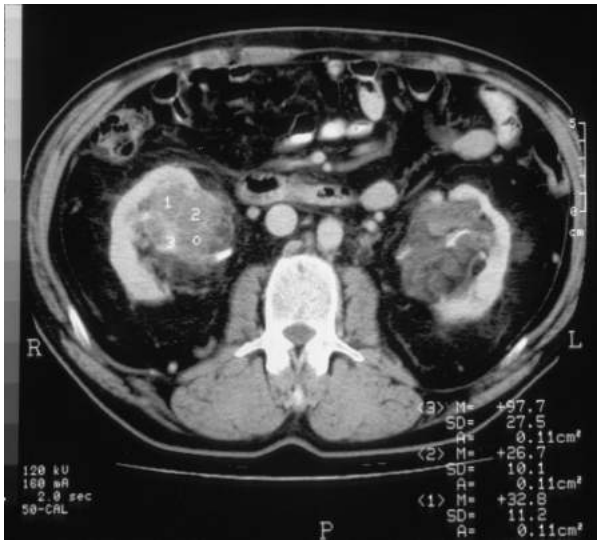

Fig. 304.1 Contrast-enhanced CT showing bilateral and multiple cysts of the renal sinus causing in this (exceptional) case severe urinary obstruction.

pyelocalyceal cyst is diagnosed. This diverticulum predisposes to stone formation and may thus be revealed by haematuria or pain.

A unique multilocular cyst with septa, not connected to the pyelocalyceal system, should evoke the diagnosis of multilocular cystic nephroma, which is a very rare, benign neoplasm.

Chronic potassium depletion such as in primary aldosteronism may induce the development of kidney cysts, predominantly in the medulla (Torres et al., 1990). Renal cysts observed in primary distal tubular acidosis could also be explained by hypokalaemia (Torres et al., 1990).

Hydatic disease may cause cysts in various organs, including the kidney. This condition is due to the larval form of the cestode *Echinococcus*, transmitted by contact with infected animals. Typical imaging aspect is a large calcified cystic lesion containing daughter cysts. Non-enhanced CT is especially helpful in demonstrating minor calcifications. Cysts are usually also found in the liver. Sensitivity of the tests used for serum anti-*Echinococcus* antibody detection is in the range of 50–80%. Those in (past and current) close contact with dogs in a known endemic area are particularly at risk (Huang and Zheng, 2012).

Hereditary disorders other than ADPKD may also be revealed in the early stage by a few kidney cysts. The most frequent are hepatocyte nuclear factor 1B (HNF1B) disease and tuberous sclerosis complex (TSC). Associated dysplastic kidney abnormalities and extrarenal features usually reveal the first condition (see Chapter 313), whereas kidney angiomyolipomas usually coexist with cysts in the second one (see Chapter 330). Cystic involvement is inconstantly present in medullary cystic kidney disease (MCKD) (see Chapter 316) as well as in the rare cases of nephronophthisis (NPHP) diagnosed in the young adult (see Chapter 317). Kidney lesions caused by von Hippel–Lindau (VHL) disease are discussed in Chapter 332. The diagnosis of VHL disease should always be considered in a patient with presumed ADPKD and renal cell carcinoma.

Glomerulocystic kidney disease (GCKD) is an uncommon condition predominantly described in children (see Chapter 305) but also reported in adults. The disease is either sporadic or familial with an autosomal-dominant transmission; some of these cases are associated with a mutation in the *HNF1B* gene (Kolatsi-Joannou et al., 2001).

The rare oral-facial-digital type 1 (OFD1) disease is discussed in Chapter 319.

In the presence of a glomerular haematuria, the very rare hereditary angiopathy with nephropathy, aneurysms, and muscle cramps (HANAC) syndrome due to a *COL4A1* gene mutation should be considered (see Chapter 320), as well as the autosomal dominant form of Alport syndrome due to a mutation in the *COL4A3A4* gene (Plaisier et al., 2010; personal observations).

Multiple cysts, unilaterally, in a normal or enlarged kidney

This relatively rare setting results from a limited number of developmental abnormalities (Table 304.2).

Unilateral renal cystic disease is a very rare entity characterized by multiple cysts with intervening normal parenchyma in one kidney or a portion of one kidney, mimicking ADPKD (Fig. 304.2). It can be distinguished from asymmetric ADPKD by several features: strictly unilateral location, negative family history, no extrarenal cysts, and no progression (Hwang et al., 1999; Baradhi and Abuelo, 2012). The pathogenesis is unknown.

In some cases, medullary sponge kidney may be limited to one kidney and involve several pyramids, such as in the subset associated with hemihypertrophy (Rommel and Pirson, 2001).

Though usually diagnosed during childhood (see Chapter 305), multicystic dysplastic kidney may only be recognized later in life. This condition is easily recognized since the affected kidney is non-functioning and replaced by a multiloculated cystic mass.

Multiple cysts, bilaterally, in normal or small-sized kidneys

All hereditary disorders listed in Table 304.1 may present with multiple bilateral cysts in normal-sized kidneys, especially early ADPKD and the rare subset of ARPKD patients reaching adulthood with autonomous kidney function (see Chapter 313). The MCKD and NPHP patients with multiple, bilateral cysts have small-sized kidneys. Multiple, bilateral cysts with normal-sized kidneys may also be found in medullary sponge kidney (see Chapter 319), acquired

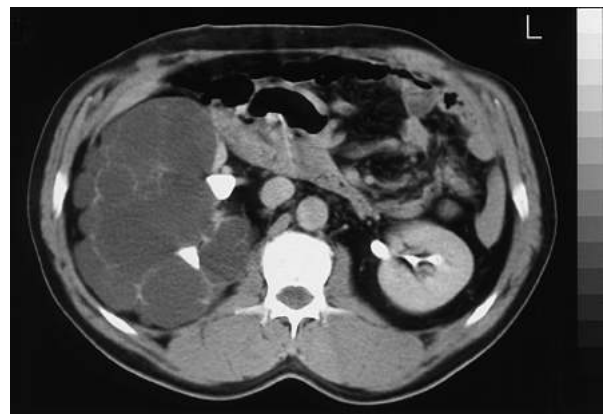

Fig. 304.2 Contrast-enhanced CT showing unilateral renal cystic disease mimicking ADPKD.

cystic kidney disease (ACKD), and lithium-induced cystic kidney disease.

The term ACKD refers to the development of cysts in the patient with long-standing chronic renal failure or maintenance dialysis (Choyke, 2000). In the early stage, cysts are small. With ongoing uraemia, the number and size of the cysts increase. The cysts can regress after successful kidney transplantation, but conversely can develop in chronically rejected kidneys. The disease may lead to renal cell carcinoma in about 2% of dialysed patients. Using a decision analysis model, Sarasin et al. found that screening provided significant benefit only to those patients with a life expectancy of 25 years or more (Sarasin et al., 1995).

Long-term lithium administration may cause not only nephrogenic diabetes insipidus and chronic interstitial nephritis but also kidney cysts, typically as multiple microcysts ranging from 1 to 2 mm and rarely > 3 mm, best recognized by MRI (Farres et al., 2003).

Multiple cysts, bilaterally, in enlarged kidneys

Multiple cysts of varying size in bilaterally enlarged kidneys is the common presentation of ADPKD, this diagnosis being by far the most frequent.

Other inherited disorders, which may account, though much less frequently, for this presentation are, by decreasing prevalence order, TSC2-PKD1 deletion syndrome (see Chapter 330), VHL disease, advanced OFD1 disease, and HNF1B disease (Faguer et al., 2011). Advanced ACKD may also present as such.

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CHAPTER 305

The child with renal cysts

Carsten Bergmann, Nadina Ortiz-Brüchle, Valeska Frank, and Klaus Zerres

Introduction

Table 305.1 summarizes the characteristics of cystic kidney diseases in children (Zerres et al., 1984; Bergmann and Zerres, 2008). A possible diagnostic algorithm is shown in Fig. 305.1.

Cystic kidney dysplasia and congenital anomalies of the kidneys and urinary tract

Cystic dysplasia (see Chapter 345) is a common finding in children. The incidence is about 1:500 with slightly more affected males than females. Due to early embryonic maldevelopment, most cases are recognized by prenatal ultrasound in the first or early second trimester. Most important is the diagnosis of additional features which can indicate a syndromic manifestation (Table 305.2). In some of these cases numeric or structural chromosomal abnormalities can be found.

Renal dysplastic elements can be present in many syndromes such as Bardet–Biedl or Meckel–Gruber syndrome (Bergmann, 2012) (see Chapter 314). While sometimes challenging, ultrasonographic differentiation from polycystic kidney disease is usually possible. In cystic-dysplastic cases, loss of the kidney's reniform character is typical. The most important genetic entity with cystic dysplastic kidneys is caused by autosomal dominant mutations in the gene encoding the transcription factor TCF2/HNF1β. Mutations in this gene can result in a wide phenotypic spectrum often summarized as renal cyst and diabetes (RCAD) syndrome (see Chapter 315). Incomplete penetrance or at least significant variable expressivity and a high fraction of new mutations have always to be kept in mind when evaluating the family history. Spontaneous mutations can be found in about 30–50% of all HNF1β cases. Mutations in many other genes have to be discussed in patients found to be negative for *TCF2/HNF1β* gene mutations. Meanwhile > 20 genes have been already identified (and many more will follow) in patients with manifestations belonging to the congenital anomalies of the kidneys and urinary tract (CAKUT) spectrum, including renal agenesis, hypo-, and dysplasia (Renkema et al., 2011) (see Chapter 345).

Autosomal recessive polycystic kidney disease

This rare condition is described in Chapter 313. Typical ultrasonographic manifestations in childhood are massively enlarged kidneys with increased echogenicity. Single cysts usually cannot be demonstrated in early childhood, but may occur with increasing age, which than can be difficult to distinguish from the autosomal dominant form of polycystic kidney disease (ADPKD) (Table 305.1). Severe early manifestations or ARPKD can often be demonstrated *in utero*. The most severely affected children frequently die shortly after birth due to pulmonary hypoplasia. In children surviving the neonatal period, the prognosis is better.

Autosomal-dominant polycystic kidney disease

With an incidence of > 1:1000, ADPKD (see Chapter 312) is one of the most frequent monogenic diseases. About 2% of all patients present with an early manifestation in childhood, sometimes manifesting prenatally as Potter sequence due to severe oligo- or anhydramnios. The clinical picture can be highly variable even within the same family and among patients carrying the same mutation. In some individuals with an early and severe disease manifestation, additional hypomorphic alleles in *trans* have been found in genes for cystic kidney diseases and other ciliopathies. Early manifestations usually do occur in *PKD1* families, but have also been reported in some families harbouring a *PKD2* germline mutation (Torres and Harris, 2012).

Congenital hepatic fibrosis/polycystic liver disease

Ductal plate malformation with hyperplasia of the bile ducts and congenital hepatic fibrosis (CHF) is an obligatory finding in ARPKD, but can also be found in many other syndromes usually of the ciliopathy spectrum such as nephronophthisis (NPHP), Joubert syndrome, and Meckel–Gruber syndrome (Bergmann et al., 2005). In single cases, CHF has also been observed in patients with ADPKD. Liver cysts often found in adult patients with ADPKD, are usually not observed in children. Autosomal dominant polycystic liver disease is a rare condition with mutations in *PRKCSH* and *SEC63* genes and in which patients usually do not show clinical symptoms during childhood (Drenth et al., 2010).

Nephronophthisis, nephronophthisis-associated ciliopathies, and medullary cystic kidney disease

Autosomal recessive NPHP (incidence about 1:50,000) (see Chapter 317) is usually defined by cysts at the corticomedullary junction. It is the most common inherited cause of end-stage

Table 305.1 Main characteristics of cystic kidney diseases

	Polycystic kidney diseases		Cystic dysplasia
	Autosomal recessive PKD	**Autosomal dominant PKD**	
Synonyms	Infantile polycystic kidneys type POTTER I	Adult polycystic kidneys type POTTER III	Type POTTER IIA (enlarged) multicystic kidneys (enlarged POTTER type IIB (hypoplastic)
Kidney lesion as part of syndrome	No	Often as POTTER type III changes	Frequent (see below)
Incidence	About 1:40,000	About 1: 1 000	Including all types 1:500–1:1000
Pathology of the kidney			
Macroscopic shape	Reniform	Reniform	Usually loss of reniform shape
Size	Enlarged, only normal at the beginning	Enlarged, only normal at the beginning	Ranging from hyperplastic to hypoplastic kidneys
Symmetry	Symmetrical	Symmetrical, at the beginning asymmetrical even over a period of years	Often asymmetrical, symmetrical involvement often in case of POTTER sequence
Microscopic location of cysts	Dilated collecting ducts	Cysts in all parts of nephron including collecting ducts	Usually complete loss of kidney architecture
Diameter of cysts	At onset up to 2 mm, with longer survival up to several cm	At onset very small, later very different, up to several cm	Different up to several cm
Connective tissue	Usually not increased	Usually not increased, in later stages slight increase	Increased
Primitive ducts	None	None	Present
Cartilage	None	None	Nearly pathognomonic but not always present
Involvement of other organs			
Liver changes	Obligatory congenital hepatic fibrosis (of different extent), Caroli syndrome	In about 1/3 of adult cases 'cystic liver,' rare in children. In rare cases congenital hepatic fibrosis	Only as part of syndromes
Associated features	Cystic pancreas (rare)	Mitral valve prolapse, berry aneurysms, cystic pancreas	As part of syndromes involvement of different organs
Main clinical manifestations	Neonatal period: respiratory distress: With prolonged survival renal insufficiency and portal hypertension (highly variable)	Onset usually 3rd–5th decade, sometimes in children, very rare in newborns with respiratory distress and renal insufficiency. Pain and enlargement of kidneys, proteinuria, haematuria, hypertension, nephrolithiasis, urinary tract infection, cerebral haemorrhage	Variable: latent (unilateral involvement) or Potter sequence. Frequently symptoms from associated abnormalities
Risk for siblings	25%	50%; increased recurrence risk for early manifestation after one affected child with early onset (in rare cases of spontaneous mutation no risk)	Unknown, usually < 10% (cases with monogenic modes of inheritance known)
Manifestation in affected family members	Often similar course in siblings. Discordant sib pairs well known	.	As part of RCDA unilateral and/or bilateral agenesis/dysplasia of kidneys
Parental kidneys	No alterations	Demonstration of one affected parent (unless parents are too young to demonstrate cystic changes in ultrasound). Rare cases of spontaneous mutations	See above

renal disease (ESRD) during childhood and early adolescence. So far, almost 20 *NPHP* genes are known. Loss of the ability to concentrate urine properly causes early polyuria and polydipsia. Disturbances of electrolytes, anaemia, and metabolic acidosis are further characteristic findings. The diagnosis is usually made by imaging techniques typically demonstrating normal-sized or hypoplastic kidneys with increased echogenicity and no or corticomedullary-localized cysts.

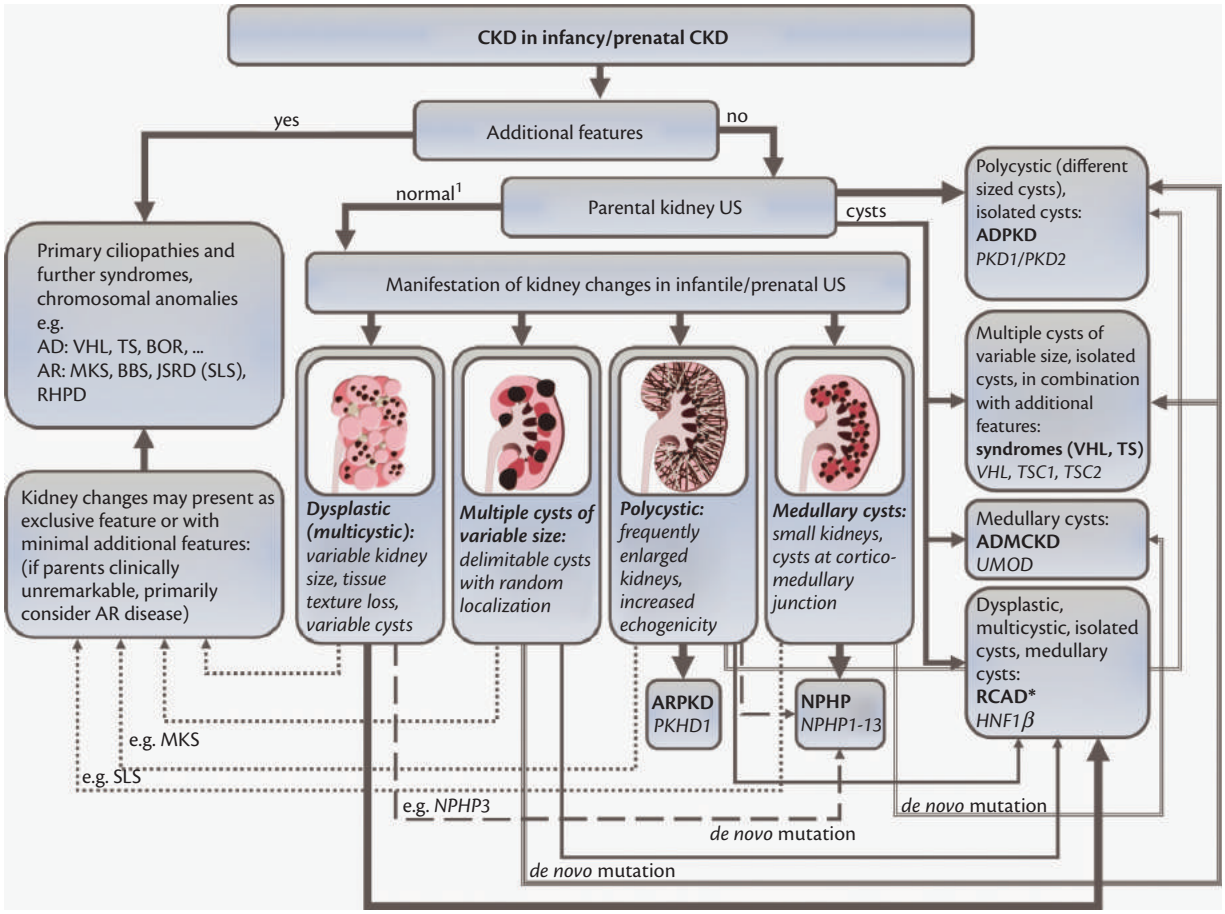

Fig. 305.1 Diagnostic algorithm to approach cystic kidney diseases (CKD) in infancy—simplified illustration of the genetic background. ADMCKD = autosomal dominant medullary cystic kidney disease; ADPKD = autosomal dominant polycystic kidney disease; ARPKD = autosomal recessive polycystic kidney disease; BBS = Bardet–Biedl syndrome; BOR = branchiootorenal syndrome; JSRD = Joubert syndrome-related disorders; MKS = Meckel–Gruber syndrome; NPHP = nephronophthisis; RCAD = renal cysts and diabetes syndrome; RHPD = renal-hepatic-pancreatic dysplasia; SLS = Senior–Løken syndrome; TS = tuberous sclerosis; US = ultrasound; VHL = von-Hippel–Lindau syndrome; arrow thickness indicates the probability of the corresponding diagnosis; = AD *de novo* mutations possible; = nephronophthisis disease spectrum; = manifestation within the scope of a syndrome; * = either AD with incomplete penetrance or as *de novo* mutation; [1] = conclusions dependent on parental age). The weight of the line and arrow (from dotted to solid and bold) pointing to final genetic diagnosis indicates the probability of causation.

Many patients with a NPHP-like renal phenotype may demonstrate additional organ manifestations such as ocular, hepatic, skeletal, and/or cerebellar findings. Senior–Løken syndrome and Joubert syndrome are two of these NPHP-associated ciliopathies (Otto et al., 2011) (see Chapter 317).

Autosomal dominant medullary cystic kidney disease (MCKD) (see Chapter 318) is comparable to NPHP with regard to kidney morphology (summarized as NPHP/MCKD complex; see Chapter 316), but usually shows a much milder clinical course leading to ESRD only in adulthood.

Glomerulocystic kidneys

Heterogeneous conditions have been described as 'glomerulocystic', making the observation not so specific. The term 'glomerulocystic' is often used for early manifestations of ADPKD as glomerular cysts are typical findings in cases with early onset ADPKD. The term should, however, be used preferentially for conditions different from ADPKD. *TCF2/HNF1β* gene mutations (see Chapter 315) are probably the most common cause of glomerular cysts and may mimic an ADPKD-like phenotype. Another cause for glomerulocystic kidneys can be urethral obstruction, also still sometimes denoted as cystic kidney disease type Potter IV.

Cystic kidneys as an important feature of syndromes

Cystic kidneys may occur as a manifestation of many genetic syndromes. An overview of some of the most common syndromes with cystic kidney disease is given in Table 305.2. Most of these syndromes follow an autosomal recessive mode of inheritance, however, some are autosomal dominantly or rarely X-linked inherited.

Non-heritable cystic kidneys/renal cysts as differential diagnosis

Solitary cysts

Solitary cysts have to be taken into consideration as a differential diagnosis of inherited conditions. Solitary cysts are less common than often thought. The average frequency is practically 0% at the age of 15–29 years and later on 1.7% (30–49 years), 11.5%

Table 305.2 Syndromes with cystic kidneys

Syndrome	Main features	Renal involvement	Genetic cause
Bardet–Biedl-syndrome	Retinopathy, obesity, mental retardation, polydactyly, hypoplastic genitalia	Most often cystic kidneys	AR (several genes)
Branchio-oto-renal (BOR) syndrome	Pre-auricular pits, hearing loss, branchial fistulas or cysts, anomalous pinna	Type II cystic kidneys in about 6% of gene carriers, renal agenesis	AD
Chromosomal disorders (e.g. trisomy 10p, 13, 17, triploidy)	According to the chromosomal disorder additional malformations, facial dysmorphias, mental retardation	Different types, often agenesis/dysplasia	Often *de novo* disorders or result of a familial translocation
Jeune thoracic dystrophy syndrome	Small thorax, short limbs, hypoplastic iliac wings, bile duct and pancreatic dysgenesis	Different types	AR (several genes)
Joubert syndrome (JS) and 'Joubert syndrome-related disorders' (JSRDs)	Cerebellar malformation, molar tooth sign, irregular breathing, retinopathy, strabismus, mental retardation	Renal dysplasia, medullary cysts	AR (several genes)
Meckel–Gruber-syndrome	Dorsal encephalocoele, polydactyly, cleft palate, malformation of brain, genital anomalies, hepatic fibrosis	Most often dysplasia, agenesis/hydronephrosis	AR (several genes)
Oral-facial-digital-syndrome type I	Oral frenula and clefts, hypoplasia of alae nasi, digital asymmetry, partial clefts in lip, tongue and alveolar ridges, mental retardation	Most often type III cystic kidneys	X-linked dominant
RCAD (renal cysts and diabetes) syndrome	Urinary tract and genital malformations, familial diabetes mellitus (MODY type 5), goat	Different types, most often dysplasia/agenesis	AD
Senior-Løken syndrome	Retinopathy, cerebellar malformations, hepatic fibrosis	Medullary cysts	AR
Tuberous sclerosis syndrome	Adenoma sebaceum, harmartomatous skin nodules, seizures, phakomata, bone lesions, mental retardation	Type III cystic kidneys, renal dysplasia, renal angiomyolipomas (40–80%)	AD (several genes), in about 80% spontaneous mutation, broad clinical spectrum, incomplete penetrant
von Hippel–Lindau syndrome	Retinal angiomata, cerebellar haemangioblastoma, phaeochromocytoma	Cystic kidneys type III, renal cell carcinoma	AD with incomplete penetrance and variable expressivity

AD = autosomal dominant; AR = autosomal recessive.

(50–69 years), and 22.1% (> 70 years), respectively. For bilateral cysts the respective figures are as follows: 1% (30–49 years), 4% (50–69 years), and 9% (> 70 years). Isolated liver cysts occur with a frequency of 2.5–4.6% (Ravine et al., 1993). Overall, solitary cysts in children not affected by ADPKD or any other cystic kidney disease are very uncommon.

Acquired cystic kidneys

Acquired cystic kidneys are not usually seen in children, perhaps because of the duration of renal disease required for their development.

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CHAPTER 306

Autosomal dominant polycystic kidney disease: overview

Albert C. M. Ong and Richard Sandford

Introduction

Based on an estimated population prevalence of between 1/400 and 1/1000, there are > 60,000 individuals with or at risk of developing complications associated with autosomal dominant polycystic kidney disease (ADPKD) in the United Kingdom. This equates to > 300,000 people in the United States and 7 million worldwide. Once diagnosed, an individual with ADPKD will require long-term medical follow-up and treatment with unknown but significant costs to national healthcare systems (Spithoven et al., 2014). A major proportion, probably two-thirds, will develop end-stage renal disease (ESRD) requiring renal replacement therapy—dialysis or transplantation. ADPKD is therefore the commonest genetic cause of ESRD. Most centres worldwide report that approximately 1 in 10 patients receiving dialysis therapy or transplantation have a diagnosis of ADPKD. Improvements in healthcare for individuals with ADPKD will therefore impact directly on patients, their families, and healthcare resources.

ADPKD may cause few symptoms until late stages when renal failure becomes symptomatic. Some patients experience symptoms from the sheer size of their kidneys. At an earlier stage, some may experience haematuria, pain from cyst haemorrhage, and infection, which can be slow to resolve (see Chapter 307). Renal stones may occur. Intracranial aneurysms are the most serious non-renal manifestation but tend to be restricted to some families, and in the absence of a family history of intracranial haemorrhage, screening is not generally recommended. Massive liver involvement sometimes causes symptoms but not usually liver dysfunction, and is much more likely to occur in women than in men.

Until recently, care for affected individuals was supportive and aimed at reducing cardiovascular morbidity and mortality, providing good blood pressure control and symptomatic relief of disease complications, and renal replacement therapy when necessary. Now the prospect of new therapies that directly target renal cyst formation and expansion and may delay progression to ESRD, based on well-designed and fully powered clinical trials, is becoming a reality. The first such trials targeting the mammalian target of rapamycin (mTOR) pathway were reported in 2010 and the landmark TEMPO study followed in 2012 (Serra et al., 2010; Walz et al., 2010; Torres et al., 2012).

The identification of cAMP- and mTOR-dependant pathways as central to the progression of renal cystic disease and drugs that directly modulate these pathways has facilitated early clinical trials in ADPKD (Chang and Ong, 2013). Key to facilitating this shift from translational research to translational medicine has been the identification of well-validated clinical trial endpoints that can be measured in a patient- and clinical trial-relevant time frame. Adoption of change in renal volume as a clinical biomarker of disease progression has been driven by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-sponsored Consortium for Radiological Imaging Studies of Polycystic Kidney Disease (CRISP) (Chapman et al., 2003). The main goal of CRISP has been to develop methods of monitoring disease progression that would shorten the period of time required to determine efficacy of treatment interventions in ADPKD patients. Using magnetic resonance imaging (MRI), changes in renal volume can now be used as a surrogate measure of disease progression being inversely correlated with change in glomerular filtration rate (GFR) (Grantham et al., 2006). Individuals with total kidney volumes > 1500 mL aged > 30 years have the fastest rate of decline in estimated GFR (Grantham et al., 2006). Baseline renal volume also provides accurate prognostic information for affected individuals and is therefore likely to have a significant impact on how ADPKD patients are managed in the future (Chapman et al., 2012).

It is well recognized that the underlying mutation, whether in the *PKD1* or *PKD2* gene, is a major predictor of disease severity. Mutations in *PKD1* occur in the majority of affected individuals and are associated with an average age at ESRD of 53 years. Mutations in *PKD2*, however, although they are found in only 15% of cases, predict development of ESRD in the eighth decade of life (Hateboer et al., 1999). This substantial difference has been explained using MRI, which has demonstrated a smaller number of cysts in *PKD2* mutation carriers, compared to *PKD1* at any given age (Harris et al., 2006). Therefore individuals with smaller kidneys and a *PKD2* mutation have a more favourable renal prognosis. The nature of the *PKD1* mutation may also have prognostic implications (Cornec-Le Gall et al., 2013). Mutation testing, in addition to guiding prognosis, may also be used in pre-symptomatic testing and diagnosis. In particular, it may be of benefit in the assessment of potential living related kidney donors, those with unusual features on conventional imaging, or those who do not meet current ultrasound-based diagnostic criteria. With the wider availability of mutation testing with a clinically useful mutation detection rate of > 70%, further indications are likely to become apparent (see Chapter 308).

Many clinical trials in ADPKD and polycystic liver disease are now being conducted to investigate the efficacy of a range of different

interventions (<http://clinicaltrials.gov>). Of particular interest are the HALT-PKD studies that aim to determine whether blockade of the renin–angiotensin–aldosterone system (RAAS) alters the progression of the cystic disease and the decline in renal function. RAAS blockade is commonly used in ADPKD although there is little evidence to show what effect, if any, it has on disease progression. These have now shown that blockade of the RAAS is effective in controlling blood pressure but does not alter the rate of decline in GFR despite being associated with a slower rate of increase in TKV in early disease (Schrier et al., 2014; Torres et al., 2014).

Therefore the evidence base upon which we develop management guidelines for ADPKD (see Chapter 309) is currently being greatly strengthened (Chapman et al., 2015). The aim will be to slow disease progression and reduce the number of individuals reaching ESRD and developing disease-related complications. Chapters 307–312 will therefore review the current state of knowledge of ADPKD from its epidemiology, presentation, diagnosis, and monitoring of progression to treatment of complications and prospects for novel therapies.

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CHAPTER 307

Autosomal dominant polycystic kidney disease: clinical features

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) affects all ethnic groups and is the commonest monogenic kidney disease, with a prevalence of 1/500 to 1/1000 (Torres et al., 2007a). It is also the commonest hereditary cause of end-stage renal disease (ESRD). Descriptions of bilateral enlarged cystic kidneys were made as early as the sixteenth century, but a systematic assessment of the clinical features was first performed by Dalgaard in 1957. Development of bilateral enlarged kidneys was recognized to precede the onset of uraemia, typically by some years (Dalgaard, 1957). The identification of *PKD1* and *PKD2* and the finding of widespread tissue expression of both polycystin proteins (Ong et al., 1999) confirmed that ADPKD is a multisystem disorder with a number of important extrarenal manifestations.

Renal manifestations

Cyst growth

The development of multiple, bilateral fluid-filled renal cysts is a cardinal feature of ADPKD. Cysts originate as saccular diverticulae from the tubule wall, which enlarge and lose continuity with the tubule lumen. Cyst growth progresses as a result of epithelial proliferation and the transepithelial secretion of solute and water. Cyst enlargement is usually accompanied by an increase in total kidney size, a feature that is helpful in differentiating ADPKD from simple kidney cysts. Thousands of cysts of varying size typically develop in each kidney, often leading to massive kidney growth.

Cyst growth and kidney enlargement precede and predict the loss of excretory kidney function in ADPKD. Compression of healthy renal parenchyma by cysts is at least partly responsible for the excretory failure; retarding cyst growth is thus a therapeutic target and surrogate outcome measure in studies of interventions to halt functional decline (Grantham et al., 2006). Gross haematuria, proteinuria, hypertension, nephrolithiasis, and pain are also associated with larger kidney volumes.

In ADPKD patients with a positive family history, multiple renal cysts are apparent on ultrasound in 85%, 96%, and 100% of those aged 15–29, 30–39, and 40–59 years respectively (Pei et al., 2009) (see Chapter 308). Rarely, ADPKD may manifest with multiple cysts and kidney enlargement in neonates or even *in utero* (Shamshirsaz et al., 2005). Patients with PKD1 develop cysts at an earlier stage than those with PKD2; age- and gender-adjusted kidney sizes of patients with PKD2 are two-thirds the size of those with PKD1 (Harris et al., 2006). Cyst and kidney growth proceed exponentially in ADPKD. A greater absolute rate of cyst and kidney growth is seen with PKD1, though the larger cyst volume means that the percentage growth rate is no different from PKD2 (Harris et al., 2006). Other factors associated with more rapid increase in kidney volume are higher urine sodium excretion, lower serum high-density lipoprotein cholesterol and lower renal blood flow (Torres et al., 2007b, 2011).

Loss of excretory renal function

Loss of excretory renal function is highly variable in ADPKD; some patients reach ESRD in their fourth decade, whilst others retain independent kidney function into their ninth decade (Hateboer et al., 1999).

Hyperfiltration at functioning nephrons initially maintains normal excretory function despite the progressive growth of cysts and loss of normal parenchyma. Impairment of maximal urinary concentrating ability is an early feature, present in 60% of children and accompanied by elevated vasopressin levels (Torres et al., 2007a). Reduced renal blood flow and increased renal vascular resistance are also early findings, which may result from activation of the renin–angiotensin system (Meijer et al., 2010). Urinary albumin excretion is elevated in young adults with ADPKD, increases with disease progression, and predicts rate of functional decline (Chapman et al., 1994; Meijer et al., 2010). However, heavy proteinuria (> 1 g/24 hours) is not a common feature of ADPKD.

By the time excretory kidney function becomes significantly impaired, kidney volume is usually markedly enlarged, with little remaining normal parenchyma. Glomerular filtration rate then declines rapidly, at approximately 5 mL/min/year (Klahr et al., 1995). Half of all ADPKD patients reach ESRD by age 60. In addition to greater kidney size, risk factors for more rapid loss of kidney function include hypertension before age 35, male gender, gross haematuria before age 30, multiple pregnancies, and black race (Johnson and Gabow, 1997).

The mutated gene is a major determinant of renal prognosis. Subjects with PKD1 develop ESRD at a median age of 54 years, versus 74 years for those with PKD2 (Hateboer et al., 1999). There is no clear genotype–phenotype correlation for truncating versus in-frame mutations, but mutations in the more 5′ region of the *PKD1* gene are associated with a slightly earlier onset of renal failure (53 vs 56 years) (Harris and Rossetti, 2010). Genetic background is

also a modifier of renal disease, but even within families there is marked variability in the age of end-stage kidney disease; patients presenting with enlarged kidneys as neonates are found in families with otherwise more typical ages of disease manifestations (Fain et al., 2005).

Loin pain

Abdominal and lower back pain are very common features of ADPKD, affecting over half of all adult patients (Bajwa et al., 2004). Pain is the commonest symptom leading to the diagnosis. Chronic loin pain may reflect distension of the renal capsule, traction on the renal pedicle, or compression of surrounding structures. The pain often fluctuates in severity and may respond poorly to analgesics. Though larger kidneys are more likely to cause chronic pain, some patients with only small cysts also complain of severe chronic pain (Bajwa et al., 2001). Musculoskeletal back pain can be a consequence of altered posture accompanying massive cystic kidneys and increased abdominal girth.

Acute episodes of loin/flank pain in ADPKD may be the result of local complications, including haemorrhage, infection, and nephrolithiasis (Table 307.1). Non-renal causes of pain should also be considered, including extrarenal complications of ADPKD such as hepatic cysts, colonic diverticulitis, and abdominal wall hernias.

Management of chronic pain in ADPKD may require a combination of behavioural modification, analgesics, and non-pharmacologic interventions such as heat, ice, or transcutaneous nerve stimulation. More invasive approaches including cyst aspiration with sclerosant injection or laparoscopic/open surgical cyst fenestration have been used in cases of refractory pain (Hogan and Norby, 2010). However, pain often recurs after these interventions. When patients are established on dialysis or have little residual function, nephrectomy is sometimes appropriate for control of pain and other local complications.

Cyst haemorrhage and haematuria

Gross haematuria is a common presenting symptom of ADPKD, reported in 42% of patients at some stage; the mean age at first episode is 30 years and there is a tendency to recurrent episodes (Gabow et al., 1992). Microscopic haematuria has a prevalence of 3–17% in cross-sectional studies (Chapman et al., 1994).

The cyst wall is highly vascularized under the influence of angiogenic factors including vascular endothelial growth factor. Cyst wall vessels are relatively fragile and prone to bleed. When haemorrhage is accompanied by cyst rupture into the collecting system, gross haematuria is the result. Such episodes are usually self-limiting, lasting less than a week and rarely causing haemodynamic compromise (Gabow et al., 1992). Bed rest and the maintenance of an adequate urine output to reduce clot formation in the collecting system are usually sufficient. In rare circumstances of persistent or severe haematuria, arterial embolization or even nephrectomy may be required. Precipitating factors for cyst haemorrhage include minor trauma and infection. Greater kidney size, impairment of excretory function, hypertension, and PKD1 are also risk factors for gross haematuria in ADPKD (Gabow et al., 1992; Hateboer et al., 1999). Other sources of haemorrhage should be considered (Box 307.1), particularly if the haematuria is prolonged or occurs for the first time at a late age. Nephrolithiasis is one potential cause of haematuria associated with ADPKD (see below). Differential diagnoses of haematuria unrelated to ADPKD should also be considered, including urological malignancies.

When haemorrhage is not accompanied by rupture into the collecting system, gross haematuria is not evident and the presenting symptom may be acute localized pain. Asymptomatic haemorrhage into cysts also seems to be a common event since computed tomography (CT) or magnetic resonance imaging (MRI) appearances consistent with haemorrhage are reported in ADPKD patients even in the absence of symptoms (Levine and Grantham, 1985). Other

Table 307.1 Approach to the ADPKD patient with abdominal pain

Cause	Clinical features	Diagnostic tests
Renal cystic change per se	Chronic pain, often fluctuating in severity, unilateral or bilateral	No diagnostic test; diagnosis based on history, in the context of known cystic change and the absence of a recognized acute precipitant
Renal cyst haemorrhage	Acute pain, localized tenderness, ± haematuria, low grade fever and leucocytosis	Ultrasound, CT, or MRI appearances may be consistent with haemorrhage, though differentiation from cyst infection is difficult
Renal cyst Infection	Acute pain, fever, raised inflammatory markers, localized tenderness	Ultrasound, CT, or MRI appearances may be consistent with cyst infection, but are not specific Blood cultures may be positive, but PET scan or culture of cyst aspirate is most sensitive
Pyelonephritis	As for cyst infection, but pain may be more diffuse or bilateral, preceding lower urinary tract symptoms may be present	Urine and blood cultures
Nephrolithiasis	Acute or recurrent episodes of loin pain; may be accompanied by haematuria or infective symptoms	CT scan most sensitive imaging modality
Renal carcinoma	Loin pain, fevers, weight loss, persisting or late onset haematuria	Serial CT or MRI may be helpful to assess change in appearance of complex cysts. Other features of malignancy, e.g. contrast enhancement, lymphadenopathy, renal vein extension may be present
Liver cysts per se	Chronic/fluctuating right upper quadrant pain	As for renal cysts
Liver cyst infection	Acute right upper quadrant pain and tenderness, fever	As for renal cyst infection
Liver cyst haemorrhage	Acute right upper quadrant pain and tenderness, ± low grade fever	As for renal cyst haemorrhage
Colonic diverticulitis	Left iliac fossa pain and tenderness, fever, diarrhoea or rectal bleeding	Typical appearances on CT scan

Box 307.1 Causes of haematuria in ADPKD

- Spontaneous haemorrhage from cyst wall vessel
- Minor trauma
- Infection
- Nephrolithiasis
- Renal carcinoma
- Other sources unrelated to ADPKD, e.g. bladder carcinoma.

patterns of haemorrhage that occur more rarely in ADPKD are subcapsular, retroperitoneal, and even intraperitoneal.

Infection

Urinary tract infection affects as many as 50% of patients with ADPKD (Delaney et al., 1985), with women more likely to be affected than men. Lower tract infections present and are managed as in the general population. Upper tract infection presents with loin pain, fever, and raised inflammatory markers. This may be caused by pyelonephritis, cyst infection, or a combination of the two. Distinguishing cyst infection from pyelonephritis is often not possible clinically, though a more localized area of tenderness is consistent with infection confined to a cyst. Loin pain caused by cyst haemorrhage is also accompanied by fever and transient leucocytosis in some cases, so may mimic infection.

Since larger cysts are not in communication with the collecting system, urine culture is frequently negative in cyst infections (Sallee et al., 2009). Gram-negative enteric organisms are the commonest pathogens identified in both pyelonephritis and cyst infection (Schwab et al., 1987; Sallee et al., 2009). Empirical antibiotic treatment should therefore be directed against such organisms. An additional consideration is the theoretical benefit of using an antibiotic with good cyst penetration. Lipophilic antibiotics such as fluoroquinolones are recommended when cyst infection is suspected.

Since ultrasound, CT, and MRI do not distinguish cyst haemorrhage from infection, they are often unhelpful in the initial investigation of suspected cyst infection. Positron emission tomography (PET) scanning may give a better indication of whether cyst infection is present (Jouret et al., 2011). When infection does not settle promptly with treatment, imaging is important in excluding complicating features such as perinephric abscess formation, nephrolithiasis, or obstruction. Cyst infection that does not settle with antibiotics may benefit from percutaneous drainage (Sallee et al., 2009), though this requires identification of the likely infected cyst. Cyst aspiration may also yield positive culture results when urine culture has been negative.

Nephrolithiasis

Nephrolithiasis is detectable on CT in up to a third of ADPKD patients and causes symptoms in 20–28% (Grampsas et al., 2000). Stone composition is most commonly uric acid or calcium oxalate. Larger kidneys are a risk factor for stone formation in ADPKD, perhaps reflecting structural distortion causing urinary stasis. Metabolic factors potentially contributing to the greater incidence of stone formation in ADPKD include hypocitraturia, hypomagnesuria, and a lower urinary pH (Grampsas et al., 2000).

Diagnosis of nephrolithiasis in ADPKD is made more difficult by the fact that associated symptoms of loin pain and haematuria are also commonly caused by cyst growth, haemorrhage, and infection. Cyst wall and parenchymal calcification complicate the radiological detection of stones; the optimal imaging modality is CT (Nishiura et al., 2009).

Renal carcinoma

The incidence of renal carcinoma is not known to be increased in patients with ADPKD. However, presentation may be different in this population, with more bilateral, metastatic, and multicentric tumours at diagnosis, a younger age of onset, and more constitutional symptoms such as fever (Bonsib, 2009). Diagnosis of carcinoma, like nephrolithiasis, is made more difficult in ADPKD by the differential diagnosis of cyst growth or haemorrhage as an explanation for loin pain, mass, and haematuria. Complex cysts resulting from previous haemorrhage may be difficult to differentiate from malignancy radiologically. Rapid growth of a complex cyst, or contrast enhancement on CT/MRI are suggestive of malignancy.

Extrarenal manifestations

Cardiovascular disease

Cardiovascular disease is the leading cause of mortality among patients with ESRD due to ADPKD. Cardiovascular mortality rate on dialysis is actually lower for ADPKD patients than for age- and gender-matched non-diabetic patients (Perrone et al., 2001). However, ADPKD is associated with early-onset vascular pathology that develops before loss of glomerular filtration rate. This reflects both renal-dependent hormonal changes and renal-independent vascular remodelling.

Hypertension

Hypertension is an early feature in ADPKD, affecting 60% of adults before significant loss of excretory renal function has occurred (Chapman et al., 2010). Hypertension is present in 20–30% of children with ADPKD and the median age of hypertension diagnosis is 32–34 years (Ecder and Schrier, 2009). Attenuation of the normal nocturnal blood pressure dip is present in normotensive young adults with ADPKD (Valero et al., 1999) and is also more common in hypertension associated with ADPKD than essential hypertension. By the time patients commence dialysis, hypertension is almost universal. Predictors of earlier hypertension in ADPKD include kidney size, PKD1, male gender, and a history of hypertension in an affected parent (Hateboer et al., 1999; Ecder and Schrier, 2009).

Half of hypertensive ADPKD patients have left ventricular hypertrophy, a predictor of cardiovascular mortality (Ecder and Schrier, 2009). Increasing blood pressure even within the normotensive range is associated with greater left ventricular mass in ADPKD (Schrier, 2009). Hypertension is also associated with earlier progression to ESRD.

Activation of the renin–angiotensin–aldosterone system plays an important role in the pathogenesis of hypertension in ADPKD. Hypertensive ADPKD patients have greater levels of plasma renin and aldosterone than matched essential hypertensive subjects (Chapman et al., 1990). Kidneys from ADPKD patients show upregulation of renin expression in the juxtaglomerular apparatus and in arterioles. Cysts also express renin,

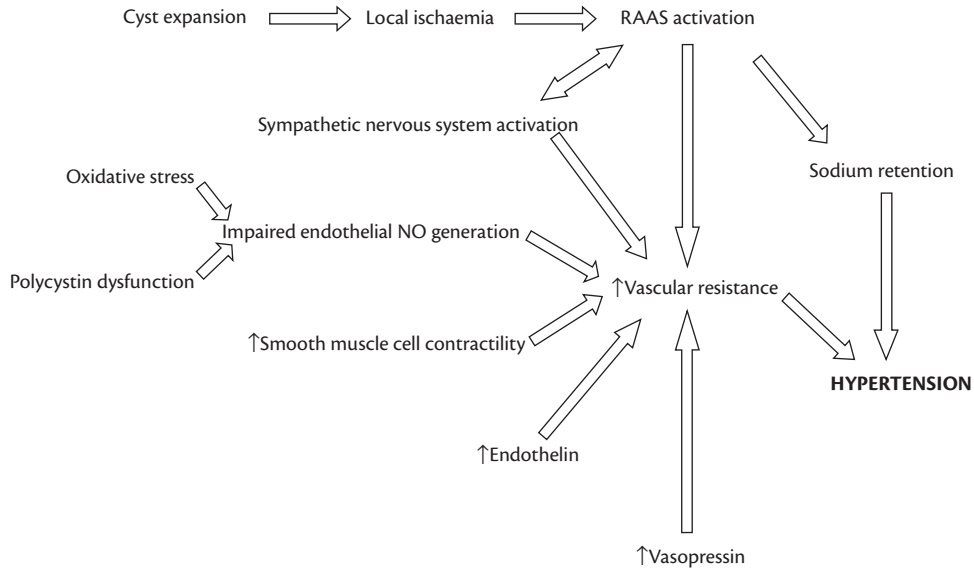

Fig. 307.1 The pathogenesis of hypertension in ADPKD. NO = nitric oxide; RAAS = renin–angiotensin–aldosterone system.

angiotensinogen, angiotensin-converting enzyme, and angiotensin II (Loghman-Adham et al., 2004). Local ischaemia resulting from parenchymal compression by expanding cysts may drive intrarenal activation of the renin–angiotensin–aldosterone system; attenuation of renal blood flow accompanies increasing kidney volume before the loss of excretory function and is associated with hypertension (Torres et al., 2007b). Even normotensive ADPKD patients have increased levels of renin and aldosterone (Chapman et al., 1990).

Other pathways that could also contribute to the early development of hypertension in ADPKD include increased vascular smooth muscle contractility, endothelial dysfunction, sympathetic nervous system activation, and greater plasma levels of vasopressin and endothelin (Fig. 307.1) (Torres et al., 2007a; Schrier, 2009).

Vascular dysfunction in ADPKD

Measures of vascular function are abnormal even in normotensive young adult ADPKD patients with preserved excretory renal function. Endothelial cells and vascular smooth muscle cells express polycystin 1 and 2; vascular dysfunction in ADPKD may be a direct result of vascular polycystin deficiency (Chapman et al., 2010). In endothelial cells polycystins play a role in shear stress sensing and nitric oxide signalling. In vascular smooth muscle cells, polycystins participate in pressure sensing and regulation of contractility. Endothelial-dependent vasodilation is impaired in subcutaneous vessels from normotensive ADPKD patients and coronary flow reserve is reduced in both normotensive and hypertensive ADPKD patients (Turkmen et al., 2008). Arterial stiffness also increases at an early stage of ADPKD (Borresen et al., 2007).

Vascular dysfunction in ADPKD may contribute to the early accumulation of atherosclerosis; carotid intima-media thickness is increased even in young, normotensive ADPKD subjects (Turkmen et al., 2008). Inflammation and increased oxidative stress are also evident in ADPKD before significant loss of excretory function (Menon et al., 2011) and are further potential contributors to cardiovascular disease pathogenesis.

Aneurysmal disease

The prevalence of intracranial aneurysms is fivefold higher in ADPKD patients compared to the general population (Pirson et al., 2002); the clinical features and management of these are described in more detail in Chapter 310.

Cerebral aneurysm rupture in ADPKD is a catastrophic but rare event, with an incidence of 1/2000 patient-years (Schievink et al., 1992). Most aneurysms therefore do not rupture; ischaemic stroke and hypertensive non-aneurysmal haemorrhage are commoner causes of neurological symptoms in ADPKD. As rupture is rare, and preventive therapy not free from risk, screening is generally only recommended if there is a family history of aneurysm rupture (see Chapter 310).

Aneurysmal disease, dolichoectasia (elongated dilation), and dissection of extracranial arteries including the coronary arteries are rare features of ADPKD (Ecder and Schrier, 2009). However, the incidence of abdominal aortic aneurysms is not increased.

Valvular disease

Mitral valve prolapse is detectable on echocardiography in a quarter of ADPKD patients and an increased prevalence is apparent from childhood (Hossack et al., 1988; Ecder and Schrier, 2009). Mitral regurgitation is also increased and can be haemodynamically significant. However, audible murmurs are less frequent than echocardiographic abnormalities and valve replacement is not commonly required. A disorder of connective tissue function underlies some of the mitral pathology in ADPKD, but hypertensive heart disease is also a potential cause of mitral regurgitation.

Aortic regurgitation resulting from valve degeneration or dilation of the aortic root and annulus may be increased in ADPKD (Hossack et al., 1988). Other valve lesions including tricuspid prolapse and tricuspid regurgitation are less consistently found to be increased. Echocardiographic screening for valvular lesions is not recommended in the absence of a murmur.

Extrarenal cystic disease

Hepatic cysts

Hepatic cysts are the commonest extrarenal feature of ADPKD and are evident on MRI in 58%, 85%, and 94% of patients aged 15–24, 25–34, and 35–46 years (Bae et al., 2006). Cysts arise from excessive proliferation of intrahepatic biliary ductule epithelium and affect

patients with both PKD1 and PKD2. Volume of cysts progressively increases with age, as for renal cystic disease. The management of massive cystic liver disease is considered further in Chapter 311.

Hepatic cyst volume is fivefold greater in women than in men and massive hepatic cystic disease in ADPKD is largely confined to women (Bae et al., 2006). Oestrogen receptors are present on the epithelial cells of cysts and oestrogen stimulates proliferation of cells from hepatic cysts *in vitro*. Postmenopausal women with ADPKD receiving hormone replacement therapy manifest a greater rate of liver enlargement than those who do not receive oestrogen (Sherstha et al., 1997). Pregnancy and oral contraceptive use are also associated with larger cysts.

Hepatic cystic disease in ADPKD is usually asymptomatic. Despite the presence of multiple cysts, hepatic parenchymal volume is usually preserved and it is very rare for impairment of liver function to develop. Large cyst volumes may sometimes give rise to dyspnoea, early satiety, chronic abdominal or back pain, and abdominal distension (see Chapter 311). Rare complications include bile duct compression causing jaundice, compression of the portal vein causing portal hypertension, or compression of the hepatic vein or inferior vena cava causing venous obstruction. Other rare associations include congenital hepatic fibrosis and cholangiocarcinoma (Torres et al., 2007a).

Hepatic cyst haemorrhage, infection, or rupture present with acute pain and must be distinguished from other causes of abdominal pain in ADPKD (see Table 307.1). Infection typically presents with fever, localized pain, leucocytosis, and elevated inflammatory markers. A mildly deranged alkaline phosphatase may also be an indicator of hepatic cyst infection. Cyst wall thickening and intracystic debris may be apparent on ultrasound, CT, or MRI scan in the setting of infection. Haemorrhage is a differential diagnosis best identified on CT scan, but PET is the most sensitive investigation for detecting changes associated with cyst infection (Sallee et al., 2009). Enteric organisms are the commonest pathogens grown from cyst aspirates or blood and empirical antibiotics must be chosen to cover such organisms. As for the treatment of infected kidney cysts, lipophilic antibiotics are generally considered advantageous to allow cyst penetration. Cyst aspiration may be indicated when features of infection are not resolving with antibiotic therapy and imaging is able to identify a likely culprit cyst.

Other extrarenal cystic disease

Pancreatic cysts are present in 5% of patients with ADPKD, but are very rarely accompanied by chronic pancreatitis (Torres et al., 2007a). Cystic change in the seminal vesicles or ejaculatory ducts is found in 50% of men with ADPKD, though whether this impairs fertility is unknown (Vora et al., 2008). Abnormalities of sperm including necrospermia and immotility are also associated with ADPKD (Vora et al., 2008). Ovarian cysts are not increased in ADPKD and female fertility is not considered to be impaired. Arachnoid cysts are present in 8% of ADPKD patients (Torres et al., 2007a), but are not proven to be of any clinical significance.

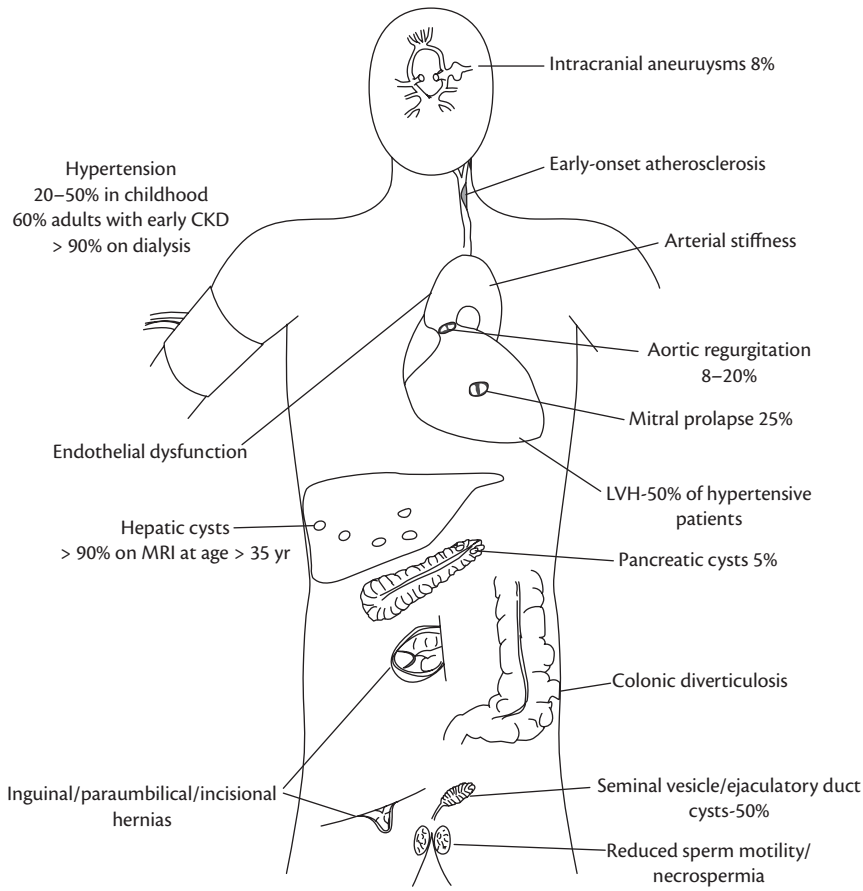

Fig. 307.2 Extrarenal features of ADPKD.

Colonic diverticular disease

The extent to which colonic diverticular disease is increased in ADPKD is unclear. Patients with ADPKD on dialysis have been found to have an increased incidence of colonic diverticula and associated complications, but this finding has not been duplicated consistently in patients without ESRD (Perrone, 1997). Altered colonic smooth muscle function resulting from polycystin mutations may predispose to diverticulum formation.

The autosomal dominant polycystic kidney disease patient on dialysis

Despite the extrarenal manifestations of ADPKD (Fig. 307.2), the mortality rate of ADPKD patients on dialysis is half that for patients with other non-diabetic causes of renal failure. The only extrarenal manifestation for which mortality is increased in ADPKD is polycystic liver disease, which accounts for 1 death per 1000 patient-years. Mortality rates due to valvular disease, diverticular disease, cerebrovascular disease, and aneurysm rupture are the same or lower in ADPKD patients on dialysis compared to non-diabetic controls (Perrone et al., 2001). This reflects the substantial comorbidity accompanying ESRD of all causes.

Peritoneal dialysis is a suitable renal replacement modality for many patients with ADPKD. Although a greater incidence of abdominal wall hernias among peritoneal dialysis patients with ADPKD has been found in some series, there is no associated increase in technique failure rate (Lobbedez et al., 2011). Since registry data reflect only patients considered suitable to commence peritoneal dialysis, it remains possible that massive polycystic kidneys limit the success of the technique by increasing intra-abdominal pressure, restricting dialysate infusion volumes and compromising solute clearances. Nephrectomy is not a contraindication to peritoneal dialysis, which has been performed successfully after bilateral nephrectomies in ADPKD, though necessitating a period of haemodialysis. The use of automated peritoneal dialysis to minimize intra-abdominal pressure may be advantageous, particularly in the setting of grossly enlarged polycystic kidneys (Alam and Perrone, 2010). Despite the association of ADPKD with diverticular disease, there is no increase in the incidence of peritonitis among ADPKD patients (Lobbedez et al., 2011).

Transplantation in autosomal dominant polycystic kidney disease patients

Survival following transplantation is at least as good for ADPKD patients as for those with other causes of renal failure (Perrone et al., 2001). However, ADPKD has implications for transplantation: The number of potential live related donors is commonly reduced by the autosomal dominant nature of the disease. At-risk family members wishing to be considered for donation must be screened formally (imaging, mutation analysis) to ensure that they do not themselves have ADPKD. Decisions regarding living donation may also be influenced by the presence or anticipation of ESRD in other family members.

Polycystic kidneys remain a potential source of infection following transplantation, which may be more severe in the context of immunosuppression. Pre-transplant nephrectomy has been performed in some cases to reduce local complications and is occasionally required in order to create space for the allograft. Pre-emptive transplantation is not possible under these circumstances unless nephrectomy is performed concomitantly with live related donor transplant. The indications, benefits, and optimal strategy for performing nephrectomy in ADPKD patients undergoing transplantation are unclear (Alam and Perrone, 2010).

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CHAPTER 308

Autosomal dominant polycystic kidney disease: diagnosis

Richard Sandford

Introduction

The diagnosis of autosomal dominant polycystic kidney disease (ADPKD) is typically made by imaging whether or not there is a family history of the disease. In the absence of a family history, features of other renal cystic diseases should also be sought and excluded before making a diagnosis of ADPKD (Table 308.1). Ultrasound is most commonly used as an initial screening and diagnostic investigation due to its wide availability and established diagnostic criteria. Increasingly, computed tomography (CT) scans and magnetic resonance imaging (MRI) are used for additional studies, as they are able to provide additional information about prognosis and progression using measurements such as renal volume, and for the investigation of complications such as nephrolithiasis, cyst haemorrhage, and infection. Molecular confirmation (genetic testing) is, becoming increasingly important for certain groups such as those with no family history, atypical features, childhood disease, or who do not meet current ultrasound diagnostic criteria. Pre-symptomatic diagnosis using genetic testing may be undertaken for reproductive planning and because relatives are increasingly offered the opportunity to become a living related kidney donor. Genetic testing to support pre-implantation genetic diagnosis or prenatal diagnosis may also be requested.

Imaging

Ultrasound scanning is the imaging modality of choice for screening and the initial diagnosis of ADPKD (Fig. 308.1). It is able to detect renal cysts > 1 cm in diameter so is less sensitive than CT or MR but is widely available, non-invasive, and inexpensive. These other imaging modalities may be used where initial results using ultrasound are equivocal or where additional information about kidney structure is required. The typical appearances seen using ultrasound scanning are bilateral enlarged kidneys containing multiple cysts of various sizes throughout the cortex and medulla (Fig. 308.1). The cysts may be simple or complex, the latter suggesting previous haemorrhage or infection. In younger individuals, the changes may be less advanced with normal-sized kidneys, few cysts, and asymmetric involvement. The presence of cysts in other organs, such as the liver (~ 90% by age 50 years) and pancreas (~ 10%), also strongly support the diagnosis of ADPKD.

Revised age- and gene-specific diagnostic criteria exist for ultrasound imaging, replacing the original Ravine criteria (Table 308.2) (Ravine et al., 1994; Pei et al., 2009). These revised criteria were developed as individuals with a *PKD2* mutation have milder disease than those with a *PKD1* mutation and hence may be falsely reassured by a normal scan especially when performed under the age of 30 years (Hateboer et al., 1999). Their positive predictive value (PPV) is 100% but their sensitivity varies according to age and genotype (Table 308.2). Using the new criteria, individuals > 15 years at 50% risk of inheriting ADPKD based on family history but < 40 years require three or more cysts, unilateral or bilateral, to confirm a diagnosis (Pei et al., 2009). This is irrespective of genotype as the PPV is 100% for individuals with mutations in *PKD1* and *PKD2*. The sensitivity for both genotypes is approximately 95%. This compares to a sensitivity of only 70% for *PKD2* patients under the age of 30 years (Table 308.2). For individuals between 40 and 59 years at least two cysts in each kidney are required for diagnosis. Again this achieves a PPV of 100% for both genotypes with a sensitivity of approximately 90%. Therefore the diagnosis can be confidently excluded in an individual with no cysts aged 30–39 years and with only one cyst aged > 40 years (Table 308.3). Under the age of 30 years a negative scan has a negative predictive value (NPV) of 90% if the genotype is not known. This would not confidently exclude disease in a potential kidney donor. For *PKD1*-associated disease the NPV is 99%, illustrating the benefit of combined imaging and genetic testing for younger individuals.

In the absence of a family history of ADPKD (10–30% of cases depending on whether family screening has been carried out), the presence of bilateral enlarged polycystic kidneys with or without liver cysts in an adult is highly suggestive of the diagnosis and provides sufficient diagnostic accuracy in most cases. Clinical management and family studies are typically based on such findings. However, if the number, size, and distribution of renal cysts or renal size are not typical for ADPKD and liver cysts are absent or the findings are in a child then a careful evaluation of the family history for features of other conditions associated with multiple renal cysts should be carried out. If diagnostic uncertainty remains then genetic testing can be carried out (Table 308.1). Typically many of these other conditions are associated with multiple renal cysts in normal or small kidneys or the pattern of distribution of cysts is suggestive of an alternative diagnosis. However, renal cysts and diabetes (RCAD), oro-facial-digital type 1 (OFD1) syndrome, and autosomal recessive polycystic kidney disease have all been described mimicking the appearances of ADPKD (see Chapter 304).

In children, imaging may reveal the typical features of ADPKD although renal size should be adjusted for age and height. Clinical presentation, family history, and parental scans are essential for the correct interpretation of images.

Table 308.1 Other diseases associated with multiple renal cysts.

Disease	OMIM #	Inheritance	Gene(s)	Extrarenal features	Renal features
Renal cysts and diabetes (RCAD) (Chapter 315)	137920	AD	*HNF1B*	Maturity-onset diabetes of the young (MODY5), abnormal liver function tests, pancreatic atrophy, genital tract anomalies	Polycystic kidneys (small normal or enlarged), renal dysplasia, glomerulocystic kidney disease, hyperuricaemic nephropathy, fetal echogenic kidneys, hypomagnesaemia
Tuberous sclerosis complex (TSC) (Chapter 330)	*TSC1* 191100 *TSC2* 613254	AD	*TSC1* and *TSC2*	Multisystem hamartomatous disease. May occur with ADPKD due to contiguous gene deletion (*PKD1* and *TSC2*)	Coexistence of renal angiomyolipomas and renal cysts diagnostic. Kidneys normal sized or enlarged
Von Hippel–Lindau disease (VHL) (Chapter 332)	193300	AD	*VHL*	Retinal and CNS haemangioblastomas, phaeochromocytoma, pancreatic cysts	Renal cysts in normal-sized kidneys (occasionally polycystic kidneys), renal cell carcinoma
UMOD nephropathy (Chapter 318)	603860	AD	*UMOD*	Hyperuricaemia	'MCKD2' (Chapter 318) in fact often shows just small kidneys, hyperuricaemic nephropathy. (MCKD1 is caused by mutations in *MUC1*.)
Acquired renal cystic disease	–	–	–	–	Multiple renal cysts in small end-stage kidneys. Increased risk of renal cell carcinoma
Autosomal recessive polycystic kidney disease (Chapter 313)	263200	AR	*PKHD1*	Oligohydramnios, pulmonary hypoplasia, hepatic ductal plate malformation, biliary ectasia, liver fibrosis.	Bilateral enlarged echogenic kidneys often detected *in utero*. Renal size may decrease with age. May mimic ADPKD in older individuals.
Simple cysts (Chapter 304)	–	–	–	–	Present in ageing population
Autosomal dominant polycystic liver disease	174050	AD	*PRKCSH, SEC63*	Polycystic liver disease without renal cysts (see Chapter 311)	–
Oro-facial-digital syndrome type 1 (OFD1) (Chapter 319)	311200	XL dominant	*OFD1*	Oral frenulae, cleft tongue, cleft palate, dysmorphic features, digital anomalies	Polycystic kidneys (may mimic ADPKD)
Nephronophthisis (Chapter 317)	256100 and others	AR	*NPHP1-15*	Multisystem disease including retinal dystrophy, liver fibrosis	Small kidneys with corticomedullary cysts

CT and MR imaging may also be used for the diagnosis of ADPKD (Fig. 308.2). They are more sensitive than conventional ultrasound: > 10 cysts in subjects under the age of 30 years using these techniques has a sensitivity and specificity of 100% (Pei et al., 2015). If negative, such an investigation can be reassuring with a negative ultrasound but the presence of a few small cysts not detected by ultrasound remains difficult to interpret. Renal volumes have been shown to be correlated with estimated glomerular filtration rate (eGFR) in ADPKD and to predict disease progression. Whilst CT and MR have been shown to have greater precision for measuring serial renal volumes, ultrasound can be used to estimate kidney volume that reflects disease severity and prognosis in an individual (O'Neill et al., 2005). A total height adjusted renal volume of > 600 mL/m has been shown to predict

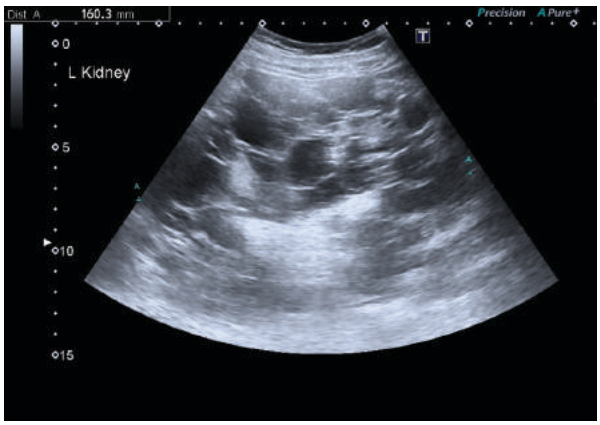

Fig. 308.1 Typical ultrasound appearances of ADPKD.

Table 308.2 Ultrasound criteria for the diagnosis of ADPKD

Age, years	*PKD1*	*PKD2*	Unknown
15–30 (≥ 3 cysts)	PPV 100% SEN 94%	PPV 100% SEN 69%	PPV 100% SEN 82%
30–39 (≥ 3 cysts)	PPV 100% SEN 97%	PPV 100% SEN 95%	PPV 100% SEN 95%
40–59 (≥ 2 cysts bilateral)	PPV 100% SEN 93%	PPV 100% SEN 89%	PPV 100% SEN 90%

PPV = positive predictive value; SEN = sensitivity.

From Pei et al. (2009).

Table 308.3 Ultrasound criteria for exclusion of the diagnosis of ADPKD

Age, years	*PKD1*	*PKD2*	Unknown
15–30 (≤ 1 cyst)	NPV 99% SPEC 98%	NPV 83% SPEC 97%	NPV 91% SPEC 97%
30–39 (≤ 1 cyst)	NPV 100% SPEC 96%	NPV 97% SPEC 94%	NPV 98% SPEC 95%
40–59 (≤ 1 cyst)	NPV 100% SPEC 94%	NPV 100% SPEC 94%	NPV 100% SPEC 94%

NPV = negative predictive value; SPEC = specificity.

From Pei et al. (2009).

progression to chronic kidney disease stage 3 and a baseline renal volume of > 1500 mL at age > 30 years is associated with a more rapid decline in eGFR (Grantham et al., 2006; Chapman et al., 2012). Therefore diagnostic imaging for ADPKD should also include a survey of all abdominal viscera and measurement of renal volumes.

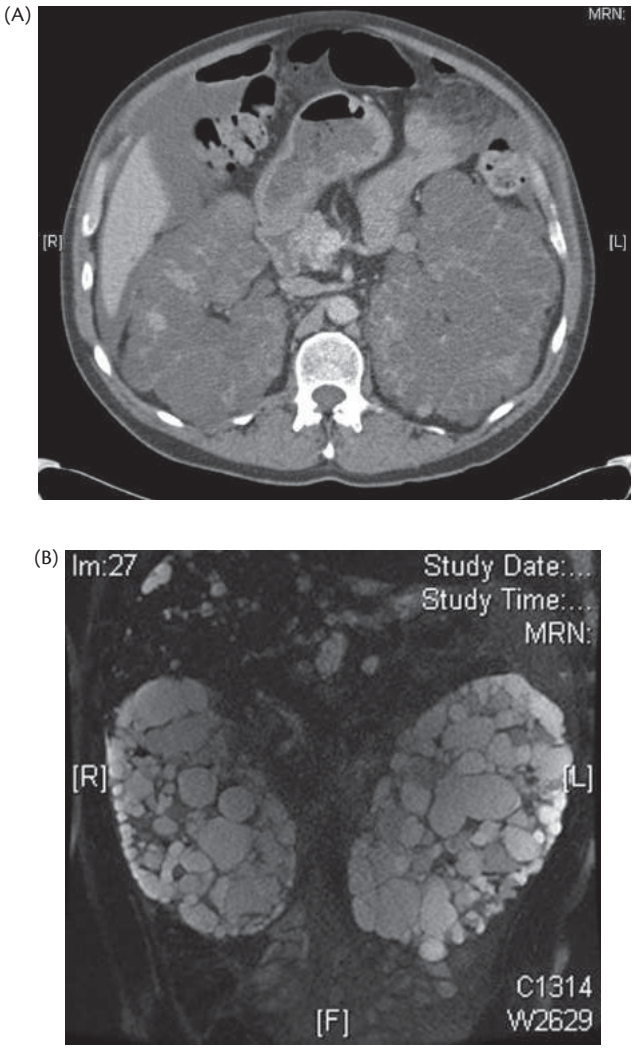

Fig. 308.2 (A) CT and (B) MR appearances of ADPKD.

Genetic testing

ADPKD is caused by mutations in *PKD1* (85%) and *PKD2* (15%). Molecular analysis of these genes in the clinical setting is becoming widely available throughout the world (<http://www.genetest.org>). An international research database of PKD mutations (<http://pkdb.mayo.edu>) is also available which describes > 2300 *PKD1* and 270 *PKD2* sequence variants of which nearly 1500 are defined as pathogenic (up to March 2015). This has greatly facilitated the interpretation of gene variants as *PKD1* is highly polymorphic. Pathogenicity can now be confidently assessed for variants although uncertainty still exists for some, especially mis-sense variants. This has permitted a pathogenic mutation detection rate of 60–90% (Cornec-Le Gall et al., 2013). If no mutation is identified or pathogenicity cannot be confidently assigned, family studies (segregation and linkage analysis) can then be offered. Linkage analysis requires multiple affected and unaffected family members to be available for analysis. It is therefore suitable for all families but can be used for diagnosis or disease exclusion if informative. Clinical guidelines for the use of molecular testing in ADPKD are still in development. However, molecular testing is likely to be targeted at individuals where there is diagnostic uncertainty, who do not fulfil diagnostic criteria, have no family history, present with early onset disease, wish to be potential donors, or are considering pre-implantation or prenatal diagnosis. Its use for prognostic testing—*PKD2* and mis-sense *PKD1* mutations predict milder disease than truncating *PKD1* mutations—remains to be clinically evaluated (Cornec-Le Gall et al., 2013). Rare case reports of mosaicism, incompletely penetrant alleles, and co-inheritance of *PKD1* and *PKD2* mutations identify additional genetic mechanisms that may influence disease severity and the interpretation of genetic testing.

Pre-symptomatic diagnosis

Pre-symptomatic diagnosis in adults may be sought for several reasons including health screening, career planning, reproductive planning, and living related kidney donation. Standard ultrasound criteria can be used and combined with genetic data if available. In some individuals, predictive genetic testing may be offered if the familial mutation is known with imaging being offered if the test is positive. Genetic counselling should be offered before screening or testing to discuss the benefits and unintended consequences of a pre-symptomatic diagnosis of ADPKD.

Genetics

Historically, polycystic kidney disease has been recognized as a distinct clinical entity for several centuries with its genetic basis being recognized at the turn of the nineteenth century (Torres and Watson, 1998). It was not until the 1980s that genetic linkage analysis in large multiplex families with classical features of the disease identified the first locus, *PKD1*, on chromosome 16p13.3 (Reeders et al., 1985). Another locus, *PKD2*, on chromosome 4p21 was eventually identified in 1993. *PKD1* and *PKD2* were finally cloned in 1994 and 1996 respectively.

PKD1 consists of 46 exons spanning approximately 50 kb of genomic DNA. Exons 1–33 are duplicated as multiple pseudogenes also on chromosome 16 with high sequence similarity. It encodes an approximately 14 kb transcript that predicts a large multidomain transmembrane protein product, polycystin-1. *PKD2* is a 15-exon

gene spanning 68 kb of genomic DNA. It encodes a novel member of the transient receptor potential (TRP) channel family, TRPP2. Polycystin-1 and TRPP2 interact to form a mechanosensitive calcium channel localized to the primary cilium. TRPP2 also functions as an endoplasmic reticulum calcium release channel whilst polycystin-1 has a role in cell–cell adhesion and communication (see Chapter 303).

Strategies have been developed that allow mutation analysis of both genes to be carried out in clinically accredited laboratories. Eighty-five per cent of cases of ADPKD are due to mutations in *PKD1* with the remainder in *PKD2*. There is no evidence to support a third locus. Mutation detection rates approaching 90% have been achieved in research laboratories. Approximately 30% of mutations are recurrent with the remainder being private, that is, unique to a single family. All types of mutation have been described in *PKD1* and *PKD2* including point mutations, spice site mutations, and small and large deletions and duplications. Large deletions spanning *PKD1* and the adjacent *TSC2* gene result in a contiguous gene deletion syndrome characterized by features typical of ADPKD and tuberous sclerosis complex (TSC) (see Chapter 330).

No clear genotype–phenotype correlations have been described in ADPKD. However, the type of mutation in *PKD1* and locus-specific effects both predict renal survival. Many studies have reported longer renal survival in individuals with a *PKD2* mutation compared to those with a PKD1 mutation (74 years vs 54 years respectively) (Hateboer et al., 1999). More recently, mutation data from > 500 families has been reported. The median age of onset of ESRD for individuals with a truncating mutation in *PKD1* was 55 years, 67 years in those with a *PKD1* mis-sense mutation, and 79 years in *PKD2* mutation carriers (Cornec-Le Gall et al., 2013). This provides some explanation for the marked clinical variability seen between ADPKD families. The variation seen within families remains unexplained and is likely to be due to additional genetic and environmental factors. Evidence for genetic factors is provided by the identification of somatic mosaicism and incompletely penetrant or hypomorphic mutations (Rossetti et al., 2009). Co-inheritance of the latter *in trans* with a pathogenic mutation may result in more severe earlier onset disease.

ADPKD is also an example of a 'two-hit' disease. Somatic mutations in the normal allele in addition to the inherited germline mutation, that is, bi-allelic inactivation, have been identified in cystic epithelial cells from polycystic kidneys and livers. However, it is not clear whether these somatic mutations are always required for cyst initiation and are driver mutations or whether they may be passenger mutations. It is also likely that other genetic alterations occur in cystic epithelia to produce the cystic phenotype of abnormal cell differentiation, proliferation, and altered fluid secretion.

Other features of ADPKD may not require a second somatic mutation and therefore haploinsufficiency may be sufficient. For example, there is no evidence to support somatic mutation in the development of intracranial aneurysms.

With the rapid introduction of next-generation sequencing technologies and tools for genomic analysis our understanding of the genetic contribution to clinical variability and the cellular mechanisms underlying cyst formation and disease progression will undoubtedly increase dramatically in the coming years. This may provide new opportunities for individuals and families to understand their disease and how it may affect them over their lifetime and identify new therapeutic targets.

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CHAPTER 309

Autosomal dominant polycystic kidney disease: management

Young-Hwan Hwang and York Pei

Introduction

Previously there has been no specific treatment proven to delay renal disease progression in autosomal dominant polycystic kidney disease (ADPKD) (Harris and Torres, 2009). However advances in experimental therapeutics (Grantham, 2003; Harris and Torres, 2009; Gallagher et al., 2010), including treatment with vasopressin receptor antagonist and mammalian target of rapamycin (mTOR) inhibitors, have raised the prospect of disease mechanism-based pharmacological modulation in ADPKD, and the first of these drugs are reaching the clinic. We will discuss the current status of promising pre-clinical and clinical studies of novel drug treatments and conclude with our perspectives on their challenges and opportunities for clinical translation.

General patient care

We encourage our patients to consume a healthy diet with high-fibre content, minimize caffeinated drinks (Belibi et al., 2002), and exercise 20–30 minutes two to three times per week. We recommend a high water intake of 3–4 L/day for patients with an estimated glomerular filtration rate (eGFR) > 30 mL/min/1.73 m^2 given the promising results of pre-clinical and clinical studies suggesting that urine dilution may suppress renal cyst growth by lowering intracellular cAMP levels (Torres et al., 2004, 2009, 2012; Wang et al., 2008). High water intake may also reduce the risk of kidney stones associated with ADPKD (see below) (Torres et al., 1993). We routinely monitor serum sodium concentrations in patients on high water intake to minimize the risk of hyponatraemia, especially for those who are on a thiazide diuretic or have an eGFR < 60 mL/min. We strongly discourage cigarette smoking as it is an important cardiovascular disease risk factor and may promote the formation of individual cysts through somatic polycystic kidney disease (*PKD*) gene mutations (Pei et al., 2001).

Normalization of hyperlipidaemia in pre-ESRD patients may reduce risk of cardiovascular complications and we recommend dietary modification and exercise as the initial step, followed by drug therapy, usually with a statin if required. Some recommend prescribing statins to this patient group in the light of the SHARP study (Baigent et al., 2011) (see Chapter 102). The lifetime risk of cardiovascular disease in patients with PKD is high, but the best time to start is not clear. We feel that routine use of a statin in ESRD should be individualized and probably targeted towards patients with both high cardiovascular risks and blood low-density lipoprotein levels.

Management of hypertension

Hypertension is a common complication of ADPKD affecting 20% of affected children at the time of diagnosis, 50% of young adults by the third decade of life before any significant reduction in GFR, and almost all patients when they reach ESRD (Chapter 307; Gabow et al., 1990; Fick et al., 1994; Ecder and Schrier, 2001).

Haemodynamic studies of normotensive young patients compared to their age-matched unaffected family members have documented extracellular volume expansion and activation of systemic renin–angiotensin–aldosterone system (RAAS) during the early course of ADPKD (Harrap et al., 1991; Barrett et al., 1994). Despite this evidence for overactivity of the RAAS (see Chapter 307), there is currently no evidence to support a renal protective effect in ADPKD by ACE inhibitors (ACEIs) or angiotensin receptor blockers (ARBs), above and beyond that of blood pressure control (Kent et al., 2007). It is also unclear whether intensive blood pressure control to < 120/80 mmHg in ADPKD is safe and effective for end-organ protection.

In accordance with the JNC 7 and Kidney Disease Outcomes Quality Initiative clinical practice guidelines (Chobanian et al., 2003; Kidney Disease Outcomes Quality Initiative (K/DOQI), 2004), we aim for a goal blood pressure < 130/80 mmHg, preferably with non-clinic readings using calibrated home blood pressure monitors. In the absence of contraindications, we generally use an ACEI as the initial antihypertensive agent, with the addition of a long-acting diuretic (e.g. chlorthalidone) if the blood pressure is not well controlled. For a third-line agent, we prefer to use a beta blocker instead of a calcium channel blocker since reduced calcium entry underpins a key defect in the pathobiology of ADPKD (Harris and Torres, 2009; Gallagher et al., 2010). This recommendation is based on theory rather than evidence and is contrary to guidelines for management of essential hypertension.

Acute kidney injury can sometimes occur in patients with ADPKD following pharmacological RAAS blockade, analogous to the setting of bilateral renal artery stenosis (Chapman et al., 1991). Careful monitoring of renal function is therefore indicated in high-risk patients (i.e. those with very large kidneys, renal impairment, or volume contraction) who are treated with an ACEI or ARB.

Management of other renal complications

Haematuria

Gross haematuria occurs in approximately 40% of patients during their clinical course and is more likely to affect those with large polycystic kidneys (Gabow et al., 1992). In some cases, precipitating events such as physical trauma and urinary tract infection can be identified. The differential diagnoses here include cyst rupture and nephrolithiasis, both may also be associated with pain. Haematuria due to cyst rupture generally resolves within a week with conservative therapy consisting of bed rest, hydration, and analgesics (Gabow et al., 1992). Rarely, severe and persistent bleeding may necessitate further intervention such as arterial embolization or even a nephrectomy. Haematuria associated with nephrolithiasis is generally microscopic, but may be grossly visible during the passage of a stone. Persistent or recurrent haematuria that is not well explained should be investigated to rule out other possible causes such as renal cell carcinoma, bladder cancer, or an underlying nephritis (Dedi et al., 2001).

Kidney pain

Flank and abdominal pain due to renal and extrarenal causes is common in patients with ADPKD. Acute pain related to the kidney may be due to infection (i.e. infected cyst or pyelonephritis), renal colic, or cyst haemorrhage. Diagnostic workup including a thorough clinical history, physical examination, urine cultures, and imaging studies usually identifies the cause of pain. Chronic pain related to massively enlarged cystic kidneys or, rarely, liver, is often described as persistent and dull, with relief only by pain medications. In this setting, mechanical back pain due to poor posture resulting from cystic kidney or liver enlargement is also common. Most patients with chronic abdominal or back pain, however, can be managed successfully by conservative measures such as ice, massage, postural exercise, and non-narcotic analgesics (Bajwa et al., 2001), although some patients suffering from persistent and disabling pain may require chronic opioid treatment. In this setting, cyst decompression surgical procedures such as cyst fenestration, transcatheter arterial embolism, or nephrectomy, should be considered, particularly for patients who are already on dialysis (Ubara et al., 2002; Cornelis et al., 2010).

Urinary tract infection

Half of patients with ADPKD will experience one or more episodes of urinary tract infection (UTI) during their lifetime (Sklar et al., 1987). Lower UTI is not much different from lower UTI in the general population (Chapter 176). By contrast, infected cysts and acute pyelonephritis are the most common causes of upper UTI and typically present with fever and flank pain (Gibson and Watson, 1998; Sallée et al., 2009). Acute onset of symptoms with diffuse flank pain favours the diagnosis of pyelonephritis, whereas more gradual onset of symptoms with localized tenderness to one kidney suggests cyst infection. Cyst haemorrhage can be associated with low-grade fever and leucocytosis and should be considered as a differential diagnosis.

The presence of white cell casts together with a positive urine culture is suggestive of pyelonephritis. However, bland sediment with negative urine culture may be seen in patients with an infected cyst that is not connected to the renal collecting system (Gibson and Watson, 1998). Blood cultures may be positive in both pyelonephritis and cyst infections (Sallée et al., 2009). Conventional imaging studies such as ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) are generally not helpful in differentiating an infected from haemorrhagic cyst. 18F-fluorodeoxyglucose positron emission tomography (PET) may be a promising modality for diagnosing cyst infection (Sallée et al., 2009). It is important to note that both pyelonephritis and cyst infection may coexist in the same patient.

As in the general population, UTI in ADPKD is typically caused by Gram-negative enteric organisms with *Escherichia coli* accounting for most cases (Sklar et al., 1987; Gibson and Watson, 1998; Sallée et al., 2009). For patients suspected to have acute pyelonephritis or cyst infection who present with minimal systemic symptoms, we recommend empirical therapy with an oral antibiotic with broad-spectrum coverage and good cyst penetration, such as a fluoroquinolone (e.g. ciprofloxacin) or trimethoprim-sulfamethoxazole (Elzinga et al., 1987, 1988), pending the results of any positive culture and antibiotic sensitivity.

For sick patients with high fever and those on oral antibiotic who fail to show symptomatic improvement within 72 hours, hospitalization for intravenous antibiotic is indicated. In the latter setting, we recommend performing a spiral CT scan to rule out other complications such as urinary tract obstruction, staghorn calculi, or perinephric abscess; and 18F-fluorodeoxyglucose PET scan to localize the cyst infection if available (Sallée et al., 2009).

When antibiotic resistance to fluoroquinolone and trimethoprim-sulfamethoxazole is suspected, an infectious disease consultation for advice is highly recommended. The duration of therapy will depend on the severity of symptoms, the initial response, and diagnostic certainty. For patients with acute pyelonephritis, we recommend a course of antibiotic therapy for 10–14 days. For patients with high suspicion of a cyst infection, we recommend a course of antibiotic therapy for 4–6 weeks, with CT-guided needle drainage of any large perinephric abscess if indicated.

Most patients with upper UTI will eventually respond to appropriate antibiotic therapy. However, surgical removal of stones may be required if nephrolithiasis is contributing to recurrent UTIs. In addition, surgical nephrectomy may be indicated for recurrent upper UTIs in patients who are renal transplant candidates to minimize the risk of post-transplant infection while on immunosuppression treatment.

Nephrolithiasis

Kidney stones occur in 25% of patients with ADPKD and more than half of the stones are composed of uric acid with the remainder mostly of calcium oxalate (Nishiura et al., 2009). The risk factors for kidney stones in this setting are not well understood, but include large kidneys, low urinary pH, hypocitraturia, and, rarely, hyperuricosuria and hypercalciuria (Grampsas et al., 2000; Nishiura et al., 2009).

Ultrasound is insensitive in this setting because of cysts obscuring the view of the collecting system and calcifications of cyst walls. By contrast, unenhanced CT scans (Chapter 14) provide much greater sensitivity for detecting small or radiolucent stones (Grampsas et al., 2000). Obstructing stones may be more difficult to recognize in ADPKD as dilation of the collecting system may be absent due to peripelvic cysts. Non-contrast helical CT scan is currently our test

of choice for evaluating patients with ADPKD who present with acute flank pain suspicious of renal colic (Dalrymple et al., 1998; Sheafor et al., 2000).

The presence of multiple large cysts can be challenging for the performance of percutaneous nephrostomy or extracorporeal shock-wave lithotripsy (ESWL). Despite these concerns, ESWL has been used successfully in patients with small stones (< 2 cm in diameter) in the renal pelvis (Nishiura et al., 2009). Percutaneous nephrolithotomy can also be used in ADPKD although experience of this is limited (Al-Kandari et al., 2009; Umbreit et al., 2010).

Dialysis and renal transplantation

Patients with ADPKD who begin RRT are on average 10 years younger than the general ESRD patient population. They exhibit a lower mortality rate on dialysis compared to patients with non-diabetic kidney diseases primarily due to a lower prevalence of cardiovascular disease (Perrone et al., 2001; Abbott and Agodoa, 2002; Kumar et al., 2008; Li et al., 2011). Both haemodialysis and peritoneal dialysis are acceptable RRTs for patients with ADPKD; some details are discussed further in Chapter 307.

Patients with ADPKD generally have a better life expectance compared to patients with non-diabetic renal disease post transplantation (Abbott and Agodoa, 2002). They experience the same complications as those without ADPKD following renal transplantation. However, several complications including new-onset diabetes mellitus, post-transplant erythrocytosis, symptomatic intracranial aneurysms, UTIs, and diverticulitis appear to be more frequent or unique to this patient population (Andreoni et al., 1999; Hamer et al., 2007; Ring and Spiegelhalter, 2007; Alam and Perrone, 2010).

Native nephrectomy may be indicated in ADPKD patients with very large polycystic kidneys (to make room for the allograft), recurrent renal cyst infection or bleeding, prior to renal transplantation (Alam and Perrone, 2010).

Novel treatments to delay renal disease progression

Recent advances in our understanding of the pathobiology of ADPKD have highlighted a critical role for the dysregulation of intracellular calcium, cAMP, and mTOR signalling in modulating cyst growth (Fig. 309.1) (Grantham, 2003; Harris and Torres, 2009; Gallagher et al., 2010).

Disruption of the polycystin complex in ADPKD has been shown to reduce intracellular calcium levels which in turn result in increased cAMP levels through its effects on adenylate cyclase and phosphodiesterase (Yamaguchi et al., 2004, 2006; Magenheimer et al., 2006). Increased cAMP levels play a central role in promoting renal cyst growth by stimulating cystic epithelial cell proliferation of (Grantham, 2003; Yamaguchi et al., 2004, 2006; Magenheimer et al., 2006). Fluid secretion into cyst lumen is also stimulated by cAMP via the cystic fibrosis transmembrane conductance regulator (CFTR) and represents an additional mechanism for cyst expansion (Magenheimer et al., 2006). These insights provided the basis for a number of pre-clinical studies which demonstrated therapeutic efficacy of renal cAMP modulation by vasopressin V2 receptor antagonists or somatostatin analogues in multiple models of PKD (Torres et al., 2004; Magenheimer et al., 2006; Yamaguchi et al., 2006; Masyuk et al., 2007; Wang et al., 2008).

A related therapeutic strategy is to target the intracellular calcium defect as exemplified by triptolide, a Chinese herbal extract which was shown to induce calcium release via a polycystin-2-dependent mechanism and reduced renal cystic disease severity in an orthologous model of ADPKD (Leuenroth et al., 2007, 2008).

By contrast, mTOR, a serine/threonine kinase which functions as a key integration centre for multiple signalling pathways of cellular energy metabolism, is aberrantly activated in ADPKD (Shillingford et al., 2006). Therapeutic mTOR inhibition with rapamycin markedly inhibited renal cyst growth in an orthologous model of ADPKD (Shillingford et al., 2010). Interestingly, both CFTR and mTOR are negatively modulated by AMP-activated protein kinase (AMPK), another key regulator of cellular energy metabolism. Metformin, an AMPK activator, also reduced renal cystic disease severity in experimental models of ADPKD (Takiar et al., 2011). More recently, the discovery that *Pkd1*$^{-/-}$ cells from knock-out mice (compared to *Pkd1*$^{+/+}$ cells) displayed a preferential shift of energy metabolism to aerobic glycolysis has identified another novel therapeutic paradigm. By targeting this metabolic pathway reprogrammed in ADPKD, treatment with a non-metabolized glucose analogue, 2-deoxyglucose, was shown to attenuate renal cystic disease severity *in vivo* by modulating both AMPK and mTOR signalling (Rowe et al., 2013). The use of metformin and 2-deoxyglucose, alone or in combination, presents an attractive therapeutic option because of their favourable side effect profiles for long-term clinical use.

Other promising therapeutic strategies identified by preclinical studies include pharmacological antagonists of cyclin-dependent kinase, B-Raf/MEK/ERK, STAT6, glucosylceramide, and histone deacetylase for targeting cellular proliferation and/or apoptosis (Bukanov et al., 2006; Sweeney et al., 2008; Cao et al., 2009; Natoli et al., 2010; Xia et al., 2010; Yamaguchi et al., 2010; Elliott et al., 2011; Olsan et al., 2011; Bukanov et al., 2012), and small molecule inhibitors of CFTR for targeting cystic fluid secretion (Albaqumi et al., 2008; Yang et al., 2008).

Defining therapeutic endpoints in ADPKD

Natural history studies of ADPKD have documented a long lag period of several decades of continuous cystic kidney enlargement and loss of normal tissue preceding any measurable decline in renal function (Chapman et al., 2003; Grantham et al., 2006). The use of sensitive biomarkers that accurately reflect renal disease severity and progression is therefore critical in the design of any therapeutic clinical trials for ADPKD. The Consortium for Radiologic Imaging Study of PKD (CRISP) demonstrated that total kidney volume (TKV) as measured by MRI is valuable for monitoring disease progression (Franz and Reubi, 1983). In this prospective, serial, observational study of 241 patients recruited between 15 to 46 years of age and eGFR of at least 70 mL/min, their mean TKV was 1060 ± 640 mL at baseline and expanded at an exponential rate of 5.3% per year.

Despite the continuous expansion of TKV, GFR as measured by iothalamate clearance remained stable in most patients for many years. Subgroup analysis of patients with baseline TKV < 750 mL,

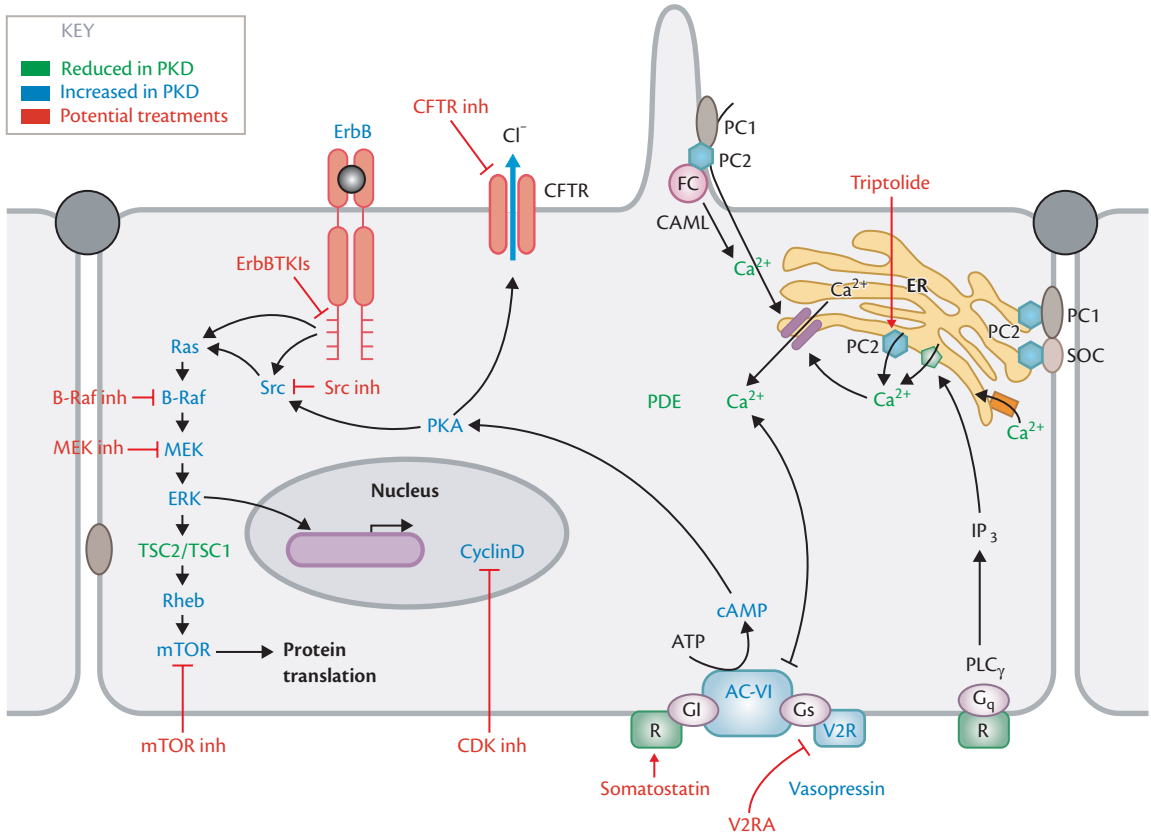

Fig. 309.1 Key mechanisms of cyst growth and molecular targets for potential treatment in ADPKD. Components and pathways that are dysregulated in PKD are indicated. Potential drug treatments targeting these defective pathways are shown in red. PC1 = polycystin-1; PC2 = polycystin-2; Ca^{2+} = intracellular calcium; ER = endoplasmic reticulum; cAMP = intracellular cyclic AMP; AC-VI = adenyl cyclase VI; PDE = phosphodiesterase; PKA = protein kinase A; V2RA = vasopressin V2 antagonist; CFTR = cystic fibrosis transmembrane conductance regulator; ERK = extracellular signal-regulated kinases; TSC1 and -2 = tuberous sclerosis complex -1 and -2; mTOR = mammalian target of rapamycin; CDK = cyclin-dependent kinase; inh = inhibitor.

750–1500 mL, and > 1500 mL indicated only the latter group was associated with a decline of GFR (i.e. −4.3 ± 8.1 mL/min/year). Subsequent follow-up of this cohort demonstrated that a significant inverse correlation between height-adjusted TKV (htTKV) and GFR which improves from baseline to the last follow-up at 8th year (i.e. r = −0.22 and −0.65, respectively) (Chapman et al., 2012). These data indicate that TKV is a sensitive biomarker of renal disease progression and provide a strong rationale for its use as a surrogate clinical outcome in therapeutic trials of ADPKD, and possibly in the future for selcting high risk patients for treatment.

Clinical trials of mechanism-based therapeutics

Multiple novel or repurposed drugs identified from preclinical studies (see above) have been or are being evaluated in clinical trials (Table 309.1; also see <http://www.pkdcure.org/research/clinical-trials/>). Here we focus mainly on randomized clinical trials (RCTs) for ADPKD that include a total sample size of at least 100 patients, a minimal treatment period of 1 year, and both kidney volume and function as outcome measures. Some are shown in Table 309.2.

Drug treatment targeting cAMP

Tolvaptan

Arginine vasopressin stimulated cAMP production in distal renal tubules and cortical collecting ducts by acting on vasopressin V2 receptor. Pharmacological V2 receptor antagonism with orally active drugs (i.e. OPC-31260 and tolvaptan) has been shown to greatly attenuate renal cyst growth in several PKD models (Torres et al., 2004; Wang et al., 2008). These pre-clinical data provide the basis for the *T*olvaptan *E*fficacy and Safety in *M*anagement of *P*KD and *O*utcomes (TEMPO 3:4) trial, in which 1445 patients, 18–50 years old, with a TKV of at least 750 mL and estimated creatinine clearance of 60 mL/min or more were randomized to tolvaptan (escalated from 45/15 mg to 90/30 mg daily, as tolerated) or placebo in a 2:1 ratio (Torres et al., 2012). Over a 3-year period and compared to placebo, tolvaptan treatment decreased the rate of TKV expansion by almost 50% (5.5%/year vs 2.8%/year, respectively; $P < 0.001$) and was associated with a slower decline in GFR (based on reciprocal of serum creatinine over time: −3.81 vs −2.61 mg/mL/year, respectively; $P < 0.001$). A composite endpoint including worsening renal function (2 vs 5/100 patient-years of follow-up, respectively) and kidney pain (2 vs 5/100 patient-years of follow-up, respectively) also favoured tolvaptan. However, more

Table 309.1 Completed randomized controlled clinical trials in ADPKD with at least 100 patients

Study drug	Dose	Study design	Eligibility	Baseline TKV (mL)	Patient recruited	Dropout rate (%)	Study duration	Primary Outcomes	Results	Trial ID publication year
Sirolimus	2 mg/day	Single centre, randomized, open label	Age 18–40 years; eGFR > 70 mL/min/1.73 m^2	955	100	Rx: 4% Ct: 4%	18 mo	TKV and eGFR	No significant difference	NCT00346918 2010
Everolimus	2.5 mg bid	Multicentre, randomized, double-blind, placebo-controlled	TKV > 1 L; eGFR 30–89 mL/min/1.73 m^2	1968	433	Rx: 33% Ct: 15%	2yr	TKV and eGFR	Modest decrease in TKV at 1st year; decrease in eGFR	NCT00414440 2010
Tolvaptan (TEMPO ¾ Trial)	45 mg/15 mg daily; increase to 90 mg/ 30 mg daily if tolerated	Multicentre, randomized, double-blind, placebo-controlled, parallel-arm	Age: 18–50 years; TKV > 750 mL; eCrCl > 60 mL/min/1.73 m^2	1692	1445	Rx: 23% Ct: 14%	3yr	TKV and slope of reciprocal serum creatinine over time	decreased TKV (−2.8% vs. −5.5% per year); Slowed decline in eGFR	NCT00428948 2012

patients on tolvaptan compared to placebo (15.4% and 5%, respectively) dropped out from the study due to serious adverse effects mostly from increased aquaresis (8.3%) and liver enzyme abnormalities (1.2%). With respect to liver toxicity, more patients on tolvaptan than placebo (4.4% and 1%, respectively) experienced at least a threefold increase of serum alanine aminotransferases and three patients on tolvaptan withdrew from the study although their liver function eventually recovered to the normal baseline values (Food and Drug Administration, 2013). These results suggest that tolvaptan is effective in slowing renal cyst growth and generally well tolerated for long-term clinical use. However, there is currently a gap of knowledge on how slowing TKV expansion by tolvaptan may impact on clinical outcomes such as delaying the need of ESRD therapy and healthcare utilization which will require further research.

Somatostatin analogues

Somatostatin analogues represent another class of drugs that act on SST2 receptors to reduce cAMP levels in hepatic cholangiocytes and renal tubular epithelial cells and have been shown to attenuate experimental hepatic and renal cystic disease in preclinical studies (Masyuk et al., 2007). Three small randomized, placebo-controlled trials of long-acting somatostatin analogues have examined the effects of somatostatin analogues on both total liver and kidney volumes in ADPKD. Two trials evaluated octreotide (40 mg every 28 days intramuscularly) for 6 or 12 months, respectively,

Table 309.2 Ongoing randomized controlled clinical trials in ADPKD with at least 100 patients

Study drug	Dose	Study design	Eligibility	Estimated recruitment	Study duration	Primary outcomes	Trial ID, YEAR of completion
Bosutinib (Src kinase inhibitor)	200 and 400 mg/day	Multicentre, randomized, double-blind, placebo-controlled	Age: 18–50 years; TKV > 750 mL; eGFR > 60 mL/min/1.73 m^2	190	2 years	TKV and safety	NCT1233869 2014
Triptolide	60 mg/day	Single centre, randomized, open-label	Age: 15–70 years; eGFR > 30 mL/min/1.73 m^2	150	3 years	TKV and eGFR	NCT801268 2013
Lanreotide (DIPAK1 Trial)	90 or 120 mg every s/c 28 days	Single centre, randomized, open-label	Age: 18–60 years; eGFR between 30 and 60 mL/min/1.73 m^2	300	30 months	TKV and eGFR TLV	NCT1616927 2017
ACEI vs. ACEI + ARB (HALT PKD Trial)	Lisinopril 40 mg, telmisartan 80 mg; standard BP goal: 120–130/70–80 vs low BP: 95–110/60–75	Multicentre, randomized, double-blind, placebo-controlled, 2×2 factorial design	Study A: age 15–49 years, eGFR > 60 mL/min/1.73 m^2 Study B: age 18–64 years; eGFR between 25 and 60 mL/min/1.73 m^2	Study A: 558 Study B: 486	4–6 years	Study A: TKV Study B: time to 50% decrease in eGFR, ESRD, or death	NCT2836862013 2014

and the third, lanreotide (120 mg every 28 days subcutaneously) for 6 months. With a combined sample size of only 77 patients, these studies documented a modest effect in reducing the total liver volume (~3–5% compared to placebo) but minimal effect on TKV (van Keimpema et al., 2009; Caroli et al., 2010; Hogan et al., 2010; Gevers et al., 2013). These studies are limited by small sample size and short treatment duration and their results should be considered as preliminary at this time. A large randomized placebo-controlled trial with a target sample of 300 patients is currently in progress to evaluate the efficacy and safety of 36-month treatment of lanreotide in ADPKD (DIPAK1, NCT00309283; also see Table 309.2).

Drug treatment targeting mTOR

mTOR signalling is aberrantly activated in ADPKD and experimental mTOR inhibition results in dramatic attenuation of renal cystic disease in an orthologous model of PKD1 (Shillingford et al., 2010). Two small retrospective studies showed that patients with ADPKD who received an mTOR inhibitor compared to ciclosporin for immunosuppression post renal transplantation had a slower rate of expansion in their polycystic kidneys (Shillingford et al., 2006) and liver (Qian et al., 2008). These data provided the rationale for two major RCTs of mTOR inhibitors in ADPKD, but both failed to demonstrate a therapeutic benefit (see Table 309.1) (Serra et al., 2010; Walz et al., 2010).

The first involved 100 patients with mild to moderate disease (eGFR 92 mL/min/1.73 m^2 and TKV of ~1 L at baseline) randomized to receive either sirolimus (target dose of 2 mg/day) or placebo for 18 months (Serra et al., 2010). Compared to placebo, sirolimus did not modify either TKV or GFR but was generally well tolerated. This study was limited by a relatively small sample size and short treatment duration, and, the dose of mTOR inhibitor used was low compared to that used in preclinical studies (Shillingford et al., 2010; Novalic et al., 2012).

The second involved 433 patients with moderate to severe disease (eGFR ~55 mL/min/1.73 m^2 and TKV of ~2 L at baseline) randomized to receive either everolimus (target dose of 5 mg/day) or placebo for 24 months (Walz et al., 2010). Compared to placebo, everolimus treatment attenuated the mean rate of increase in TKV at the end of first year (8.2% vs 5% per year, respectively; $P = 0.02$) and less so by the end of second year (15.8% vs 11.3% over 2 years, respectively; $P = 0.06$). However everolimus treatment was associated with a greater annual decrement in eGFR compared to placebo (−5.5 vs −3.5 mL/min; $P < 0.001$). Overall, only 67% of patients on everolimus compared to 85% of patients on placebo completed the study largely due to a high withdrawal rate in the former group (21% vs 7%, respectively) from serious adverse effects (e.g. cytopenia, oral ulcers, dyslipidaemia, myalgia, and pedal oedema).

Several potential explanations have been advanced for the unexpected failure of these studies to slow disease progression (Grantham et al., 2010; Watnick and Germino, 2010). First, the inclusion of patients with a wide range of disease severity (i.e. TKV at baseline ranged from 308 mL to 7.7 L) coupled with a high drop-out rate in the treatment group might have compromised the randomized design of the study, giving rise to spurious results. Second, many patients in this study had advanced disease and might have passed the 'point of no return' for any therapeutic benefit. Under this scenario, renal fibrosis in late stages of ADPKD might drive GFR decline despite a reduction of kidney volume. Third, the decreased GFR might result from mTOR inhibition interfering with glomerular hyperfiltration which normally preserves GFR despite renal cystic disease progression.

Collectively, these two RCTs suggest that the dosage of the current generation of mTOR inhibitors required for long-term treatment of ADPKD is associated with an unfavourable therapeutic index that limits their clinical utility. Perhaps newer mTOR inhibitors with increased potency or target tissue bioavailability and reduced systemic toxicities may be developed (Wander et al., 2011; Shillingford et al., 2012).

Ongoing therapeutic trials

Three RCTs are currently in progress to evaluate the effects of bosutinab, triptolide, and lanreotide in ADPKD and the key features of their study design are summarized in Table 309.2. Bosutinab is a Src/Abl tyrosine-kinase inhibitor which has been shown to attenuate renal cyst growth and biliary ductal abnormalities in PKD models (Sweeney et al., 2008; Elliott et al., 2011). Triptolide is a compound extracted from the traditional Chinese medicine herb, Lei Gong Teng ('Thunder God Vine') and has been to induce calcium release via a polycystin-2-dependent mechanism and reduced renal cystic disease severity in an orthologous model of ADPKD (Leuenroth et al., 2007, 2008). Lanreotide is a long-acting somatostatin analogue that is expected to reduce renal cAMP levels in hepatic cholangiocytes and renal tubular epithelial cells by acting on SST2 receptors (van Keimpema et al., 2009). In addition, the HALT-PKD trial is a large RCT to test the treatment effects of an ACEI (lisinopril) versus ACEI (lisinopril) and ARB (telmisartan) and two levels of blood pressure control (≤ 110/75 vs ≤ 130/80 mmHg) on renal disease progression in a 2 × 2 factorial design.

Future perspectives

Recent advances in our understanding of basic disease mechanisms in ADPKD have led to the identification of multiple molecular drug targets for clinical trials. Indeed, there are now > 10 distinct classes of novel or re-purposed drugs that target key signalling pathways involved in renal cyst growth (Fig. 309.1). From these preclinical studies, candidate drugs that exhibit a favourable therapeutic index such as triptolide (Leuenroth et al., 2007, 2008), metformin (Takiar et al., 2011), 2-deoxyglucose (Rowe et al., 2013), leflunomide (Olsan et al., 2011), glucosylceramide synthase inhibitors (Natoli et al., 2010), and somatostatin analogues (van Keimpema et al., 2009; Caroli et al., 2010; Hogan et al., 2010) are particularly promising since they are most amenable for long-term clinical use.

For future pre-clinical studies, there is a need to use experimental models that better mimic human disease yet in a timely manner. Additional biomarkers beyond TKV may be helpful. Genetics could be a part of this, and mutation-based prognostic testing in ADPKD may soon become practical due to the lowering of costs by target re-sequencing (Rossetti et al., 2012). Specifically, patients and their families with hypomorphic *PKD1* and *PKD2* mutations are generally expected to have an excellent renal prognosis, while those with truncating *PKD1* mutations are at high risk for early progression to ESRD (Pei et al., 2012; Cornec-Le Gall et al., 2013).

The landmark TEMPO¾ study provides the first proven mechanism-based drug treatment for ADPKD and will have important implications for patient care. However, given that tolvaptan treatment is expected to be expensive it is currently unclear on how best to maximize its clinical benefits. It may be reasonable to initially target patients with similar TKV as in the TEMPO trial. In that case, protocols for measuring TKV routinely need to be established.

When multiple drugs have been validated in RCTs to affect renal disease severity in ADPKD, combination therapy using two or more of these drugs at lower dosages may provide synergism to maximize therapeutic efficacy and minimize drug-related toxicities.

Further reading

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CHAPTER 310

Management of intracranial aneurysms

Yves Pirson and Dominique Chauveau

Epidemiology

An association between autosomal dominant polycystic kidney disease (ADPKD) and intracranial aneurysm (ICA) (Pirson, 2010) has been known for many years (Fig. 310.1). Additional associated vascular abnormalities include dolichoectasias, thoracic aortic and cervicocephalic artery dissections, and coronary artery aneurysms.

Several studies, especially in transgenic animal models, have provided convincing evidence that these vascular abnormalities are caused by alterations in the arterial wall linked to mutations in *PKD1* or *PKD2* (Bichet et al., 2006).

An asymptomatic ICA is found in 8–9% of patients with ADPKD (i.e. a rate two to three times above that found in the general population (Pirson et al., 2002; Irazabal et al., 2011; Vlak et al., 2011)). Just as in the general population, 85% of them are found in the anterior circulation (Irazabal et al., 2011). Of note, most of them are small, the majority < 6 mm in diameter (Pirson et al., 2002; Irazabal et al., 2011). The only clinical characteristic clearly associated with the presence of ICA is a family history of ICA: the prevalence of unruptured ICA among ADPKD patients with a family history of ICA, subarachnoid haemorrhage (SAH), or both was indeed estimated at 16–21% versus 6% among ADPKD patients without such a history (Pirson et al., 2002; Irazabal et al., 2011).

Familial clustering is poorly explained. Patients with mutations in the 5′ region of *PKD1* are more likely to have ICA than are patients with 3′ mutations, especially in those with ICA rupture before 40 years and in families with multiple cases of ICA or other vascular events (Rossetti et al., 2003). But the absence of ICA in other relatives with ADPKD within such families indicates that other factors, genetic or non-genetic (see below), also play a role in the development of ICA.

Presentation

Fortunately, as in the general population, most ICAs remain asymptomatic. Some of them may produce symptoms via three mechanisms: rarely compression of adjacent structures or focal brain ischaemia caused by embolism, and more often SAH caused by rupture. The profile of the patient with ADPKD admitted for ICA rupture, the clinical presentation, and guidelines for management have been reviewed elsewhere (Chauveau et al., 1994; Pirson et al., 2002; Pirson, 2010). Age at the time of ICA rupture averages 41 years. This is close to that observed in other familial forms of ICA, but a decade lower than that reported in the sporadic form. Occasionally, SAH is the presenting manifestation of ADPKD.

The cardinal feature of SAH is a sudden intense headache, often described as a blow or an explosion inside the head, the worst ever experienced by the patient. Up to 38% of patients admitted for SAH have a history of acute and transient headache in the days or weeks before rupture (Gambhir et al., 2009). This 'warning headache' is most likely caused by a first, limited leak from the ICA. The first diagnostic step to assess the possibility of SAH is cerebral computed tomography scan. Once established, SAH should be further investigated and managed under the direction of a neurovascular team.

Screening

Given the unpredictable and often severe prognosis of ICA rupture—with a combined mortality-morbidity rate of 35–55%—screening and prophylactic repair of unruptured ICA has to be considered. The goal of screening is to identify patients with a risk of ICA rupture that outweighs the risk of a prophylactic procedure, surgical or endovascular.

In the general population, the risk of rupture of asymptomatic ICAs has been best documented in the International Study of Unruptured Intracranial Aneurysms (ISUIA): the yearly rate of rupture was very small (0.05%) for ICAs measuring < 10 mm in diameter without a previous history of SAH versus 0.5% in those with a previous history of SAH (Wiebers et al., 2003). The risk markedly increased with the size of ICA. The risk appeared higher in Finland and in Japan (Ishibashi et al., 2009; Vlak et al., 2011). In ADPKD patients with an unruptured ICA, the largest survey was performed at the Mayo Clinic, USA, in 38 patients during 316 patient-years: of a total of 45 ICAs (median diameter 3.5 mm) only two grew, with no progression to rupture (Irazabal et al., 2011), suggesting that growth and rupture risks are not higher than in the general population. Factors known to affect the risk of non-PKD ICA rupture are reportedly a family history of SAH, smoking, and hypertension (Rinkel, 2008; Broderick et al., 2009). In the absence of adequately powered studies in ADPKD patients as well as or in the familial form of ICA unlinked to ADPKD, it makes sense to take into account these factors in the management of ADPKD patients.

The risks of prophylactic repair of ICA for non-PKD patients were reported by ISUIA (Wiebers et al., 2003). The 1-year mortality and combined mortality-morbidity rates for surgical and endovascular repair were 2.3% and 12.1%, respectively, for open surgery and 3.4% and 9.8%, respectively, for interventional radiology.

Li et al. (2012) recently designed a decision tree analysis model to calculate the outcome (gain or loss of quality-adjusted life years (QALYs)) of single screening versus not screening. The two main

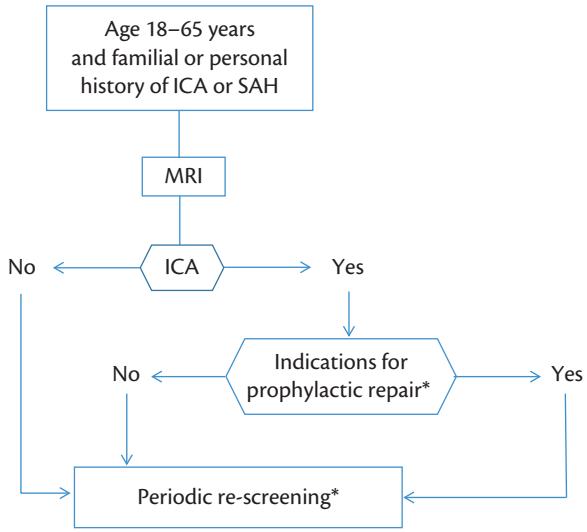

Fig. 310.1 Screening for ICA in ADPKD patients.
* See text for details. ICA = intracranial aneurysm. SAH = subarachnoid haemorrhage.

variables were ICA prevalence and risk of rupture, such as that quoted by ISUIA. Sensitivity analyses were performed to determine the effects of altering various factors on outcomes. Extrapolating these analyses to the ADPKD population and choosing to perform screening at the age of 30, screening results in a gain of QALYs when the 5-year risk of rupture exceeds 8.5%, that is, a rate far beyond that reported in an ADPKD population (Irazabal et al., 2011). This study reinforces our previous recommendation against widespread screening in ADPKD patients (Pirson, 2010).

There is, however, evidence to suggest a familial trend in likelihood of rupture in ADPKD (Belz et al., 2001; Irazabal et al., 2011); furthermore ADPKD patients with previous rupture are more likely to develop *de novo* ICA and to rupture (Belz et al., 2003). We accordingly recommend screening in patients with a family or personal history of ruptured ICA. Additional acceptable indications are preparation for major elective surgery, high-risk occupations (e.g., airline pilots), and patient anxiety despite adequate information (Torres et al., 2007). There is little to be gained from screening after the age of 65 since remaining life expectancy is insufficient to see benefit (Li et al., 2012). The continuous improvement in the outcomes of prophylactic repair of ICA could, in the future, enlarge current indications; moreover, the results of newer endovascular techniques, such as stent-assisted coiling or flow-diversion stents are expected (Seibert et al., 2011).

Practical aspects of screening

Screening is preferably performed by high-resolution, three-dimensional, time-of-flight magnetic resonance imaging; it can be done without gadolinium allowing patients with a low glomerular filtration rate to be screened. When an asymptomatic ICA is found, a recommendation for whether to intervene depends on its size, site, morphology, history of SAH from another aneurysm, the patient's age and general health, whether the ICA is coilable or clippable, and finally the experience of the neuroradiologist or the neurosurgeon (Torres et al., 2007; Pirson, 2010). Since the risk of a new ICA or enlargement of an existing one is very low in those with small (< 6 mm) ICAs (Belz et al., 2003; Irazabal et al., 2011) conservative management is usually recommended. Repeat imaging is advisable annually and at less frequent intervals once the stability of the ICA has been documented. Elimination of tobacco use and aggressive treatment of hypertension are strongly recommended. The risk of developing an ICA after an initial negative study is small at about 3% at 10 years in patients with a family history of ICA (Schrier et al., 2004; Irazabal et al., 2011). Therefore, rescreening after 5–10 years seems reasonable.

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CHAPTER 311

Management of cystic liver disease

Tom J. G. Gevers and Joost P. H. Drenth

Introduction

Hepatic cyst formation is the most common extrarenal manifestation in autosomal dominant polycystic kidney disease (ADPKD), with an overall prevalence of 67–83% (Nicolau et al., 1999; Bae et al., 2006). A large proportion of ADPKD patients are asymptomatic and have only a few hepatic cysts. However, some patients develop polycystic liver disease (PLD), arbitrarily defined as the presence of > 20 hepatic cysts (Drenth et al., 2010b; Gulick et al., 2011). Polycystic liver ADPKD patients require a different approach than those with predominantly renal manifestations (Gulick et al., 2011).

Natural history

The size and number of hepatic cysts likely increase with age, with prevalence rates of 58% (15–24 years), 85% (25–34 years), and 94% (35–46 years) (Bae et al., 2006). The persistent progression may lead to PLD with severe hepatomegaly with liver volumes of 2–10 L (normal 1.5 L) (van Keimpema et al., 2009; Caroli et al., 2010; Hogan et al., 2010). The disease typically becomes symptomatic in the fourth decade of life (Everson et al., 2004; Bae et al., 2006).

Gender is the most defining risk factor for growth of hepatic cysts (Chapman, 2003). Although men and women have equal lifetime risk, females develop larger and greater numbers of hepatic cysts (Gabow et al., 1990; Everson, 1993). Indeed, female sex hormones are an established risk factor as patients who use exogenous oestrogens and have had multiple pregnancies are more severely affected (Gabow et al., 1990; Chapman, 2003; Alvaro et al., 2006). One year of oestrogen use in postmenopausal ADPKD patients selectively increases total liver volume by 7%, whereas total kidney volume remains unaffected (Sherstha et al., 1997). In clinical practice, exogenous oestrogens should be discouraged in all PLD patients. Other risk factors include degree of renal dysfunction, severity of renal disease, and renal cyst volume (Gabow et al., 1990; Harris et al., 1996; Bae et al., 2006; Hoevenaren et al., 2008).

Symptoms

Symptoms arise when liver volume increases (Sherstha et al., 1997; Everson et al., 2004). Compression of adjacent abdominal and thoracic organs may lead to abdominal pain, abdominal distention, early satiety, shortness of breath, nausea, and vomiting (Doty and Tompkins, 1989; Vauthey et al., 1991; Torres and Harris, 2009). Complications of cysts are uncommon and typically occur in severe PLD. Strategically located cysts may cause hepatic venous outflow obstruction or compression of the portal or inferior caval vein. In addition, bile duct compression may lead to obstructive jaundice (Dmitrewski et al., 1996; Torres and Harris, 2009). Abdominal pain can arise from bleeding cysts or from infection (Chauveau et al., 2000; Arnold and Harrison, 2005; van Keimpema et al., 2011). Parenchymal liver volume is generally preserved even in severe PLD, and liver function remains normal in the majority (Fick and Gabow, 1994). However, serum concentrations of alkaline phosphatase (AP) and especially gamma-glutamyl-transferase (gGT) can become elevated (Arnold and Harrison, 2005; Bistritz et al., 2005). In severe PLD, elevated AP (30–47%) and gGT (60–70%) occurs (van Erpecum et al., 1987; Que et al., 1995; Kabbej et al., 1996; Gigot et al., 1997).

Management options

The principal aim of treatment of PLD is to reduce symptoms by decreasing liver volume. There are five treatment modalities available for the management of symptomatic PLD:

- Aspiration and sclerotherapy (radiological)
- Fenestration (surgical)
- Segmental hepatic resection (surgical)
- Liver transplantation (surgical)
- Potential drug therapies:
  - Somatostatin analogues
  - mammalian target of rapamycin (mTOR) inhibitors.

The choice of a therapeutic option depends on (1) size and location of the liver cysts, (2) the extent of PLD, and (3) relation of cysts with symptoms (van Keimpema and Hockerstedt, 2009; Drenth et al., 2010a). In addition, local experience and available expertise determines which procedure is best suitable.

Radiological management

Aspiration-sclerotherapy is the preferential treatment for a dominant and large, likely symptomatic, superficially located cyst. The cyst size should be > 5 cm (Drenth et al., 2010b). With the procedure, one or several cysts are punctured under radiological guidance, and aspirated to ensure complete fluid evacuation (van Keimpema et al., 2008a). A sclerosant is injected to destroy the epithelial lining curtailing fluid production (Saini et al., 1983; Bean

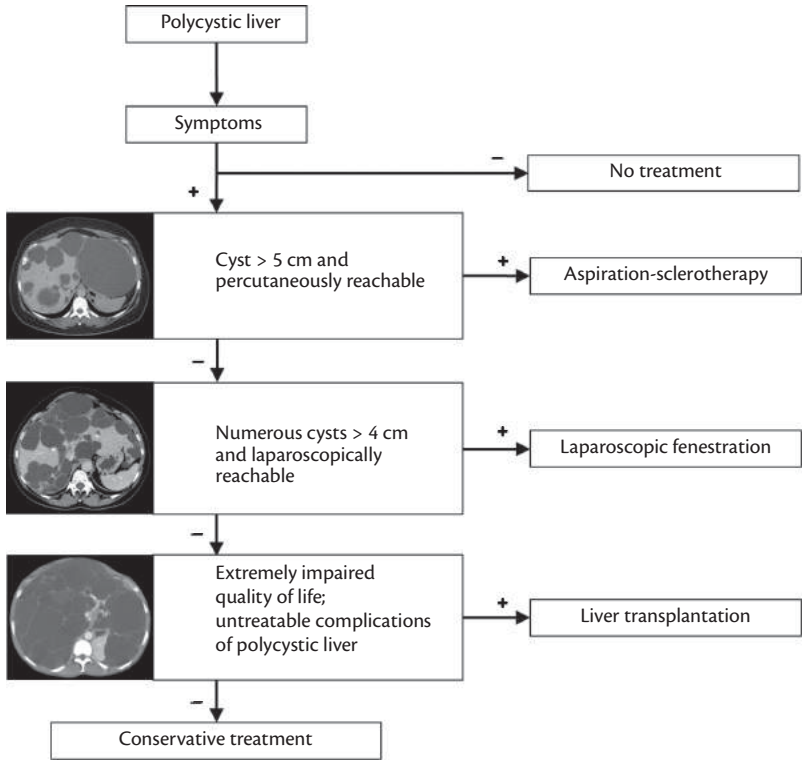

Fig. 311.1 Suggested algorithm for treatment strategy in polycystic liver disease.

and Rodan, 1985; van Keimpema and Hockerstedt, 2009). Ethanol is most commonly used, but minocycline and tetracycline are alternatives (Tokunaga et al., 1994; Yamada et al., 1994). Cysts recur in approximately 20% of treated patients, and in these cases, multiple sessions may be needed (Drenth et al., 2010b).

Surgical management

Surgical options for treatment of polycystic livers include fenestration, segmental hepatic resection, and liver transplantation.

Fenestration is a technique that combines aspiration and surgical deroofing of multiple cysts in a single procedure to achieve volume reduction (Russell and Pinson, 2007). The main indication for fenestration is a patient with multiple liver cysts (> 4 cm) accessible by laparotomy or laparoscopy (van Keimpema et al., 2008c). Although immediate symptom relieve is achieved in 92% of cases, recurrence of symptoms (22%) and cysts (24%) occur (Drenth et al., 2010b). Complications of fenestration occur in approximately 23% and include ascites, pleural effusion, arterial or venous bleeding, and biliary leakage (van Keimpema et al., 2008c; Drenth et al., 2010b). Laparoscopy leads to a lower complication rate (29% vs 40%) and is therefore preferred over laparotomy (Martin et al., 1998). The main disadvantage of a laparoscopic approach is that cranially located liver cysts are difficult to reach.

Segmental hepatic resection is considered in severe symptomatic patients with cyst-rich liver segments and at least one predominantly normal liver segment. The procedure is often combined with fenestration for deroofing of cysts in the remnant segment (Russell and Pinson, 2007). The distribution of cysts in the liver dictates the extension of the resection, although at least 25% normal liver parenchyma must be present to have a beneficial postresectional outcome (Schindl et al., 2005). Symptom relief is achieved in 86% of patients (Drenth et al., 2010b). However, the procedure is associated with considerable morbidity (51%) and mortality (3%) (Drenth et al., 2010b). The complications are similar to that of fenestration (Vauthey et al., 1991; Martin et al., 1998; Russell and Pinson, 2007; van Keimpema et al., 2008c; Drenth et al., 2010b). Furthermore, the risk of subsequent adhesions may complicate a possible future liver transplantation (Starzl et al., 1990; Swenson et al., 1998; Pirenne et al., 2001). Thus, segmental hepatic resection should be reserved for massive PLD patients not eligible for other treatment options.

The only curative option for PLD is transplantation (Everson et al., 2004). Candidates for liver transplantation are patients with extremely impaired quality of life or with untreatable complications such as portal hypertension and nutritional compromise (Russell and Pinson, 2007). Countries using the Model for End-Stage Liver Disease (MELD) criteria include exceptions for PLD, as these patients rarely have a significant MELD score because of preserved liver synthetic function (van Keimpema and Hockerstedt, 2009).

The 1- and 5-year survival in ADPKD patients with liver transplantation alone is 93% and 92%, respectively, whereas data for a combined liver and kidney transplantation are 86% and 80%, respectively (Drenth et al., 2010b). The overall morbidity after liver or combined liver-kidney transplantation is 41%, but with time, 91% of patients report improved quality of life (Kirchner et al., 2006; Drenth et al., 2010b). A combined liver–kidney transplantation should be considered in ADPKD patients with end-stage renal disease. In these cases, a liver and kidney from the same donor is preferable, as it protects the kidney graft from rejection and improves kidney graft survival (Shaked et al., 1993; Rasmussen et al., 1995).

Medical management

There are new medical drug options under investigation that target PLD. Fluid secretion and cell proliferation in hepatic cysts is stimulated by production of cyclic adenosine monophosphate (cAMP) (Torres and Harris, 2009; Strazzabosco and Somlo, 2011). Somatostatin analogues may revert the process of hepatic cyst growth by inhibiting cAMP (van Keimpema et al., 2008b; Gevers and Drenth, 2011). Recent trials showed that two long-acting somatostatin analogues, lanreotide and octreotide, decrease PLD liver volume. A 6–12-month treatment decreases liver volume by −5% to −2.9%, while this increased with 0.9–1.6% in the placebo group (van Keimpema et al., 2009; Caroli et al., 2010; Hogan et al., 2010). These observations suggest that somatostatin analogues affect the natural course of the disease. The most important question to address in future studies is whether this beneficial effect is maintained with prolonged therapy.

The mTOR inhibitor sirolimus reduced PLD volume by 12% after renal transplantation in ADPKD while volume increased by 14% with tacrolimus (Qian et al., 2008). Although the effect of sirolimus on polycystic liver volume is impressive, formal randomized trials need to confirm this observation. In addition, the toxicity of mTOR therapy may preclude its long-term use (Watnick and Germino, 2010).

Conclusion

There are several therapeutic options available for treatment of ADPKD patients with symptomatic PLD. How to select a treatment greatly depends on localization and size of cysts and we suggest an algorithm to facilitate the choice of therapy (Fig. 311.1).

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CHAPTER 312

Autosomal dominant polycystic kidney disease in children and young adults

Carsten Bergmann and Klaus Zerres

Diagnosis in childhood

An approach to the child with renal cysts is described in Chapter 305.

In a study by the Denver Polycystic Kidney Disease Research Group, approximately 60% of children < 5 years of age, and 75–80% of children 5–18 years of age with a *PKD1* mutation had renal cysts detectable by ultrasound (Gabow et al., 1997). Bear and colleagues proposed a rate of false negative ultrasonographic diagnosis of about 35% below the age of 10 years (Bear et al., 1992). In general, the finding of even one renal cyst should alert the clinician to the possibility of ADPKD, because simple cysts are extremely rare in childhood (McHugh et al., 1991). Ravine et al. found nil prevalence in individuals aged 15–29 years (Ravine et al., 1993). Thus, in children with a 50% risk of autosomal dominant polycystic kidney disease (ADPKD), the finding of one cyst is considered practically diagnostic.

About 95% of all carriers show ultrasonographic evidence of ADPKD at the age of 20 years and almost every patient at age 30. Where an earlier diagnosis is essential, magnetic resonance imaging is more sensitive than ultrasound (see Chapter 308), but if a positive genetic diagnosis has been made in the family, genetic testing is likely to be definitive.

Extrarenal ADPKD features other than hypertension are only rarely observed in children.

Early-onset autosomal dominant polycystic kidney disease

Clinical symptoms do not usually arise until the middle decades; however, there is striking phenotypic variability not only interfamilially but even within the same family, indicating that modifying genes, environmental factors, and/or other mechanisms discussed below considerably influence the clinical course in ADPKD (Persu et al., 2004; Paterson et al., 2005; Bergmann et al., 2011). In line with this, a small proportion of ADPKD patients present with an early-manifesting clinical course.

Early manifestation in ADPKD has usually been defined as clinical symptoms (e.g. arterial hypertension, proteinuria, and impaired renal function) occurring before the age of 15 years. Among these are cases with significant peri-/neonatal morbidity and mortality, sometimes indistinguishable from those with severe ARPKD (see Chapter 313). Conflicting data exist on the incidence of very early-manifesting ADPKD cases that may be attributable to inheritance of two defective *PKD* genes (e.g. Vujic et al., 2010). While most authors propose a figure of about 2% (Kaariainen, 1987; Sedman et al., 1987), Sweeney and Avner even suggest a prevalence of up to 5% (Sweeney and Avner, 2006). Given the prevalence rates for ADPKD (1/400–1/1000) and autosomal recessive polycystic kidney disease (ARPKD) (1/20,000), one can figure out that among children with polycystic kidney diseases in departments of paediatric nephrology, the total number of patients with early-onset ADPKD is about the same to those with ARPKD.

Families with early-manifesting offspring have a high recurrence risk of almost 50% for the birth of a child with a similar clinical course (Zerres et al., 1993). Increased risk extends also to offspring of affected siblings of the respective parent carrying the same germline mutation (Ross and Travers, 1975; Gal et al., 1989).

Conclusive data of underlying mechanisms are still lacking and a matter of ongoing research. Most seriously discussed mechanisms are anticipation, imprinting, and the segregation of modifying genes. Anticipation denotes the progressively earlier appearance and increased severity of a disorder in successive generations. While this mechanism has been well established for many neurological diseases (e. g. fragile X-syndrome, spastic paraplegia, and myotonic dystrophy), only weak arguments exist for ADPKD. Fick et al. suggested an unstable mutation as a plausible explanation responsible for 53% of the informative 86 families of their study that demonstrated anticipation defined as a 10-year-earlier onset of end-stage renal disease (ESRD) in offspring, when compared to their affected parent (Fick et al., 1994). However, the type of mutations identified so far provides no hint for unstable DNA in ADPKD. Furthermore, several groups failed to find any evidence of anticipation (Geberth et al., 1995; MacDermot et al., 1998). In the study by Geberth et al. (1995), the median difference for age at renal death in 74 parent–offspring pairs was 0 years, ranging from −26.3 to + 27.2 years. There was no deviation from normal (Gaussian) distribution according to the Shapiro–Wilk test. Moreover, the proposal of anticipation was rebutted by MacDermot et al. who described a family in which the clinical presentation of ADPKD

was impossible to reconcile with anticipation (MacDermot et al., 1998). The gene carrier in generation I showed more severe clinical symptoms than his offspring in generation II, whereas in generation III the affected father of two affected fetuses presenting *in utero* was asymptomatic.

Similarly inconsistent data are available for imprinting mechanisms. Imprinting denotes the differential expression of genetic material depending on whether the genetic material has been inherited from the mother or father. Bear et al. were first to postulate an influence of genetic imprinting on disease progression in 10 Newfoundland pedigrees linked to *PKD1* (Bear et al., 1992). Age of onset of ESRD was significantly earlier in persons inheriting the disease from their mothers than from their fathers (50.5 vs 64.8 years, P = 0.004). A statistically significant predominance of affected mothers transmitting the mutant gene was corroborated by two larger series of families with early-onset ADPKD (Zerres et al., 1993; Fick et al., 1994). In our survey of 79 children with early-onset ADPKD out of 64 families, a statistically significant maternal predominance was observed (M:F = 41:23) (Zerres et al., 1993). In the study by Fick et al. (1994), the mutation was transmitted maternally in 65% of the 52 parent–offspring pairs with ESRD anticipation. While these findings are in line with a genetic imprinting effect in terms of an earlier onset and accelerated progression of ADPKD in case of a maternally inherited gene, it cannot fully explain early-onset ADPKD in every case because paternal transmission has been frequently observed too (MacDermot et al., 1998; own unpublished data).

Segregation of a modifying allele being inherited from the unaffected parent is an intriguing hypothesis and was recently shown to be present in some families with early and severe polycystic kidney disease (Rossetti et al., 2009; Bergmann et al., 2011). The recurrence risk of about 25% for similarly early-onset ADPKD in sibs (Zerres et al., 1993) fits this hypothesis and is further supported by the low incidence of *in utero* presentation of ADPKD in second-degree relatives in these families. Single-case reports in the literature of second-degree relatives also affected by early-onset ADPKD may thus be explained by chance segregation of a modifying gene in these families (Ross and Travers, 1975; Gal et al., 1989). However, two additional pedigrees with very unusual transmission of early-onset ADPKD may raise some suspicion as to the accuracy of this hypothesis too (Kaariainen, 1987; Fick et al., 1993). In one of these families, an affected mother had four offspring with *in utero* onset polycystic kidney disease by two different unrelated husbands (Kaariainen, 1987). In the other pedigree, early-onset of ADPKD was reported in mother and daughter (Fick et al., 1993). Thus, in these families every unaffected parent would have been expected to be a carrier of a rare modifying allele by chance to fit this theory. Conclusively, the basis of early-onset ADPKD is complex and most probably depends on different mechanisms that require further examination.

Clinical spectrum of children with autosomal dominant polycystic kidney disease

The clinical spectrum of children with ADPKD varies widely and can range from fetuses with prenatal ultrasonographic evidence of massively enlarged kidneys and oligo-/anhydramnios who may die perinatally from respiratory insufficiency to renal cysts noted more or less accidentally on ultrasound in fully asymptomatic children. In general, the single most useful investigation in the evaluation of a child with early onset of cystic renal disease of unknown underlying disease entity might be ultrasound of the parents (Ogborn, 1994). If ADPKD is clinically suspected and the parents are < 30 years of age, the grandparents should be considered for renal ultrasound (Bear et al., 1984). It is noteworthy that a negative family history does not exclude the possibility of ADPKD, since the affected parent may have a clinically silent disease. Given the autosomal dominant mode of inheritance, the recurrence risk for a further affected child is 50%. In case of normal parental renal ultrasound at age 30 and trusted paternity, a spontaneous mutation has to be discussed and the recurrence risk is negligible except for the rare case of germline mosaicism in one parent. However, as a matter of course, in those cases primarily differential diagnoses like ARPKD (see Chapter 307) or other cystic kidney diseases should be taken into consideration (see Chapter 305).

Two large longitudinal studies on ADPKD children conducted at the University of Colorado, United States, demonstrated that severe renal enlargement at a young age and/or hypertension were risk factors for accelerated renal growth (Fick-Brosnahan et al., 2001; Shamshirsaz et al., 2005). To use renal enlargement as a marker for disease progression is clinically relevant because many symptoms, for example, pain, haematuria, proteinuria, stones, and hypertension, are associated with large kidneys. Furthermore, these authors could confirm that a large cyst number in early childhood is a predictor for faster structural progression. Conclusively, larger kidneys are associated with increased morbidity and more rapid progression to ESRD.

Intriguingly, in children with ADPKD, renal involvement is oftentimes asymmetric (including asymmetric kidney enlargement) and even unilateral in a small minority at early stages of the disease (Fick-Brosnahan et al., 1999). As with ARPKD, the kidneys can present as large and hyperechoic bilateral masses with decreased corticomedullary differentiation. While the ultrasonographic kidney pattern in ADPKD and ARPKD often becomes quite similar and hard to distinguish with ongoing disease (Nicolau et al., 2000; Avni et al., 2002; Nahm et al., 2002), the radiographic features in the early disease course are often easier to distinguish. Unlike ARPKD, in which the cysts are usually fusiform and tiny often impressing as pepper-salt pattern on ultrasound, ADPKD kidneys are frequently characterized by macrocysts even in small children.

While a significant proportion of adult ADPKD patients experience at least one episode of gross haematuria that is known to be a risk factor for the progression of renal disease (Gabow et al., 1992a, 1992b; Johnson and Gabow, 1997), haematuria occurs in only about 10% of affected children at a mean age of 9 years (Fick-Brosnahan et al., 2001).

Arterial hypertension in the first months of life may occur more often in patients with ARPKD, however, it is also common in paediatric ADPKD patients even with normal renal function (Sedman et al., 1987; Gagnadoux et al., 1989; MacDermot et al., 1998). Hypertension should be identified as early as possible and treated, particularly in children < 12 years of age with > 10 renal cysts (Avner, 2001). This is consistent with data emerging from clinical studies of adult ADPKD. The precise pathogenesis of hypertension in PKD still remains to be elucidated; at least in part it appears to be mediated by activation of the intrarenal renin–angiotensin–aldosterone system, reduced renal blood flow, and increased sodium retention (Chapman and Schrier, 1991; Harrap et al., 1991).

Intracranial aneurysms in polycystic kidney disease are discussed in Chapter 310. The general recommendation is to screen

only in families with a history of aneurysm rupture. Aneurysm rupture is exceptionally rare among children and adolescents with ADPKD. Mariani et al. pointed out that there is only a small chance of detecting an intracranial aneurysm before the age of 30 and, thus, do not recommend screening before the third decade (Mariani et al., 1999).

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CHAPTER 313

Autosomal recessive polycystic kidney disease

Carsten Bergmann and Klaus Zerres

Classification and differential diagnosis

An approach to the child with renal cysts is described in Chapter 305.

In their seminal studies, Osathanondh and Potter systematically classified renal cystic diseases into four distinct types (Osathanondh and Potter, 1964). Potter syndrome type I is referred to as autosomal recessive polycystic kidney disease (ARPKD), type II as renal cystic dysplasia, type III as autosomal dominant polycystic kidney disease (ADPKD), and type IV occurs when a long-standing obstruction in either the kidney or ureter leads to cystic kidneys or hydronephrosis. Types II–IV in particular can be part of many syndromes. While this classification still has an impact for pathoanatomical descriptions, it has been replaced by the genetic nomenclature.

Cystic kidneys are an important feature of numerous genetic syndromes described in Chapter 305. These include the mainly recessively inherited ciliopathies Jeune, Bardet–Biedl, Joubert, and Meckel–Gruber syndromes as well as the dominant disorders tuberous sclerosis (TSC), von Hippel–Lindau (VHL), disease and branchio-oto-renal (BOR) syndrome.

Accurate diagnosis is essential both in the management of patients with cystic kidneys and in counselling their families. When an effort is made to classify the wide array of different entities with renal cysts, it might be helpful to first distinguish between acquired and inherited forms. Knowledge about the family history and the clinical picture, together with the location and morphology of the cysts, and any possible extrarenal manifestations should help in making a diagnosis. Sometimes, cytogenetic studies and microarray-based comparative genomic hybridization may be useful to exclude rearrangements or aberrations such as large deletions or duplications.

ARPKD is transmitted in an autosomal recessive fashion, in other words virtually all individuals who have inherited two unfavourable, mutated *PKHD1* germline alleles will develop the disease. The parents usually each carry a heterozygous *PKHD1* mutation and are invariably healthy without developing cysts. The same applies to all other individuals bearing only one defective allele and an intact *PKHD1* gene copy *in trans*, that is, there is no heterozygote manifestation in ARPKD. The gene's name *PKHD1* is an abbreviation that stands for polycystic kidney and hepatic disease 1 and is also sometimes used as disease name pointing to the fact that liver changes in terms of ductal plate malformation (DPM) with hyperplastic biliary ducts and congenital hepatic fibrosis (CHF) are obligatory in ARPKD.

Epidemiology and morphology

ARPKD is much rarer than its dominant counterpart ADPKD (see Chapter 306) with a proposed incidence among Caucasians of about 1/20,000 live births corresponding to a carrier frequency of approximately 1/70 in non-isolated populations. Isolated populations may have considerably higher prevalences. For Finland, Kääriäinen and colleagues reported an incidence of 1/8000, for instance (Kääriäinen et al. 1988). Some severely affected babies may die pre- or perinatally without a definitive diagnosis, making it difficult to give exact figures on the disease prevalence. Furthermore, the patient cohorts published vary largely and range from severely affected, perinatally demised patients mainly seen by gynaecologists and pathologists to mild and moderately affected patients followed by paediatricians and their adult colleagues. Notably, among all children with polycystic kidney disease in departments of paediatric nephrology, the total number of patients with ARPKD equals the quantity of individuals affected with early-onset ADPKD. In contrast, when only considering perinatally demised patients with polycystic kidney disease, ARPKD outnumbers ADPKD.

Renal pathology

Renal cysts are fluid-filled, epithelia-lined, dilated saccular lesions that generally arise from tubular segments. Usually, ARPKD can be reliably diagnosed pathoanatomically (Zerres et al., 2003), but histological changes may vary depending on the age of presentation and the extent of cystic involvement. Principally, in affected neonates the kidneys retain their reniform contour and are symmetrically, massively enlarged (up to 10 times the normal size) with many tiny cysts (Fig. 313.1, Table 313.1). Macroscopically, the cut surface demonstrates cortical extension of fusiform or cylindrical spaces arranged radially throughout the renal parenchyma from medulla to cortex (Fig. 313.2). Invariable histological manifestations are fusiform dilations of renal collecting ducts and distal tubuli lined by columnar or cuboidal epithelium that usually remain in contact with the urinary system (unlike ADPKD), whereas glomerular cysts (as in ADPKD) or dysplastic elements (e.g. cartilage; as in Meckel–Gruber syndrome or some other syndromic ciliopathies) are usually not evident in ARPKD kidneys (Fig. 313.3). During early fetal development, a transient phase of proximal tubular cyst formation has been identified that is largely absent by birth, however (Nakanishi et al., 2000). With advancing clinical course the kidney structure might increasingly resemble the pattern observed in ADPKD with renal cysts that vary considerably in size and

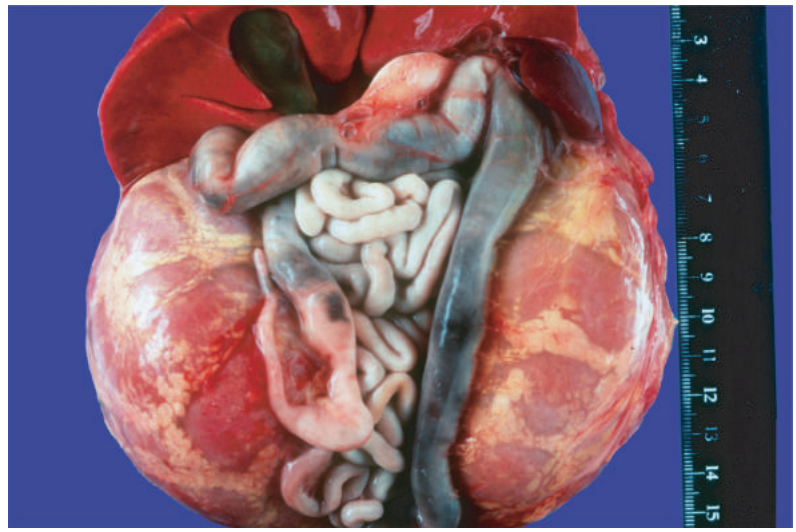

Fig. 313.1 Abdominal situs of an ARPKD patient with symmetrically enlarged kidneys that maintain their reniform configuration.

Table 313.1 Characteristics of autosomal recessive (ARPKD) and dominant (ADPKD) polycystic kidney disease

	ARPKD	**ADPKD**
Synonyms	Infantile polycystic kidney disease Potter type I	Adult polycystic kidney disease Potter type III
Incidence	~ 1:20,000	1:500–1000 (~ 2% early manifesting)
Pathology of kidneys: Macroscopy Location of cysts Ultrasound and diameter of cysts Pathology of liver	Massively, symmetrically enlarged kidneys (reniform) Dilated collecting ducts and distal tubuli At onset typical pepper-salt pattern in ultrasound, increased echogenicity of renal parenchyma throughout cortex and medulla due to tiny, sometimes invisible cysts (usually < 2 mm); with advancing age up to several cm similar to ADPKD pattern Mandatory: ductal plate malformation/congenital hepatic fibrosis with hyperplastic biliary ducts and portal fibrosis (may impress as Caroli disease)	Generally enlarged (also reniform), but usually to a lesser extent Cysts in all parts of the nephron (including glomerulus) Cysts of different size in cortex and medulla (usually several larger cysts in adults); at onset often small, however, sometimes already several cm early in childhood 'Liver cysts' common in adults, but rare in children. Occasionally, ductal plate malformation/congenital hepatic fibrosis
Associated anomalies	Rarely pancreatic cysts and/or fibrosis; single case reports with intracranial aneurysms	Pancreatic cysts and/or cysts in other epithelial organs; intracranial aneurysms in ~ 8%, familially clustered
Main clinical manifestations	Peri-/neonatal period: Respiratory distress (in 30–50%). With prolonged survival renal insufficiency, portal hypertension, and other variable co-morbidities	General onset 3rd–5th decade with arterial hypertension, proteinuria, haematuria, and/or renal insufficiency, ~ 2% early manifestation in childhood (rarely with perinatal respiratory distress)
Risk for siblings	25%	50% (except for rare cases of spontaneous mutation with virtually no risk)
Risk for own children	< 1% (unless unaffected parent is related to his/her affected partner, or ARPKD is known in the unaffected partner's family)	50% (also for patients with a spontaneous mutation)
Manifestation in affected family members	Often similar clinical course in siblings (in ~ 20% extensive intrafamilial variability)	Variable, however, often similar within the same family; in case of early manifestation ~ 50% recurrence risk
Parental kidneys	No alterations	Except for rare cases of spontaneous mutation, usually one parent is affected and shows renal cysts (be careful when parents are too young for definite clinical diagnosis/ < 30–40 years)
Prognosis	In perinatal cases with respiratory distress usually poor, for those surviving the neonatal period much better with renal death in ~ 15% in childhood, often severe complications (e.g. oesophageal varices) due to portal hypertension, if possible transplantation (often combined kidney-liver TX)	In early manifesting cases often better than in ARPKD. In 'adult' cases, chronic renal failure in ~ 50% by age of 60 years; median age of ESRD onset (54 vs 74 years in PKD1 vs PKD2)

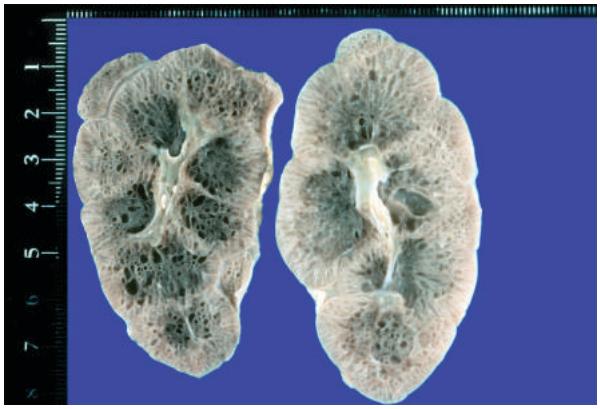

Fig. 313.2 Cross-section of ARPKD kidneys reveals the cortical extension of fusiform or cylindrical spaces arranged radially throughout the renal parenchyma from medulla to cortex.

appearance, often also accompanied by some degree of interstitial fibrosis (Avni et al., 2002).

Liver pathology

Liver changes are invariably present from early embryonic development onwards. Defective remodelling of the ductal plate leads to dysgenesis of the hepatic portal triad with hyperplastic biliary ducts and CHF (Fig. 313.4) (Desmet, 1998). At later stages, fibrous septa may link different portal tracts by intersecting the hepatic

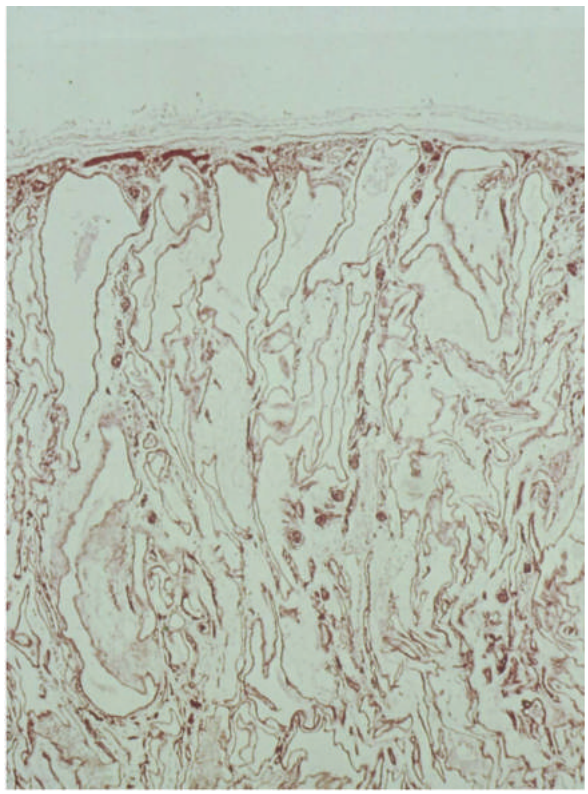

Fig. 313.3 Microscopically, fusiform dilations of renal collecting ducts and distal tubuli lined by columnar or cuboidal epithelium can be observed in ARPKD. These dilated collecting ducts run perpendicular to the renal capsule.

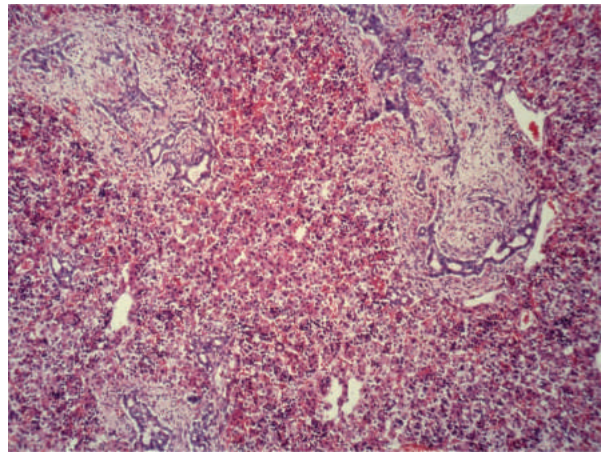

Fig. 313.4 Obligatory hepatobiliary changes in ARPKD subsumed as ductal plate malformation (DPMs) and characterized by dysgenesis of the hepatic portal triad with hyperplastic biliary ducts and congenital hepatic fibrosis (CHF).

parenchyma often leading to portal hypertension; however, the remaining liver parenchyma usually develops normal. Only cholestasis parameters such as gamma-glutamyl transferase are sometimes elevated, whereas other liver enzymes are characteristically within normal ranges.

Biliary anomalies may develop at any stage of the physiologic involution-remodelling process, and the timing or stage of development determines the resulting clinical and histological phenotype. Typically, cysts that arise from small interlobular bile ducts are detached from the biliary tree, while those that stem from malformation of medium- and large-sized bile ducts usually maintain connections.

While there are overlaps between each subgroup, bile duct hamartomas, ARPKD, and other ciliopathies (e.g. Bardet–Biedl, Joubert, and Meckel-Gruber syndromes) are mainly manifestations of ductal plate malformation (DPMs) of the small interlobular bile ducts, whereas medium-sized intrahepatic ducts are generally afflicted in ADPKD and polycystic liver disease. Caroli disease is usually the result of DPMs of large intrahepatic bile ducts, while choledochal cysts are thought to represent large extrahepatic DPMs (Desmet, 1998).

Clinical features and management

While ADPKD is usually a disease of adults with < 5% of patients displaying an early manifesting clinical course, ARPKD is typically an infantile disease. Despite dramatic advances in neonatal and intensive care over the past decades, the short-term and long-term morbidity and mortality of ARPKD remain substantial.

The clinical spectrum is much more variable than generally presumed (Adeva et al., 2006). Ages at diagnosis and initial clinical features are listed in Table 313.2 that summarizes results of various clinical studies on ARPKD. Notably, these studies differ widely by their selection criteria of patients and their mode of analysis of data. Patients from most of these surveys were recruited from paediatric departments, some from specialized single centres. As a consequence, individuals with an early lethal form of ARPKD were underrepresented. Most of the individuals in the study by Roy et al. (1997) had previously been reported by Kaplan et al.

Table 313.2 Summary of findings obtained in clinical studies of ARPKD patients

	Gunay-Aygun et al. (2010, 2012)	Bergmann et al. (2005)	Guay-Woodford and Desmond (2003)	Capisonda et al. (2003)	Roy et al. (1997)	Zerres et al. (1996)	Gagnadoux et al. (1989)	Kaplan et al. (1989)	Kääriäinen et al. (1988)	Cole et al. (1987)
Patients (n)	73 (63 unrelated patients)	186 (164)	166	31	52	115	33	55	73 (18 neonatal survivors)	17
Age at diagnosis	42% perinatal 58% non-perinatal (median, 2.9 y)	23% prenatal, 31% <1 m, 16% 1–12 m, 30% > 1 y	46% prenatal, 27% < 1 m, 11% 1–12 m, 16% > 1 y	32% prenatal, 23% < 1 m, 19% 1–12 m, 26% > 1 y	85% < 1 y, 15% > 1 y	10% prenatal, 41% < 1 m, 23% 1–12 m, 26% > 1 y	33% < 1 m, 55% 1–18 m, 12% 6–11 y	42% < 1 m, 42% 1–12 m, 16% < 1 y	72% < 1 m, 6% < 1 y, 22% > 1 y	100% 1–12 m (inclusion criteria)
Renal function	25% of perinatally symptomatic patients ESRD by 11 y, 25% of non-perinatally symptomatic patients ESRD by 32 y	86% GFR < 3rd centile for age median age CRF 4.0 y, 29% ESRD (by 10 y), 58% ESRD (by 20 y)	42% GFR < 3rd centile for age 13% ESRD	51% GFR < 80 mL/min/1.73 m^2 16% ESRD	33% ESRD (by 15 y)	72% GFR < 3rd centile for age 10% ESRD	42% GFR < 80 mL/min/1.73 m^2 21% ESRD	58% SC > 100 μmol/mL	82% GFR < 90 mL/min/1.73 m^2	35% GFR < 40 mL/min/1.73 m^2 29% ESRD
Kidney length	Perinal patients: 6.3 ± 3.3 SD Non-perinal patients: 4.5 ± 3.7 SD	92% > 2 SD	NA	NA	NA	68% > 2 SD	100% > 2 SD	NA	NA	NA
Hypertension (% on drug treatment)	NA	76% (80% M/72% F) medication started at median age of 3 y (53% during first 6 m)	65%	55%	60% (by 15 y)	70%	76%	65%	61%	100% (drug treatment or BP > 95th centile)
Growth retardation	NA	16% < 2 SD (23% M/10% F)	24% < 2 SD	NA	NA	25% < 2 SD	18% < 4 SD	NA	6% < 2.5 SD	NA
Anaemia	NA	14% (9% M/19% F)	NA	NA	NA	NA	NA	NA	NA	NA
Evidence of portal hypertension[a]	64% splenomegaly, 30% oesophageal varices	44% (41% M/47% F)[a] 38% splenomegaly, 15% oesophageal varices 2% ascites	15%	37%	23% (8/35)	46%	39%	47%	50% (hepatomegaly)	35%
Survival rate	NA	1 y 85%, 5 y 84%, 10 y 82%	1 y 79%, 5 y 75%	1 y 87%, 9 y 80%	NA	1 y 89%, 3 y 88%	1 y 91%	1 y 79%, 10 y 51%, 15 y 46%	1 y 19%	1 y 88%
Death rate in the first year of life	NA	15%	8%	13%	26%	9%	9%	24%	22%	12%

BP = blood pressure; ESRD = end-stage renal disease; F = female; M = male; m = months; NA = not available; SC = serum creatinine; SD = standard deviation; y = years.

[a]Based on sonographic evidence of hepatomegaly, splenomegaly, and directional reversal of portal vein flow, or clinical, radiological or endoscopic evidence of oesophageal varices or ascites.

[b]Manifestation of clinical signs of congenital hepatic fibrosis was positively correlated with age. In 87%, increased echogenicity of the liver has been reported (89% M/84% F). Cystic changes of the liver probably representing Caroli disease with dilated larger intrahepatic bile ducts have been noted in 27 individuals equalling 16% of the total cohort (17% M/15% F). Within this survey, only two boys exhibited impaired hepatocellular function (1%) underscoring that liver function is usually retained in ARPKD. In six patients (4 M/2 F) liver transplantation (LTX) was performed (mean age 13.8 years). In four cases it was done in parallel with NTX (combined LNTX).

who exclusively included patients with pathoanatomically proven CHF (Kaplan et al., 1989). Kääriäinen et al. analysed mainly data obtained from Finnish death registers (Kääriäinen et al., 1988). Inclusion criteria of the study by Cole et al. were diagnosis within the first year of life and survival of the neonatal period (Cole et al., 1987).

Perinatal features

The majority of cases are identified late in pregnancy or at birth. Severely affected fetuses display a 'Potter' oligohydramnios phenotype with pulmonary hypoplasia, a characteristic facies, and contracted limbs with club feet. As many as 30–50% of affected neonates die shortly after birth from respiratory insufficiency. Respiratory distress in these severely affected children is mainly caused by two reasons: first, mechanistically related by the massively enlarged kidneys that push the diaphragm upwards and lead to thoracic compression. Second, the critical degree of pulmonary hypoplasia as a consequence of oligo-/anhydramnios due to *in utero* renal dysfunction. In contrast, end-stage renal disease (ESRD) itself is only very rarely a cause for neonatal demise. Hyponatraemia related to a urine dilution defect is often present in the newborn period, but usually resolves over time (Kaplan et al., 1989; Zerres et al., 1996; Guay-Woodford and Desmond, 2003).

Outcomes

Advances in mechanical ventilation and other supportive measures as well as further improvements in renal replacement therapies have increased the survival rates of ARPKD patients with many of them now reaching adulthood. A few may even be clinically asymptomatic until advanced adulthood and have a normal life expectancy (Adeva et al., 2006). These very mildly afflicted individuals are exceptions to the rule. Usually, a wide range of associated co-morbidities evolve in ARPKD, such as systemic hypertension, ESRD, and clinical manifestations of CHF (Roy et al., 1997; Guay-Woodford and Desmond, 2003; Bergmann et al., 2005). Therefore, ARPKD is still a disease with a severely diminished life expectancy and an important cause of renal- and liver-related morbidity and mortality in children.

In a study by our group on almost 200 ARPKD patients with known *PKHD1* mutational status, the survival rate of those patients who survived the newborn period was 94% at 5 years and 92% at 10 years of age (Bergmann et al., 2005), while Dell and Avner reported a 10-year survival rate of 82% for patients who survived the first year of life (Dell and Avner, 1993).

Imaging

By ultrasound, children with ARPKD typically have characteristic bilateral, large hyperechoic kidney masses with poor corticomedullary differentiation. Cysts are usually fusiform and tiny often impressing as a so-called pepper-salt pattern at early stages. Macrocysts are uncommon in small infants, although they may be observed with advanced clinical course when ultrasonographic patterns of ARPKD and ADPKD oftentimes adjust and become difficult to distinguish (Nicolau et al., 2000; Avni et al., 2002; Nahm et al., 2002). Data for kidney length measured by ultrasound related to age revealed that 92% had a kidney length above or on the 97th centile for age, respectively + 2 standard deviation (SD) scores (Bergmann et al., 2005).

Renal disease

In our survey that included mainly patients from tertiary hospitals with departments of paediatric nephrology, chronic renal failure was first detected at a mean age of 4 years (Bergmann et al., 2005). Infants with ARPKD may have a transient improvement in their glomerular filtration rate (GFR) due to renal maturation in the first 6 months of life (Cole et al., 1987). However, subsequently, a progressive but highly variable decrease in renal function occurs. The management of children with declining renal function should follow the standard guidelines established for chronic renal insufficiency in other paediatric patients (Warady et al., 1999). In our study (Bergmann et al., 2005), ESRD occurred in 29% of patients at 10 years and 58% at 20 years, which is much lower than figures reported by previous studies that proposed rates of approximately 50% of ARPKD patients progressing to ESRD within the first decade of life (Cole et al., 1987; Roy et al., 1997).

Renal transplantation is the treatment of choice for individuals with ESRD. In case of massively enlarged kidneys, native nephrectomies may be warranted to allow allograft placement. In a few cases reported in the literature, uni- or bilateral nephrectomy led to respiratory improvement, better enteral nutrition, and more effective peritoneal dialysis. Early renal replacement therapy by renal transplantation after bilateral nephrectomy was reported in two infants at the age of 9 and 15 months of life (Spechtenhauser et al., 1999; Prelog et al., 2006), but is not usually an option in the neonatal period. We described a female newborn with ARPKD and huge kidneys in whom unilateral nephrectomy was performed as rescue therapy and haemodialysis was performed for several days before the girl died from complications of pulmonary hypertension (Arbeiter et al., 2008). Overall, uni- or bilateral nephrectomy should be considered in ARPKD patients with massively enlarged kidneys to be performed early with consecutive renal replacement therapy.

Liver disease

In keeping with a generally prolonged survival in ARPKD, the hepatobiliary complications may come to dominate the clinical picture in some patients. A serious, potentially lethal complication in ARPKD is ascending cholangitis that may cause fulminant hepatic failure. It always requires diligent evaluation with aggressive antimicrobial treatment. ARPKD patients may not display the typical clinical findings of cholangitis; thus, every patient with unexplained recurrent sepsis, particularly with Gram-negative organisms, should be critically evaluated for this diagnosis (Kashtan et al., 1999).

While hepatocellular function is usually preserved, sequelae of portal hypertension may lead to haematemesis or melaena due to bleeding oesophageal varices and/or hypersplenism. Primary management of variceal bleeding may include endoscopic approaches, such as sclerotherapy or variceal banding. In other patients, portosystemic shunting or a combined liver and kidney transplantation might be considered a viable therapeutic option.

Currently, there is no consensus as to the indication of combined liver and kidney transplantation (CLKT) and data on long-term outcome are scarce. We recently analysed in detail a cohort of eight ARPKD patients with known *PKHD1* mutational status undergoing CLKT in a single specialized centre (Brinkert et al., 2013). Patient survival after CLKT was 100% and liver and

kidney graft survival was 72% and 88%, respectively. Liver and kidney function were stable in all patients with median estimated GFR (eGFR) of 95 mL/min/1.73 m^2 (range 68–133 mL/min/1.73 m^2). Further data demonstrated significantly better growth in these patients after CLKT. In accordance with our results, Chapal and colleagues conclude that pre-emptive liver transplantation might be a therapeutic option in ARPKD patients with severe portal hypertension and/or Caroli disease evaluated for renal transplantation (Chapal et al., 2012). Although the decision for a combined transplant against the background of normal liver synthesis remains difficult, our data and those of others document a potentially favourable outcome of CLKT if performed in specialized institutions.

Although most patients show a comparable degree of severity with regard to liver and kidney involvement, there is no direct correlation between the severity of liver and kidney disease. A recent study by Gunay-Aygun et al. found spleen volume to have an inverse correlation with platelet count and prothrombin time (Gunay-Aygun et al., 2013). Platelet count was the best predictor of spleen volume and the severity of portal hypertension, but did not correlate with renal function. Single ARPKD patients even present with an organ-specific phenotype, that is, either an (almost) exclusive renal phenotype or a predominant or mere liver phenotype. In accordance, it could be demonstrated that *PKHD1* mutations can cause isolated CHF or Caroli disease (Rossetti et al., 2003; Bergmann et al., 2005). Two transgenic mouse models for *Pkhd1* display an isolated liver phenotype without any renal involvement (Moser et al., 2005).

Hypertension and cardiovascular disease

Arterial hypertension usually develops in the first few months of life and affects up to 80% of children with ARPKD (Table 313.1). Hypertension can be difficult to control in these children and may require multidrug treatment. To prevent sequelae of hypertension (e.g. cardiac hypertrophy and congestive heart failure) and deterioration of renal function, careful blood pressure monitoring is essential and systemic hypertension needs to be early and aggressively treated.

Angiotensin-converting enzyme inhibitors are regarded as the treatment of choice. Further appropriate drugs that are generally effective include angiotensin II receptor inhibitors, calcium channel blockers, beta blockers (particularly in those patients with signs of CHF and portal hypertension), and diuretics (especially loop agents) (Guay-Woodford and Desmond, 2003; Jafar et al., 2005).

Hypertension at least in part appears to be mediated by activation of the intrarenal renin–angiotensin–aldosterone system (RAAS), reduced renal blood flow, and increased sodium retention (Chapman and Schrier, 1991; Harrap et al., 1991). However, there is controversial data concerning whether or not the RAAS is activated in the first place, or at least inappropriately with respect to the prevailing blood pressure and sodium state (Ritz, 2006). Several studies postulated that the demonstrated link between renal structural severity and hypertension is likely due to dysregulated sodium balance and upregulation of the RAAS machinery (Kaplan et al., 1989; Chapman et al., 1990; Watson et al., 1992; Wang and Strandgaard, 1997), but the pathophysiology of hypertension in ARPKD is not clearly understood.

In contrast to ADPKD, patients with ARPKD are thought not to be at risk of intracranial aneurysms.

Phenotypic variability among affected siblings

About 20% of ARPKD multiplex pedigrees exhibit gross intrafamilial phenotypic variability with peri-/neonatal demise in one and survival into childhood or even adulthood in another affected sib (Deget et al., 1995). An even higher proportion of 20 out of 48 sibships (42%) was present in a study cohort among families with at least one neonatal survivor per family representative for the spectrum of patients usually followed by departments of paediatric nephrology (Bergmann et al., 2005). Adjusted for differing family sizes, the risk for perinatal demise of a further affected child in this study was 37% (22 perinatally deceased children from a total of 59 patients excluding the moderately affected index cases).

Some caution is therefore warranted in predicting the clinical outcome of a further affected child in a family with an increased risk for ARPKD. It is reasonable to recommend to all of those families to deliver in an experienced, well-equipped clinic with paediatric intensive care unit and interdisciplinary teams of obstetricians and paediatricians.

Phenotypes cannot be simply explained on the basis of the underlying *PKHD1* genotype. Modifying alleles, environmental factors, and other mechanisms such as epigenetics potentially influence the clinical course. Understanding these is a matter of ongoing research (Garcia-Gonzalez et al., 2007; Hopp et al., 2012). Recently, we and others could show that in some polycystic kidney disease families with variable expressivity only the severely affected patients harboured further mutations in addition to their expected familial germline defect that were assumed to aggravate the phenotype (Rossetti et al., 2009; Bergmann et al., 2011).

Genetic diagnosis

Marquardt is thought to be the first who postulated genetic heterogeneity of polycystic kidney disease when stating: 'In surviving individuals, cystic kidneys are inherited dominantly. In non-viable individuals, cystic kidneys are recessive' (Blyth and Ockenden, 1971).

Given its autosomal recessive mode of inheritance, the recurrence risk for subsequent pregnancies of parents of an affected child is 25%. Overall, males and females seem to be equally affected. Unaffected siblings have a two-thirds risk of being a carrier for ARPKD. However, most healthy siblings, other close relatives, and patients themselves seeking genetic counselling for their own family planning usually can be reassured, given that the risk of ARPKD for their own children will be comparably low when the partner is neither related to the index family nor a case of ARPKD is known in the partner's pedigree. (For offspring of patients 1:140; for offspring of patients' healthy siblings 1:420; for offspring of patients' healthy uncles/aunts 1:560, when using a heterozygosity rate of 1:70, respectively).

The *PKHD1* gene

The main gene mutated in ARPKD is *PKHD1* that is amongst the largest disease genes characterized to date in the human genome, extending over a genomic segment of about 470 kb with evidence for 86 exons (Onuchic et al., 2002; Ward et al., 2002). The longest *PKHD1* transcript contains 67 exons with an open reading frame (ORF) composed of 66 exons (ATG start codon in exon 2) that

encodes a protein of 4074 amino acids (aa). The gene is highly expressed in fetal and adult kidney and at lower levels in the liver (Onuchic et al., 2002; Nagasawa et al., 2002). Weak expression is present in other tissues too, among them pancreas and arterial wall.

The biology of the molecule is considered with that of other genes involved in cystic diseases in Chapter 303.

Genetic diagnosis

The large size of *PKHD1* has posed significant challenges to DNA-based diagnostic testing in times of Sanger sequencing, but has now much improved with the utilization and implementation of new sequencing technologies (next-generation sequencing (NGS)) in routine diagnostic settings. Other challenges are set by the extensive allelic heterogeneity with a high level of missense mutations and private mutations in 'non-isolate' populations (Bergmann et al., 2003, 2004a, 2004b, 2005; Furu et al., 2003; Rossetti et al., 2003; Losekoot et al., 2005; Sharp et al., 2005). Mutation detection rates of about 80% for the entire clinical spectrum of ARPKD patients ranging from individuals with perinatal demise to moderately affected adults have been shown (Bergmann et al., 2004b, 2005; Losekoot et al., 2005; Sharp et al., 2005). The power of *PKHD1* mutation analysis is further strengthened by the observation that in > 95% of families screened at least one mutation could be identified. However, the molecular defect still remains to be determined in a considerable proportion of cases. One major cause of missing mutations is the limited sensitivity of screening methods used in some of the older studies.

Genotype–phenotype correlations

Genotype–phenotype correlations can be drawn for the type of mutation rather than for the site of individual mutations (Bergmann et al., 2003). All patients carrying two truncating mutations display a severe phenotype with peri- or neonatal demise while patients surviving the neonatal period bear at least one missense mutation. The converse does not apply and some missense changes are as devastating as truncating mutations. Loss of function probably explains the usually uniform and early demise of patients carrying two truncating alleles. An assumption that a truncated ORF will always constitute a null mutation has also been postulated for other disorders (Muntoni et al., 2003). This uniformity is probably attributable to ablation of the message by nonsense-mediated decay. As regards fibrocystin, a critical amount of the full-length protein seems to be required for normal function that obviously cannot be compensated by alternative isoforms which might be generated by reinitiation of translation at a downstream ATG codon as a possible mechanism for the evasion of NMD. No significant clinical differences could be observed between patients with two missense mutations and those patients harbouring a truncating mutation in *trans*, thus, the milder mutation obviously defines the phenotype (Bergmann et al., 2005).

Patients with no discovered *PKHD1* mutation

In patients without a detectable *PKHD1* mutation, misdiagnosis of *PKHD1*-linked ARPKD has to be considered. As mentioned above, polycystic kidney disease has become much more complex in recent times with increasing evidence for a genetic network and mutations in multiple cilia-related disease genes that may mimic ARPKD. First, it is known that about 2% of ADPKD patients with a mutation in *PKD1* or *PKD2* show an early and severe phenotype with considerable perinatal morbidity and mortality that can be clinically indistinguishable from ARPKD. Furthermore, mutations in *PKD1* and *PKD2* can also be inherited in a recessive way (Bergmann, 2012). Finally, the phenotype of ARPKD can also be mimicked by mutations in *HNF1B* (see Chapter 315) and genes typically causing other ciliopathies (Chapters 314, 316) (e.g., nephronophthisis, but also other usually more syndromic ciliopathies). Therefore, a certain kind of alertness is important to circumvent possible pitfalls in genetic diagnostics.

Genetic heterogeneity and prediction of the pathogenicity of missense variants remain challenging. When only *PKHD1* sequencing data is available, caution is required for clinical decisions especially when only novel or rare missense changes are found. Due to these different aspects, novel genetic diagnostic testing approaches based on NGS allow simultaneous investigation of all genes known for cystic and polycystic kidney disease and other ciliopathies. This kind of analysis provides a thorough and complete result of all genes of interest and avoids otherwise possible misdiagnoses, especially with regard to prenatal testing.

Prenatal diagnosis

Given the recurrence risk of 25%, frequently devastating early manifestations of ARPKD and oftentimes comparable clinical courses among affected siblings, many parents of ARPKD children seek early and reliable prenatal diagnosis (PND) to guide future family planning. Typically, ARPKD patients are identified by ultrasound only late in pregnancy or at birth. However, even with state-of-the-art technology, fetal sonography at the time when termination of pregnancy (TOP) is usually performed frequently fails to detect enlargement and increased echogenicity of kidneys or oligohydramnios secondary to poor fetal urine output (Zerres et al., 1988; Reuss et al., 1990; Zerres et al., 1998). Therefore, an early and reliable PND for ARPKD in 'at-risk' families is only feasible by molecular genetic analysis.

Ethical considerations around prenatal diagnosis are discussed in Chapter 302.

Indirect, haplotype-based linkage analysis has often been performed for ARPKD in the past in terms of PND. However, due to the aforementioned reasons this is nowadays usually regarded as too risky without knowledge of the *PKHD1* mutational status and should only be performed in those families in which the diagnosis has been proven. Interested families should also be informed about the possibility of preimplantation genetic diagnosis at some single diagnostic centres and that this procedure always requires a lot of coordination and work-up in advance.

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CHAPTER 314

Bardet–Biedl syndrome and other ciliopathies

Lukas Foggensteiner and Philip Beales

Introduction

Cilia are complex and highly conserved microtubule-based structures found on most vertebrate cells (Chapter 303). Primary cilia are present as a single non-motile structure on the cell surface and are involved in mediating diverse chemical and mechanical interactions with the environment. These cilia consist of an axoneme comprised of nine pairs of microtubules originating from a basal body within the cytoplasm in a configuration described as 9 + 0. The axoneme is surrounded by a lipid bilayer and protrudes from the cell surface to interact with the cellular milieu. A large number of ciliary proteins are present, supporting and facilitating axonal transport and sensory functions. Motile cilia are similar but smaller in size and are pre-set in large numbers on mucosal epithelial cells. Their motility is derived from an additional central pair of microtubules giving them a 9 + 2 configuration. Motile cilia are functionally different from primary cilia and will not be considered in this chapter. Motile and primary cilia do, however, share some functional proteins and phenotypic overlap in primary/motile ciliary disorders is described.

The ciliary genes are highly conserved across the evolutionary spectrum, suggesting a fundamental biological role for these organelles, and mutations in these genes can give rise to a diverse but overlapping pattern of abnormality in many experimental systems. In humans, the first disease to be linked to a ciliary protein was autosomal dominant polycystic kidney disease, characterized by renal cysts and renal failure. Other diseases linked to ciliary proteins were soon identified and now a significant range of singe-gene disorders are known to be associated with mutations in genes primarily expressed in the primary cilia. These conditions are collectively referred to as ciliopathies. In most of these conditions the mechanism by which the mutations disrupt ciliary function and cause disease remain poorly understood (see Chapter 303). This chapter deals with the rarer ciliopathies.

The phenotypic features of ciliopathies is diverse and variable even within families but an overlapping pattern of organ involvement across the range of conditions has emerged, presumably reflecting the underlying function of the primary cilia in different organ systems (Fig. 314.1).

Bardet–Biedl syndrome

In the 1920s, the French physician George Bardet and the Hungarian Arthur Biedl published separate papers describing the same syndromic condition which included polydactyly, obesity, retinitis pigmentosa, and hypogonadism. Several decades earlier, Lawrence and Moon in London had described patients with retinitis pigmentosa and paraplegia and these conditions were grouped together as Lawrence–Moon–Bardet–Biedl syndrome. Although this nomenclature has persisted in the literature, the separate terms Bardet–Biedl syndrome (BBS) and Lawrence–Moon syndrome are now preferred. Whether they represent genetically and phenotypically distinct conditions, however, remains to be established.

Clinical features

BBS is a highly pleiotropic, autosomal dominantly inherited, multisystem condition with prevalence in Western populations of 1/125,000 to 1/175,000. Early descriptions of the condition identified retinitis pigmentosa, polydactyly, obesity, hypogonadism, diabetes, renal involvement, and learning difficulties as prominent characteristics. Few patients, however, have all of these features and many other rarer manifestations of the condition may be present (Beales et al., 1997, 1999) (Table 314.1).

Patients typically present with clinical features at around the age of 3 years but in one large series the mean age at diagnosis was 9 years of age. Rod-cone dystrophy and pigmentary retinopathy is perhaps the most consistent feature and is present in > 90% of patients and severe visual impairment is therefore common in patients by early adulthood. Night blindness is usually evident by age 7–8 years and legal blindness occurs in the mid teens. However, individuals with proven disease-causing mutations have been described with no ocular phenotype.

Polydactyly is common, affecting some 70% of patients, and may occur in the hands or feet. Most affected individuals have corrective surgery in childhood therefore a clinical history must always be sought from carers when the diagnosis of BBS is being considered. Brachydactyly is also common with short, broad, and stubby fingers.

Mean height of BBS patients is reported as a few centimetres below the population mean in several series. Obesity is usual with a mean body mass index in one series of 31.5 kg/m^2 in males and 36.6 kg/m^2 in females. The aetiology of the obesity remains undefined but hyperphagia is observed in many individuals and leptin resistance is seen in murine BBS models (Rahmouni et al., 2008). Type 2 diabetes is present in 5–10% of adults with BBS and may be related to the obesity although an underlying state of insulin resistance has not been excluded.

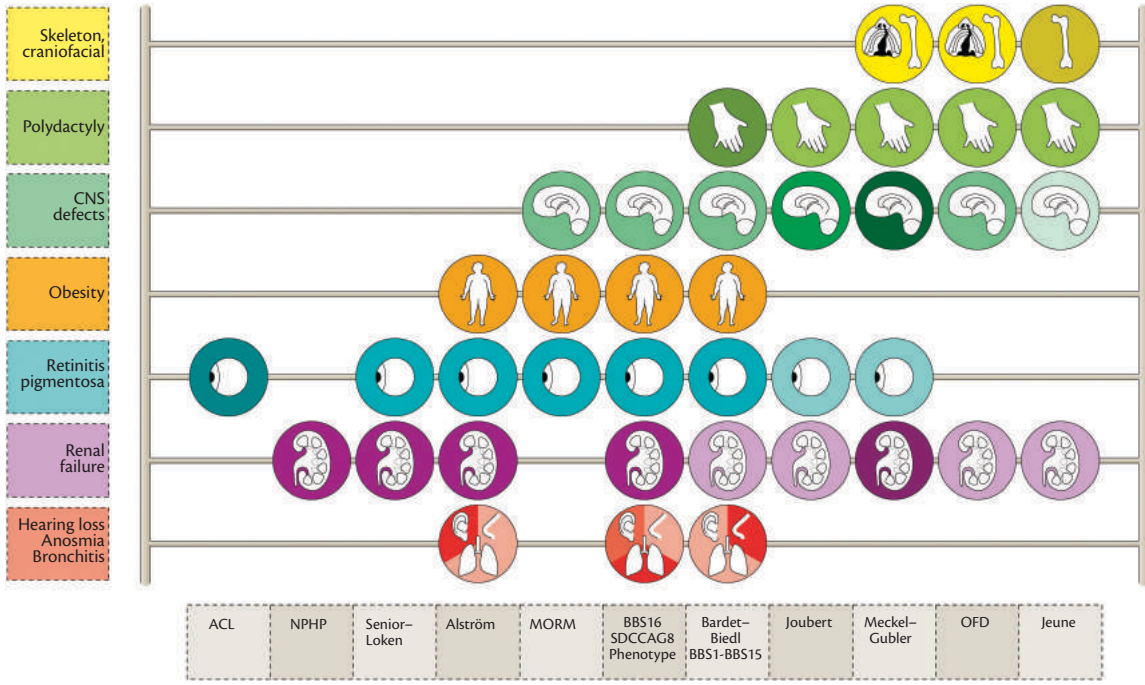

Fig. 314.1 The 'ciliopathy abacus'.

Hypogonadism is common in males and may require testosterone therapy. Females tend to have delayed menarche and irregular menstrual cycles.

Learning difficulties are a prominent feature of BBS and may be severe but are more commonly mild to moderate with a significant minority of patients able to gain employment. More subtle behavioural abnormalities are also reported which include abnormal affect, emotional immaturity and volatility, and obsessive–compulsive features.

Renal involvement in BBS ranges from benign structural renal abnormalities to end-stage renal disease (ESRD). No comprehensive survey of renal morphology in affected individuals has been published; however, diverse renal abnormalities including renal cysts, fetal lobulation of the kidneys, renal agenesis, vesicoureteric reflux, and dysplastic kidneys have all been described and some structural renal abnormality may affect up to 25% of individuals (Beales et al., 1999). ESRD is perhaps less common than expected, affecting about 10% of individuals in one large series (Jenkins et al., 2011).

Patients with a significant renal phenotype typically develop declining renal function in childhood with ESRD before the age of 20 years. Haematuria and proteinuria are typically absent so renal biopsy is rarely performed and little information on renal histology in this condition is available. All modalities of dialysis and transplantation are reported and have been successful in BBS. Patients with normal renal function may have urinary concentrating defects, polyuria, or symptoms of an irritable bladder.

Hypertension is seen in approximately 30% of patients and frequently requires treatment. Evidence of truncal and limb ataxia is present in some 40% of patients. Patients and carers often report poor coordination, unsteadiness, and general clumsiness and formal examination may reveal an ataxic gait, past pointing, and dysdiadokokinesia. Neurological abnormalities in BBS are rarely disabling, however.

Many other less common features of BBS are recognized. Otitis media causing hearing impairment affects some 20% of children but improves as patients mature. Asthma affects 25% of children and may require bronchodilators. Chronic respiratory disease is, however, uncommon in adults.

Pathophysiology of BBS

BBS was first linked to ciliary dysfunction following the identification of BBS8 which localizes to ciliated structures and to basal bodies and centrosomes in cells (Ansley et al., 2003). Subsequently, many of the remaining BBS proteins also localize to cilia. These highly conserved cellular structures project from the apical surface of most vertebral cells and fall into two classes: motile and immotile (primary) cilia (Tobin and Beales, 2007; Baker and Beales, 2009). Motile cilia are organized in a '9 + 2' microtubule configuration in which nine microtubule pairs surround a central doublet. These cilia generate flow or movement of fluid. Immotile cilia are thought to function mainly as a sensory organelle regulating signal transduction pathways and have a '9 + 0' configuration—similar in structure to the motile cilia but without the central pair or dynein arms rendering them sessile (Tobin and Beales, 2007; Baker and Beales, 2009; Waters and Beales, 2011).

Conserved across many phyla, cilia (and flagella) require the importation of protein building blocks and other signalling molecules from the cell body. To do so, they utilize intraflagellar transport (IFT), a system of molecular motors (e.g. kinesins) which carry cargo from the docking zone in the basal body to the cilial tip along the microtubular scaffold. At the tip, cargo proteins destined for the base use a different molecular motor, dynein, to make the journey back to the base of the cilium (for further detail see Chapter 303).

Amongst some of the roles reported, BBS proteins appear to facilitate and coordinate IFT and can be divided into two functional

Table 314.1 Bardet–Biedl syndrome phenotypes

Phenotype	Prevalence	Comments
Limb abnormalities		
Polydactyly	69%	Affecting hands or feet
Brachydactyly	46%	
Syndactyly	8%	Usually 2nd and 3rd toes
Ocular abnormalities		
Rod-cone dystrophy	92%	Characterized by retinitis pigmentosa
Other abnormalities	7%	Including strabismus, astigmatism, cataracts
Obesity		
Overweight	72%	Females > males
Obese	52%	
Renal abnormalities		
Stage 5 chronic kidney disease	10%	Age at ESRD usually < 20 years
Structural renal abnormalities	24%	Including renal cysts, lobulation, scarring, dysplastic kidneys, vesicoureteral reflux, unilateral renal agenesis
Abnormal urinary sediment	<1%	Proteinuria or haematuria not typical
Urinary concentrating defect		Polyuria
Cognitive abnormalities		
Learning difficulties	62%	Usually mild to moderate
Behavioural problems	33%	Includes obsessive–compulsive features and hyperactivity
Genital abnormalities (males)		
Hypogonadism	89%	Infertility is usual
Maldescent of testes	13%	
Delayed puberty	31%	
Diabetes	6%	Type 2 diabetes. Association with obesity phenotype
Neurological abnormalities		
Truncal and limb ataxia	40%	
Abnormal gait	33%	
Dental abnormalities	27%	Includes high arched palate, dental crowding, and micrognathia
Obstructive sleep apnoea	?	Associated with obesity but also evidence of central sleep apnoea (citation)
Anosmia	50%	Typically characterized by reduced olfaction (citation)
Hearing	23%	Mainly conductive and associated with chronic otitis media
Asthma	24%	Typically mild and of early onset

groups; BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, and BBS9 form the BBSome complex which associates with RAB8 to promote cilia maintenance and ciliogenesis (Nachury et al., 2007; Loktev et al., 2008; Jin and Nachury, 2009). BBS6, BBS10, and BBS12 belong to the chaperonin-like superfamily and appear to play a role in BBSome assembly (Seo et al., 2010).

The molecular pathogenesis of the pleiotropic effects of BBS has been elucidated only in part. The retinal degeneration arises secondary to IFT defects in the cilium connecting the inner to outer segments of the photoreceptor (Nishimura et al., 2004; Mockel et al., 2011). The polydactyly most likely arises from defective Hedgehog signalling which is dependent on intact functional cilia. The obesity observed in BBS appears to be multifactorial in origin. Mouse models demonstrate an association between BBS, increased food intake, and decreased physical activity; and there is evidence of peripheral leptin resistance (Rahmouni et al., 2008). Marion et al. (2009) found that primary cilia are transiently present in differentiating pre-adipocytes and contain receptors for Wnt and hedgehog pathways suggesting that dysregulation of adipogenesis may in part contribute to the development of obesity in BBS. Hyposmia/anosmia may be attributed to defective ciliated olfactory epithelium and subfertility in part to defective cilia in sperm cells/oviducts.

McKusick–Kaufman syndrome

McKusick–Kaufman syndrome (MKKS) is a rare recessive condition characterized by polydactyly and hydrocolpos in females or genital tract abnormalities in males (Robinow and Sha, 1979). Other congenital features reported include cardiac malformations and ano-rectal abnormalities. The phenotype may resemble BBS

in early childhood but patients do not develop retinitis pigmentosa, renal involvement, or diabetes. Genetically, however, BBS and MKKS syndrome overlap as both may be associated with mutations in *BBS6* as well as other known BBS genes (Schaefer et al., 2011).

Alström syndrome

Alström syndrome is a rare autosomal recessive ciliopathy affecting < 1:1,000,000. First described by Carl Henry Alström in 1959 (Alström et al., 1959), the condition is now known to be caused by mutations in the *ALMS1* gene (Li et al., 2007). The syndrome is characterized by the progressive development of severe multiorgan pathology that usually presents in infancy and evolves during childhood. The phenotype is variable, however, and few individuals have all the recognized complications of the condition. The most common childhood manifestations of Alström syndrome are dilated cardiomyopathy (DCM), rod-cone dystrophy, sensorineural hearing impairment, obesity, and insulin resistance (Marshall et al., 2005). As children age, many develop type 2 diabetes, short stature, and hypogonadism. The pulmonary, hepatic, and renal phenotypes develop in later childhood and renal failure is a common cause of death in this condition. In contrast to BBS, polydactyly is not a recognized feature of Alström syndrome and significant cognitive impairment is unusual.

Clinical features

DCM can occur at any age but is seen most typically during infancy (Makaryus et al., 2007); however, onset, progression, and clinical outcome of the DCM vary, even within families. Approximately 40% of affected infants have a transient but severe DCM with onset in early infancy with a significant mortality (Hoffman et al., 2005; Marshall et al., 2005). Those that survive, however, may see a significant improvement in cardiac function during early childhood. A subset of patients develop DCM later in childhood or early adulthood and these individuals seem to have more relentlessly progressive disease with a significant mortality in later life.

Obesity, insulin resistance, and type 2 diabetes are a prominent part of the Alström syndrome phenotype. Obesity develops in childhood, often starting in infancy and insulin resistance develops concurrently. Most individuals eventually develop type 2 diabetes but the age of onset is highly variable, even within families. Other endocrine abnormalities are also reported, including primary hypogonadism and growth hormone/insulin-like growth factor axis dysfunction.

Rod-cone dystrophy develops in the majority of individuals with Alström syndrome and may present in the first few weeks of life. It is usually relentlessly progressive leading to total blindness in late childhood or early adulthood.

Sensorineural hearing impairment, often complicated by chronic otitis media (glue ear), is common and most patients benefit from hearing aids. Approximately 10% of individuals become profoundly deaf.

Chronic respiratory illness is common and includes recurrent upper respiratory tract infections, chronic asthma, sinusitis/bronchitis, and frequent episodes of pneumonia. The chronically inflamed airways are hyper-reactive and highly sensitive to triggering or irritating factors. In some individuals, as inflammation continues, moderate to severe interstitial fibrosis develops. Pulmonary disease can become severe and include chronic obstructive pulmonary disease and pulmonary hypertension, secondary to pulmonary fibrosis. Respiratory infections are a significant cause of morbidity and mortality in Alström syndrome.

Most patients with Alström syndrome have detectable liver involvement. This may first present with hepatic steatosis associated with raised transaminase and gamma-glutamyl transferase levels. A proportion develops hepatitis or hepatic fibrosis, declining liver synthetic function, and liver failure. Although this can occur in childhood (Quiros-Tejeira et al., 2001), severe liver dysfunction usually occurs in early adult life. Portal hypertension, leading to oesophageal varices and upper gastrointestinal haemorrhage is a cause of death in some patients (Marshall et al., 2005, 2007). Liver histology reveals varying degrees of steatohepatitis, hepatic fibrosis, and cirrhosis. Hepatic transplantation has been successful in Alström syndrome.

Renal abnormalities are a significant clinical feature of Alström syndrome. A recent survey of 161 Alström patients showed renal insufficiency to be present in 80 (50%) patients, age range between 5 and 42 years. Renal function declined as patients aged, and ESRD was the cause of death in seven patients (Marshall et al., 2005). The cause for renal insufficiency is not fully understood but recent analysis of *Alms1* knockout mice reveals stunted cilia on renal tubular epithelial cells and an abnormal calcium signalling response to mechanical stimuli. As mice aged, they developed specific loss of cilia from the kidney proximal tubules associated with foci of apoptosis or proliferation (Li et al., 2007; Girard and Petrovsky, 2011). It is therefore likely that mutations in the *ALMS1* gene disrupt ciliary function in tubular epithelial cells leading to renal disease. Histological examination of biopsy and autopsy material from Alström patients reveals widespread interstitial fibrosis and tubular atrophy. In addition, nearly all patients with Alström syndrome are insulin resistant or frankly diabetic and 30% are hypertensive, both of which may contribute to renal dysfunction.

General urologic disturbances and abnormal voiding patterns were also reported in nearly 50% of Alström patients presenting as pre-micturition discomfort, difficulty initiating voiding, poor flow, long voiding intervals, retention, urgency, and urge incontinence, suggesting a variable decrease in bladder sensation and activity or, conversely, overactivity. Many other abnormalities are associated with Alström syndrome including hypogonadism, hypothyroidism, hyperlipidaemia, short stature, and developmental delay.

The Alström gene (*ALMS1*) has been identified (Hearn et al., 2002), and genetic confirmation of the diagnosis is now available. Although very rare, Alström syndrome represents an opportunity for preventive medicine particularly if diagnosed early. It is one of the inherited groups of deaf-blind syndromes with severe and characteristic multisystem complications. The high prevalence of childhood type 2 diabetes in patients with Alström syndrome distinguishes this syndrome from other monogenic causes of childhood obesity, such as BBS. Photophobia and nystagmus are often evident in infancy. Thus early onset of visual and hearing impairment accompanied by one or more other features of the syndrome (e.g. cardiomyopathy, obesity, insulin resistance, and renal impairment) should heighten index of suspicion.

No disease-specific therapy is available but surveillance for potential target organ involvement and treatment of life-threatening multisystem complications is paramount. These include cardiac failure, renal failure, hepatic failure, severe hypertriglyceridaemia leading to pancreatitis, urological dysfunction, and restricted pulmonary

function. Early organ transplant should also be considered and genetic counselling should be offered to all Alström patients.

Joubert syndrome

Joubert syndrome is a clinically and genetically heterogeneous condition caused by mutations in at least 10 separate genes expressed in the primary cilium. The disease is rare with a reported prevalence of < 1/80,000 and is inherited as an autosomal dominant single-gene disorder. Classifications of Joubert syndrome recognize that this term describes a range of overlapping conditions with some common features and the term Joubert syndrome and related disorders (JSRD) has been proposed to encompass this spectrum of clinical presentations (Gleeson et al., 2004; Brancati et al., 2010). Subtypes of JS include *pure JS* with only central nervous system (CNS) involvement, *JS with ocular defects, JS with renal defects, JS with occulo-renal defects, JS with oro-facial-digital defects*, and *JS with hepatic defects*. This classification is supported by emerging genotyping studies which suggest a correlation between disease causing mutations and phenotype. The defining feature of JSRD is a central nervous system developmental abnormality causing hypodysplasia of the cerebellar vermis, an abnormally deep interpeduncular fossa at the level of the isthmus and upper pons, and horizontal, thickened, and elongated superior cerebellar peduncles. This gives rise to a distinct radiological feature on cerebral magnetic resonance imaging scanning referred to as the molar tooth sign (MTS). Clinically almost all patients with JSRD display generalized hypotonia in infancy which evolves into central ataxia, ocular apraxia, developmental delay, and varying degrees of impaired cognitive function. Many other structural CNS abnormalities are also associated with the MTS including hydrocephalus and white matter cysts.

Retinal involvement in JSRD ranges from congenital retinal blindness, also known as Leber congenital amaurosis to lesser degrees of retinal dystrophy (Sturm et al., 2010), optic disc drusen, and colobomas.

Renal involvement in JSRD is common, affecting 25% of individuals, and clinically resembles nephronophthisis (NPHP) with progressive interstitial fibrosis associated with the development of small cysts at the corticomedullary junction. Renal failure may develop in early childhood but more typically becomes evident in the early teenage years culminating in ESRD by the end of the second decade of life. Renal failure can be managed in the conventional way with dialysis and transplantation. Patients with features of Joubert syndrome and cystic dysplastic kidneys have been described as having Dekaban–Arima syndrome. It is likely, however, that the renal pathology in these cases was consistent with NPHP and that this does not represent a distinct disease.

Joubert syndrome is a genetically heterogeneous disorder with disease-causing mutations described in > 10 different genes expressed in the primary cilium including *NPHP-1* which encodes nephrocystin-1. A pattern of genotype/phenotype correlation is emerging (Valente et al., 2008) with mutations in *NPHP-1, RPGRIP1L*, and *CEP290* being associated with a renal phenotype.

Oral-facial-digital syndrome

Oral-facial-digital syndromes (OFDS) are a group of overlapping conditions with a phenotype that includes oral, facial, and digital manifestations as well as polycystic renal disease and other systemic features (Gurrieri et al., 2007). OFDS type I is an X-linked dominant condition with a prevalence of 1/250,000 caused by mutations in the *OFD1* gene and includes a renal cystic phenotype which can lead to ESRD. The condition is only seen in females as affected males die in early gestation. It is described in more detail in Chapter 319.

Jeune syndrome

In 1955, Jeune described a recessive condition characterized by osteochondrodysplasia with associated skeletal abnormalities, polydactyly, and variable renal, hepatic, pancreatic, and retinal complications. Also described as asphyxiating thoracic dystrophy, it often leads to death in infancy due to a severely constricted thoracic cage and respiratory insufficiency (Keppler-Noreuil et al., 2011). Subject that survive into later childhood may develop a cystic renal phenotype and renal failure. Although Jeune syndrome is genetically heterogeneous, a subset of individuals are homozygous for mutations in the *IFT80* gene which encodes an intraflagellar transport protein that is essential for ciliary function (Beales et al., 2007).

Meckel–Gruber syndrome

Meckel–Gruber syndrome (MKS) is a rare autosomal recessive disorder characterized by the presence of an encephalocoele, polydactyly, multicystic dysplastic kidneys, and biliary ductal dysplasia. MKS is a lethal syndrome which usually results in intrauterine death or neonatal death. (Alexiev et al., 2006) The condition is genetically heterogenous with at least six MKS genes (*MKS1–6*) described to date. Furthermore, mutations in the *NPHP3* gene, which can also be responsible for NPHP and Senior–Loken syndrome (Bergmann et al., 2008; Shaheen et al., 2011) can also give rise to a MKS-like syndrome illustrating the degree of phenotypic and genetic overlap in the spectrum of ciliopathies. Most of the MKS genes are expressed in the primary cilium and disruption of ciliary function is likely to underlie the pathophysiology of the condition.

Leber congenital amaurosis

Leber congenital amaurosis (LCA) is a rare recessive condition that causes severe visual impairment or blindness in early childhood but has no extraocular features. It is a genetically heterogeneous condition caused by mutations in at least 14 genes, several of which have also been implicated in other non-syndromic or syndromic retinal diseases, such as retinitis pigmentosa and Joubert syndrome (Ahmed and Loewenstein, 2008; den Hollander et al., 2008). Several of the causative genes are now known to be expressed in the primary cilia of photoreceptor cells and are involved in ciliary transport processes. Successful treatment of LCA with gene therapy has been reported (Chung and Traboulsi, 2009).

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CHAPTER 315

Hepatocyte nuclear factor-1B

Coralie Bingham

Biology

The hepatocyte nuclear factor-1B gene (*HNF1B*; HGVS approved gene name *TCF2;* OMIM #189907 <http://omim.org/entry/189907>) is located on chromosome 17q12. It codes for a transcription factor protein. HNF1B is a member of the homeodomain-containing superfamily of transcription factors and is closely related to HNF1A. The HNF1A and HNF1B proteins show > 80% sequence homology, bind to the same DNA sequence, and can exist as both homodimers and heterodimers (Mendel et al., 1991).

During mouse embryonic development, HNF1B is expressed earlier than HNF1A, which is activated only during organogenesis (Cereghini et al., 1992). HNF1B is essential for visceral endoderm specification (Barbacci et al., 1999). In adult animals, HNF1A and HNF1B are expressed in the liver, kidney, pancreas, and gut. HNF1A is predominantly expressed in the liver and HNF1B in the kidney. HNF1B alone is expressed in the lungs, thymus, and genital tract (Lazzaro et al., 1992).

In the neonatal rat kidney, HNF1B is expressed in the proximal and distal convoluted tubules and loop of Henle, which are derived from the embryonic metanephrogenic mesenchyme, and in the collecting ducts, which are derived from the ureteric bud (Lazzaro et al., 1992). In female mice, HNF1B expression has been demonstrated in the oviduct and the uterus, which are derived from the Müllerian ducts. In males, HNF1B is expressed in the epididymis, vas deferens, and seminal vesicles, which are derived from the Wölffian duct, and in the prostate and testes (Reber and Cereghini, 2001).

Human metanephroi express HNF1B at pre-glomerular stages (54–56 days of gestation) through to 91 days of gestation. *In situ* hybridization studies have shown that HNF1B transcripts are predominantly expressed in fetal collecting duct branches, with lower levels of expression in the metanephric mesenchyme (Kolatski-Joannou et al., 2001).

Liver-specific inactivation of the *Hnf1b* gene in mice produces animals with severe jaundice caused by abnormalities of the gall bladder and intrahepatic bile ducts (Coffinier et al., 2002). Renal specific inactivation of the *Hnf1b* gene in mice leads to the development of cystic kidneys. In addition, HNF1B upregulates transcription of uromodulin (*Umod*), polycystic kidney and hepatic disease 1 (*Pkhd1*), and polycystic kidney disease (*Pkd2*) in the mouse (Gresh et al., 2004).

Mutations in *HNF1B*

The initial description of mutations in *HNF1B* causing developmental renal disease arose from the study of monogenic diabetes. Maturity-onset diabetes of the young (MODY) is a monogenic form of young-onset (usually diagnosed before 25 years) type 2 diabetes inherited as an autosomal dominant trait. Heterozygous mutations in the *HNF1A* gene are the commonest cause of MODY (Bingham and Hattersley, 2004). The *HNF1B* gene was a candidate gene for MODY because *HNF1A* and *HNF1B* are closely related. The first description of an *HNF1B* mutation causing disease was in 1997 in a Japanese family with early-onset diabetes and renal disease including renal cysts (Horikawa et al., 1997). Heterozygous mutations and whole and partial gene deletions in the *HNF1B* gene have now been widely reported; presentation with renal disease is much more common than presentation with diabetes. Mutations and deletions account for similar numbers of cases. There is an autosomal dominant pattern of inheritance although the mutations and particularly the whole-gene deletions arise *de novo* in around 50% of cases (Edghill et al., 2006a, 2008).

Renal phenotype of *HNF1B* mutations

Developmental renal disease is the most consistent phenotype seen with *HNF1B* mutations. Renal abnormalities are frequently detected on antenatal ultrasound scans emphasizing the importance of this gene in renal development (Bingham et al., 2000; Adalat et al., 2009). Large bright kidneys, multicystic dysplastic kidneys, and hydronephroses may be seen on antenatal scans (Adalat et al., 2009).

In children and adults, a wide range of abnormalities have been reported including renal cysts, renal dysplasia, multicystic dysplastic kidneys, hydronephrosis and hydroureter, megabladder and hydronephrosis as part of the prune belly syndrome, single kidney, and horseshoe kidney; renal cysts are the most common phenotype (Bingham and Hattersley, 2004; Murray et al., 2008; Adalat et al., 2009).

The renal calyces and papillae may be an abnormal shape including pelviureteric junction obstruction. The renal pelvis and calyces are derived from the embryonic ureteric bud which is known to be a site of high HNF1B expression (Bingham and Hattersley, 2004).

There is variable renal histology including glomerulocystic kidney disease (dilatation of the Bowman's spaces and primitive glomerular tufts in at least 5% of the cysts); cystic renal dysplasia (an absence of normal nephrons with unspecific cysts) and oligomeganephronia (reduced number and hypertrophy of glomeruli) (Lindner et al., 1999; Bingham et al., 2000, 2001).

Patients with an *HNF1B* mutation have renal function which ranges from normal to dialysis dependence or transplantation (Bingham and Hattersley, 2004). There have been cases of fetal loss with severe cystic renal dysplasia (Bingham et al., 2000).

Longitudinal studies have suggested that the early enlarged kidneys seen in the fetus or in childhood fail to grow and the kidneys become relatively smaller as the subject ages. Small hypoplastic kidneys are seen in some adults (Waller et al., 2002; Adalat et al., 2009). There is no correlation between the type of mutation and the severity of the renal phenotype. Affected members of the same family may also have variability in renal phenotype.

Heterozygous mutations and whole and partial gene deletions in the *HNF1B* gene have now been shown, in multiple large studies, to be the most common known genetic aetiology of developmental renal disease. *HNF1B* mutations/deletions were found in 24% patients with unexplained renal disease (Edghill et al., 2006a, 2008), 29% of those with fetal bilateral hyperechogenic kidneys (Decramer et al., 2007), 31% of children with renal disease presenting *in utero* or on postnatal scans (Ulinski et al., 2006), 23% of children with renal malformations (Adalat et al., 2009), 22% of children with hypoplasia and cysts (Weber et al., 2006), and 20% of fetal, paediatric, and adult subjects with developmental renal disease (Heidet et al., 2010). In children, *HNF1B* mutations may be associated with renal disease alone; however, there is multisystem involvement (described in subsequent sections) in many patients which becomes apparent with ageing.

In addition to the importance of *HNF1B* in developmental renal disease, biallelic *HNF1B* inactivation as a result of monoallelic germline and somatic mutations has been reported in chromophobe renal tumours in adults (Rebouissou et al., 2005).

Diabetes phenotype of *HNF1B* mutations

The majority of *HNF1B* mutation carriers have extrarenal phenotypes, most commonly diabetes. The association of *HNF1B* mutations with renal cysts and diabetes is termed the renal cysts and diabetes (RCAD) syndrome (Bingham and Hattersley, 2004). The diabetes may present as MODY type 5, C-peptide is persistently detectable, and there is an absence of pancreatic autoantibodies (Raile et al., 2009). The median age of presentation with diabetes is 20 years (range 15 days–61 years); insulin treatment is usually required (Edghill et al., 2008). *HNF1B* mutations are a rare cause of neonatal diabetes (Edghill et al., 2006a). Diabetes may also develop after renal transplantation when stress and the use of glucocorticoids and tacrolimus may be additional triggers (Waller et al., 2002). Diabetes is associated with pancreatic atrophy and exocrine dysfunction which highlights the importance of *HNF1B* in human pancreatic development. In some patients there is agenesis of the pancreatic body and tail (Haldorsen et al., 2008; Raile et al., 2009). Insulin secretion is reduced due to beta-cell dysfunction and there is insulin resistance with insufficient endogenous insulin secretion (Pearson et al., 2004). Despite being insulin resistant, patients are typically not obese. Low birth weight is observed in babies with an *HNF1B* mutation, consistent with reduced insulin secretion *in utero* (Edghill et al., 2006b).

Genital tract malformations

During female embryologic development, the two Müllerian (paramesonephric) ducts develop into the main genital duct. The caudal Müllerian ducts fuse to form the corpus and cervix of the uterus and vagina. The most common uterine malformations are caused by a failure of the Müllerian ducts to fully fuse; these include septate and bicornuate uterus, unicornuate, and uterus didelphys. Müllerian duct aplasia leads to vaginal aplasia and rudimentary or absent uterus. Women with *HNF1B* mutations may have uterine malformations resulting from Müllerian duct fusion defects or aplasia (Bingham et al., 2002; Oram et al., 2010). Uterine malformations are associated with renal tract malformations. Mutations of the *HNF1B* gene are found in women with both renal and uterine malformations (Oram et al., 2010). Genital tract malformations have also been reported in men with *HNF1B* mutations, these include epididymal cysts, asthenospermia and infertility, bilateral agenesis of the vas deferens, undescended testes, and hypospadias (Bellanné-Chantelot et al., 2004).

Hyperuricaemia and gout

Hyperuricaemia and young-onset gout are common features in patients with *HNF1B* mutations. Hyperuricaemia is probably a result of altered urate transport by the kidney. Some families with an *HNF1B* mutation, hyperuricaemia, young-onset gout, and renal disease fit the criteria for familial juvenile hyperuricaemic nephropathy (Bingham et al., 2003).

Hypomagnesaemia

Hypomagnesaemia is a further feature in some patients with *HNF1B* mutations. Patients are usually asymptomatic from the hypomagnesaemia although tetany has been reported. HNF1B regulates transcription of FXYD2 which is involved in the tubular handling of magnesium. HNF1B therefore also has a role in the regulation of tubular function (Adalat et al., 2009).

Abnormal liver function tests

Elevated liver enzymes are frequent in patients with *HNF1B* mutations. This is characterized by elevation of alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transpeptidase without jaundice or liver insufficiency (Bingham and Hattersley, 2004). Liver histology has rarely been examined and has usually been normal or shown changes of mild cholestasis (Bellanné-Chantelot et al., 2004). However, there have been isolated reports of more severe liver phenotypes including congenital jaundice, severe cholestasis with pruritus, and bile duct hypoplasia (Kitanaka et al., 2004; Raile et al., 2009).

Other features

Other features have been described in some patients with *HNF1B* deletions; these include severe developmental delay, autism, epilepsy, coloboma, and cataract (Raile et al., 2009; Loirat et al., 2010).

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CHAPTER 316

Nephronophthisis and medullary cystic kidney disease: overview

John A. Sayer

Nephronophthisis

The conditions nephronophthisis (NPHP) and medullary cystic kidney disease (MCKD) share some common clinical and histological features that relate to features of interstitial renal disease. These overlapping descriptions were based on both historical clinical descriptions (Smith and Graham, 1945) and histological studies where the pathological features are shared (Zollinger et al., 1980). However, it is now known that these conditions have a different inheritance pattern and distinct molecular pathogenesis, therefore they should be considered as separate or different conditions.

NPHP (Fanconi et al., 1951) is an autosomal recessive condition characterized by polyuria, polydipsia secondary to urinary concentration defects, and progressive renal failure leading to end-stage renal failure (ESRF) within the first three decades of life (Hildebrandt and Zhou, 2007). Renal ultrasound findings demonstrate normal- or reduced-sized kidneys, with loss of corticomedullary differentiation and sometimes corticomedullary cyst formation. Thus NPHP is often described as a cystic kidney disease (Wolf and Hildebrandt, 2011), although cysts are not always present.

The diagnosis of NPHP may be made using a renal biopsy with a classical histological triad of renal tubular atrophy, interstitial fibrosis, and tubular basement membrane disruption, including thickening and attenuation or disintegration (Zollinger et al., 1980). In fact, NPHP literally means a disintegration of nephrons (Wolf and Hildebrandt, 2011). These histological features mean that NPHP may also be termed an inherited interstitial kidney disease. The molecular pathogenesis now reveals that NPHP is part of a ciliopathy phenotype (see Chapter 303) and all the genes associated with NPHP express their protein products in the primary cilium/basal body complex (Hildebrandt and Zhou, 2007).

NPHP may present rarely in an infantile form, or more typically as a juvenile form (Table 316.1). Infantile NPHP usually represents a severe phenotype with early-onset ESRF (Fig. 316.1), before the age of 5 years (Simms et al., 2009). There may be antenatal findings of oligohydramnios and a fetal/neonatal renal ultrasound scan may show enlarged cystic kidneys. It is important to consider infantile NPHP, autosomal recessive polycystic kidney disease, or an early presentation of autosomal dominant polycystic kidney disease in the differential diagnosis of bilateral enlarged cystic kidneys in a neonate. Infantile NPHP has been associated with mutations in *NPHP2* (Otto et al., 2003), *NPHP3* (Tory et al., 2009), and *NEK8* (Otto et al., 2008).

Juvenile NPHP is the more typical presentation, where salt wasting, polyuria, and polydipsia are seen in children at 4–6 years of age. There is an insidious decline in renal function, with the median onset of ESRF being 13 years of age (Fig. 316.1) (Wolf and Hildebrandt, 2011). A key feature of NPHP is the presence of extrarenal manifestations which occur in 10–15% of patients. These include other ciliopathy features, such as retinal dysplasia and degeneration leading to early and severe visual loss (within 2 years of age) or later-onset night blindness leading to complete visual loss by 10 years of age. Other extrarenal manifestations include neurological disorders, notably cerebellar vermis aplasia presenting as ataxia (Joubert syndrome –see Chapter 317), liver fibrosis and biliary duct proliferation, skeletal dysplasia, and cardiac malformations (including situs inversus). There are numerous genetic causes of NPHP (mutations in genes *NPHP1–16*, see Chapter 317); however, many patients remain undiagnosed, suggesting further genes will be discovered. There are no specific treatments for NPHP and care remains supportive, using renal replacement therapy and ideally transplantation where possible.

Medullary cystic kidney disease

Medullary cystic kidney disease (MCKD) is an autosomal dominantly inherited kidney disease (Bleyer, 2009). MCKD types 1 and

Table 316.1 Nomenclature used for inherited interstitial nephropathies. Autosomal dominant interstitial kidney disease (ADIKD) is superseding 'MCKD' in modern literature

Classification	Inheritance	Comments
Infantile NPHP	Autosomal recessive	Onset of NPHP before 2 years of age
Juvenile NPHP/ NPHP	Autosomal recessive	Typical NPHP, median age of ESRF 13 years
ADIKD – MCKD type 1	Autosomal dominant	Associated with *MUC1* mutations (see Chapter 318)
ADIKD – MCKD type 2	Autosomal dominant	Also called UMOD-associated kidney disease; uromodulin storage disease; familial juvenile hyperuricaemic nephropathy (see Chapter 318)
ADIKD – REN-associated kidney disease	Autosomal dominant	May mimic hyperuricaemic nephropathy Also called REN-related kidney disease

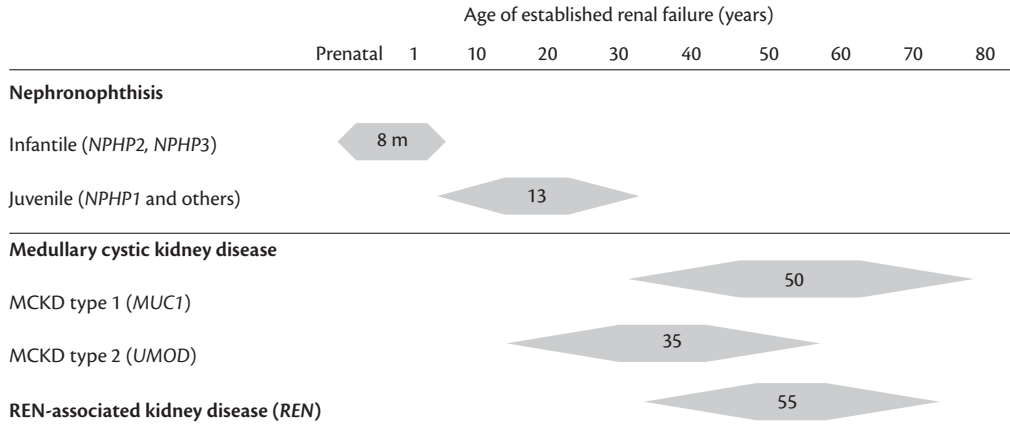

Fig. 316.1 Range of ages of established renal failure (ESRF) in nephronophthisis and medullary cystic kidney disease. Median ages of ESRF are shown. m = months.

type 2 are based on different disease loci rather than any specific phenotypic differences. The clinical features include insidious decline in renal function in early adult life (sometimes commencing from teenage years) with typically an absence of significant haematuria and proteinuria. ESRF occurs between 30 and 80 years of age (Fig. 316.1). Renal ultrasounds scans may reveal cortical or corticomedullary cyst formation in normal or slightly small kidneys. Histologically the condition resembles NPHP with an interstitial nephropathy. Extrarenal manifestations are restricted to gout and sometimes childhood anaemia.

There remains considerable confusion in nomenclature regarding MCKD, and the term Autosomal Dominant Interstitial Kidney Disease (ADIKD, see Chapter 318) is becoming preferred (Table 316.1). Fortunately, the molecular pathogenesis is now known for both MCKD type 1 and type 2.

MCKD type 1 is the less severe form of the disease, with a median age of ESRF of 50 years (Stavrou et al., 2002; Kiser et al., 2004). Urine is bland and renal cysts may be seen but are not required for the diagnosis. Gout may occur in the context of renal failure but is much less common than in MCKD type 2. Renal biopsy may show tubular atrophy and interstitial fibrosis. There may be secondary focal global sclerosis of glomeruli which should not be misinterpreted as the primary renal disease (Bleyer et al., 2005). The histology of MCKD has been described as resembling NPHP with tubular basement membrane thickening and lamellation (Stavrou et al., 2002).

A key clinical and diagnostic feature of MCKD is a dominant pattern of renal disease within the family and this should prompt a review of other affected family members (Bleyer, 2009). Recently mutations in *MUC1*, encoding the mucoprotein mucin-1, have been shown to segregate with MCKD type 1 (Kirby et al., 2013).

MCKD type 2 accounts for approximately one-third of cases of MCKD and has a more severe phenotype. Like MCKD type 1, there is an insidious decline in renal function, an absence of significant haematuria and proteinuria, and ESRD is reached between 30 and 50 years of age, median age 35 years (Scolari et al., 1999). On renal ultrasound scans there may be corticomedullary cyst formation and a key extrarenal feature is hyperuricaemia and gout, which may occur in around half of patients with MCKD type 2. Mutations in *UMOD* underlie MCKD type 2 (Hart et al., 2002; Wolf et al., 2003). *UMOD* encodes the mucoprotein uromodulin, also known as Tamm–Horsfall protein (Scolari et al., 2004).

Aside from MCKD types 1 and 2 there are rarer forms of autosomal dominantly inherited interstitial nephritis, and cases which are not associated with a known disease locus. Mutations in *REN*, encoding renin, are a rare cause of an autosomal dominant interstitial nephritis (Zivna et al., 2009). Patients with *REN* mutations may have childhood anaemia and hyperuricaemia.

There are no specific treatments for MCKD; however, gout can be controlled using allopurinol, febuxostat, and other agents. Although it is not known whether uric acid-lowering therapies impact positively upon the rate of decline in renal function/disease progression, patients appear to benefit.

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CHAPTER 317

Nephronophthisis

John A. Sayer and Roslyn J. Simms

Introduction

Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease and a leading genetic cause of end-stage renal failure (ESRF) in children and young adults (Hildebrandt et al., 2009). The incidence varies worldwide, ranging from 1/50,000 to 1/900,000 (Hildebrandt and Zhou, 2007) and is more common in areas where consanguineous marriage is prevalent. Originally, three variants of NPHP were described: infantile, juvenile, and adolescent, predominantly differentiated by the timing of onset of ESRF. It remains useful to differentiate infantile from other forms of NPHP.

Clinically, children with NPHP typically present at approximately 6 years of age with polyuria, nocturia or secondary enuresis, polydipsia, and lethargy, secondary to anaemia (Ala-Mello et al., 1996). Renal ultrasound may show normal or reduced kidney size (depending on the stage of presentation), with increased echogenicity, loss of corticomedullary differentiation, and corticomedullary cysts (Blowey et al., 1996) (Fig. 317.1). Renal histology reveals a diagnostic triad of tubular basement membrane disruption, tubulointerstitial fibrosis, and corticomedullary cysts (Zollinger et al., 1980; Waldherr et al., 1982).

NPHP is clinically heterogeneous and is often part of a multisystem disorder with extrarenal manifestations occurring in 10–15% of patients, most frequently including retinal degeneration (Hildebrandt et al., 2009).

The number of genes identified to cause NPHP continues to grow and currently includes > 20 genes which accounts for the diagnosis in approximately 40% of patients with NPHP (Chaki et al., 2012; Halbritter et al., 2013). To date, the protein products of all of the causal genes of NPHP are expressed in the primary cilium/basal body complex. Localization of the protein products of the mutated genes in NPHP is considered important in understanding the molecular pathogenesis of NPHP and explains why NPHP and related syndromes are described as ciliopathies (Hildebrandt and Otto, 2005). The concept of NPHP as a ciliopathy is discussed in Chapter 303.

Currently there are no effective specific therapies for NPHP and the management of affected patients focuses on symptoms of progressive kidney disease, timely delivery of renal replacement therapy, genetic counselling, appropriate investigation, and referral to other specialties to manage any extrarenal features.

Natural history

NPHP was originally described in an individual patient in 1945 as a medullary cystic kidney disease (Smith and Graham, 1945). In 1951, the description of NPHP was further defined as a familial disorder (Fanconi et al., 1951) with progressive chronic tubulointerstitial nephritis leading to ESRF (Salomon et al., 2009). Today NPHP is recognized to have an autosomal recessive pattern of inheritance resulting in cystic kidney disease and is the leading genetic cause of ESRF in children and adolescents (Hildebrandt and Zhou, 2007). Although conventionally, two homozygous or compound heterozygous mutations in a single gene will be identified as the genetic mistake in NPHP (Wolf and Hildebrandt, 2011), oligogenicity, whereby heterozygous mutations in at least two different recessive genes which independently do not cause disease, but in combination do so (Leitch et al., 2008), has been reported in NPHP (Hoefele et al., 2007).

In the United Kingdom, NPHP accounts for 6.5% of children with ESRF (Lewis et al., 2009). NPHP is derived from a Greek term, which literally means 'disintegration of nephrons' (Wolf and Hildebrandt, 2011) and describes part of its histology since kidney cysts develop by replacing normal renal tissue.

Epidemiology

NPHP is a rare disorder with a variable incidence worldwide estimated to be from 1/50,000 in Canada, to 1/61,000 in Finland to 1/900,000 in the United States (Hildebrandt and Zhou, 2007). A worldwide cohort of 447 patients with isolated NPHP were described in 2013 and included patients from the United Kingdom and across Europe (Halbritter et al., 2013). NPHP is more prevalent in areas with high rates of consanguineous marriage (Soliman et al., 2010); in an Egyptian cohort, 75% were offspring from consanguineous marriage.

Clinical features

Infantile and juvenile NPHP variants have been described, with distinct clinical presentations (Simms et al., 2009). Both forms of NPHP are clinically heterogeneous and may present with a diverse range of extrarenal features (Table 317.1) which may form part of a ciliopathy syndrome (Table 317.2) (Simms et al., 2011).

Infantile NPHP is characterized by enlarged dysplastic cystic kidneys (Otto et al., 2003) which may be detected on antenatal ultrasound performed to investigate fetal oligohydramnios. Infantile NPHP is rare; in 2011 it was reported to affect 18 patients from 12 families identified from a worldwide cohort of patients with NPHP (Chaki et al., 2011). Mutations in *inversin* or *NPHP2* and *NPHP3* are the predominant genetic causes for infantile NPHP (Otto et al., 2003; Halbritter et al., 2013), however homozygous mutations in *NEK8/NPHP9* have also been reported (Otto et al., 2008). Clinically, infantile NPHP leads to hypertension and ESRF

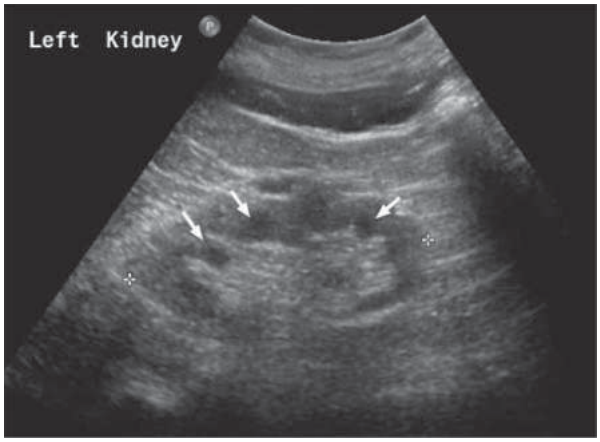

Fig. 317.1 Ultrasound showing corticomedullary cysts (arrows) in patient with nephronophthisis.
Reproduced from Simms, R. J., Eley, L., and Sayer, J. A. (2009). Nephronophthisis. *Eur J Hum Genet*, 17(4), 406–16.

before the age of 4 years (Chaki et al., 2011) with additional predominantly dysplastic manifestations including optic nerve atrophy, hydrocephalus, situs inversus, ventricular septal defects, aortic coarctation, and asthma (Chaki et al., 2011).

Children with more typical (juvenile) NPHP typically present at approximately 6 years of age with polyuria, nocturia or secondary enuresis (caused by an inability to concentrate urine), polydipsia (Simms et al., 2011), developmental delay, short stature, and generalized lethargy, secondary to anaemia (Ala-Mello et al., 1996). The median age of onset of ESRF is 12 years, however this may be delayed until adulthood (Hildebrandt et al., 2009). Mutations in *NPHP1* are the leading cause of juvenile NPHP and account for 20–25% of patients (Hildebrandt et al., 2009; Halbritter et al., 2013).

The most typical NPHP-associated extrarenal feature is retinal degeneration (Sayer et al., 2006), however other recognized conditions include cerebellar vermis hypoplasia (Joubert syndrome (JS)), occipital encephalocoele and hepatic fibrosis (Meckel syndrome (MKS)), situs inversus, bronchiectasis, skeletal defects, and Bardet–Biedl syndrome (BBS) leading to a multisystem disorder (Hildebrandt et al., 2009) (Table 317.2).

Table 317.1 Extrarenal manifestations that may be identified in patients with nephronophthisis

Body system	Effects
Eye/retinal	Retinitis pigmentosa, oculomotor apraxia, retinal coloboma, nystagmus, ptosis
Neurological	Learning difficulties and developmental delay, cerebellar vermis aplasia, hypopituitarism, encephalocoele, ataxia
Hepatic	Deranged liver function tests, fibrosis, biliary duct proliferation
Skeletal	Cone shaped epiphyses, short ribs, polydactyly, skeletal dysplasia, scoliosis
Cardiac	Situs inversus, ventricular septal defect
Respiratory	Bronchiectasis, neonatal tachypnoea/episodic hyperpnoea
Gastrointestinal	Ulcerative colitis

Table 317.2 Ciliopathy syndromes associated with nephronophthisis

Syndrome	Features
Joubert (JS)	Cerebellar vermis aplasia/hypoplasia
Cogan	Oculomotor apraxia, nystagmus, difficulty with saccades
Senior–Løken	NPHP and retinitis pigmentosa
Meckel	Occipital encephalocoele, hepatic fibrosis, enlarged cystic kidneys
Bardet–Biedl	Retinal dystrophy, learning difficulties, obesity, polydactyly, hypogonadism, NPHP, renal dysplasia, focal segmental glomerulosclerosis
RHYNS	Retinitis pigmentosa, hypopituitarism, NPHP, skeletal dysplasia
Boichis	Liver fibrosis, biliary duct proliferation
Mainzer–Saldino	Cone-shaped epiphyses, NPHP
Jeune/asphyxiating thoracic dystrophy	Short rib thoracic dysplasia
Sensenbrenner	Skeletal dysplasia
Ellis van Creveld	Ectodermal dysplasia
Alström	Retinitis pigmentosa, hearing impairment, obesity, DM2
Arima/ cerebro-oculo-hepato-renal	Leber's congenital amaurosis, colobmoa, cerebellar vermis aplasia, infantile polycystic kidneys, liver steatosis/fibrosis

DM2 = type 2 diabetes mellitus; JS = Joubert syndrome and related disorders; NPHP = nephronophthisis

Diagnosis

A diagnosis of NPHP by the nephrologist requires a degree of clinical suspicion and should be considered in a patient presenting with the following suggestive features:

- History (polyuria, polydipsia, enuresis), family history (autosomal recessive pattern), or parental or more distant consanguinity.
- Examination findings including hypertension, retinal pigmentation/blindness/abnormal eye movements, polydactyly, short stature (secondary to salt wasting, dehydration and renal insufficiency)
- Investigations: blood tests showing renal impairment, anaemia, deranged liver function tests; urine concentrating defect (<400 mOsm/kg in early morning urine); bland urine with absence of (or minimal) proteinuria and haematuria; renal ultrasound with small/normal sized kidneys, poor corticomedullary differentiation, and cysts.

The diagnostic triad for NPHP on a renal biopsy is corticomedullary cysts, tubular basement membrane disruption, and tubulointerstitial nephropathy with tubular atrophy and interstitial fibrosis (Zollinger et al., 1980; Waldherr et al., 1982). However, diagnostic

molecular genetic testing is available for some NPHP genes in selected laboratories and avoids the need for a renal biopsy.

We suggest that following appropriate genetic counselling and consent, blood should be taken for DNA extraction and genetic testing (Simms et al., 2009). Details of relevant genetic testing laboratories can be found at < http://www.ukgtn.nhs.uk> (United Kingdom) and < http://www.eurogentest.org> (Europe). If *NPHP1* mutations are not detected, further NPHP-associated genes should be screened. The presence of extrarenal manifestations sometimes provides a guide for the most appropriate genes to screen, however high-throughput 'NextGen' sequencing platforms and whole-exome/whole-genome sequencing allows multiple ciliopathy genes to be screened simultaneously and has been performed in cohorts of patients (Otto et al., 2011; Chaki et al., 2012; Halbritter et al., 2013).

Genetics

NPHP is an autosomal recessive condition, thus two mutations in a single gene are sufficient to cause disease. Table 317.3 shows a full list of the currently identified causal genes of NPHP, their estimated frequency, and associated extrarenal features. The variable phenotypes and extent of extrarenal features which may be associated with mutations in the NPHP causal genes listed in Table 317.3 highlights the genetic pleiotropy.

Since the list of currently identified causal genes for NPHP only accounts for 40–50% of patients, it is expected that further genes will be discovered. Furthermore, in some patients with NPHP, only a single causal heterozygous mutation has been identified (Hoefele et al., 2007; Chaki et al., 2011), meaning the second disease-causing mutation remains unidentified. Failure to identify a second mutation in autosomal recessive disorders emphasizes that several NPHP-related causal genes are as yet undiscovered. The frequency of this concept of unidentified NPHP genes was highlighted in 2011 following analysis of 18 NPHP-associated ciliopathy genes by DNA pooling and next-generation sequencing failed to identify a causal gene in 90 of 120 affected patients (Otto et al., 2011).

The gene mutated and the nature of the mutation are both considered to influence the extent of extrarenal organ involvement. For example, two null mutations in *NPHP6* are associated with dysplastic development of multiple organs, whereas missense mutations tend to 'rescue' to a milder phenotype such as NPHP (Chaki et al., 2011). Further evidence of the allelic nature of NPHP-related ciliopathies was identified with *NPHP11*, when missense mutations were found in patients with NPHP and truncating mutations associated with causing the more severe, embryonically lethal, Meckel syndrome (Otto et al., 2009).

Curiously, the nature and severity of the phenotype of patients with NPHP is highly variable, even within families. Although it is postulated that modifier genes (Hoefele et al., 2007; Benzing and Schermer, 2012) may influence the intrafamilial variation, few genotype–phenotype correlations have been identified (Chaki et al., 2011).

Oligogenicity, whereby heterozygous mutations occur in at least two different recessive genes, has been reported in patients with NPHP (Hoefele et al., 2007; Leitch et al., 2008; Davis et al., 2011). Triallelism is a type of oligogenicity, and specifically describes the inheritance pattern when three mutant alleles at two different loci have been identified in an individual with a disease phenotype (Katsanis et al., 2001). Although triallelism was originally associated with BBS (Katsanis, 2004), it has been reported in patients with NPHP-related disorders, when considering the impact of modifier genes (Hoefele et al., 2007). Table 317.4 details the genetic mutations in which triallelism and oligogenicity have been reported in patients with NPHP (Hoefele et al., 2007; Tory et al., 2007; Otto et al., 2008; Davis et al., 2011) and associated ciliopathies (Baala et al., 2007; Leitch et al., 2008; Khanna et al., 2009; Coppieters et al., 2010; Hildebrandt et al., 2011; Hopp et al., 2011).

The link between cystic kidney disease including NPHP and the associated extrarenal manifestations such as retinal degeneration is considered to be explained by the ciliary localization of the protein products of the mutated causal genes. In retinal photoreceptors, the connecting cilium, which is structurally analogous to primary cilia in renal epithelia (Hildebrandt et al., 2009), mediates photosensation by facilitating trafficking of rhodopsin (Hildebrandt and Otto, 2005).

Table 317.3 Genes mutated in nephronophthisis, their estimated percentage frequency and associated clinical features

Gene locus	Gene symbol	Chromosome	Extrarenal clinical features	Mutation
NPHP1	*NPHP1*	2q13	RP, OMA, JS	~ 20%
NPHP2	*INVS*	9q31	RP, hypertension, LF, VSD, situs inversus	1–2%
NPHP3	*NPHP3*	3q22.1	LF, RP, situs inversus, MKS	1–2%
NPHP4	*NPHP4*	1p36.22	RP, OMA, LF	3–4%
NPHP5	*IQCB1*	3q21.1	Severe RP	3%
NPHP6	*CEP290*	12q21.32	JS, MKS, severe RP, BBS	3–5%
NPHP7	*GLIS2*	16p13.3	–	< 1%
NPHP8	*RPGRIP1L*	16q12.2	JS, MKS	1%
NPHP9	*NEK8*	17q11.1	–	< 1%
NPHP10	*SDCCAG8*	1q44	BBS	< 1%
NPHP11	*TMEM67*	8q22.1	JS, MKS	3%
NPHP12	*TTC21B*	2q24.3	JS	1%
NPHP13	*WDR19*	4p14	–	1%
NPHP14	*ZNF423*	16q12	JS	< 1%
NPHP15	*CEP164*	11q23.3	JS	< 1%
NPHP16	*ANKS6*	9q22.33	Situs inversus, LF	<1%
NPHP17	*IFT172*	2p23.3	Short rib thoracic dysplasia	<1%
NPHP18	*CEP83*	12q22	LF, RP	<1%
NPHP19	*DCDC2*	6p22.3	LF	<1%
NPHP1L	*XPNPEP3*	22q13	Cardiomyopathy, seizures	< 1%
JBTS3	*AHI1*	6q23.3	JS, RP	1%
SCA10	*ATXN10*	22q13.31	Spinocerebellar ataxia	< 1%

BBS = Bardet–Biedl syndrome; JS = Joubert syndrome; LF = liver fibrosis; MKS = Meckel syndrome; OMA = oculomotor apraxia; RP = retinitis pigmentosa; VSD = ventricular septal defect.

Table 317.4 Oligogenic mutations identified in patients with nephronophthisis and associated disorders

Gene	Second gene	Ciliopathy
NPHP1 (hom)	*NPHP3* (het)	NPHP
	NPHP4 (het)	NPHP
	NPHP6 (het)	NPHP, SLS
	RPGRIP1L (het)	NPHP, SLS, JS
	AHI1 (het)	NPHP, JS
INVS (het)	*NPHP3* (het)	NPHP
IQCB1 (hom)	*NEK8* (het)	NPHP
IQCB1 (het)	*RPGRIP1L* (het)	SLS
NPHP6 (hom)	*NPHP4* (het)	MKS
	TMEM67/MKS3 (het)	BBS
	AHI1 (het)	NPHP, SLS
	PKHD1 (hom)	MKS
NPHP6 (het)	*NPHP4* (het)	SLS
	RPGRIP1L (het)	JS
	CC2D2A (het)	MKS
	B9D1 (het)	MKS
RPGRIP1L (het)	*NPHP6* (het)/*IQCB1* (het)	SLS
	NPHP3 (comp. het)	SLS
	MKS1 (het)	BBS
CC2D2A (hom)	*PKDH1* (het)	MKS
CC2D2A (het)	*NPHP3* (het)	MKS
TTC21B (het)	*NPHP4* (het)	NPHP
	C2ORF86 (het)	MKS

BBS = Bardet–Biedl syndrome; het = heterozygous; hom = homozygous; JS = Joubert syndrome; MKS = Meckel syndrome; NPHP = nephronophthisis; SLS = Senior–Løken syndrome.

The concept of ciliopathies is discussed further in Chapter 303.

Differential diagnosis

The distinguishing features of NPHP include its pattern of autosomal recessive inheritance and normal or small kidney size. These features differentiate NPHP from other inherited cystic kidney diseases such as:

- autosomal recessive cystic kidney disease: enlarged cystic kidneys, hepatic fibrosis, biliary duct anomalies (Sweeney and Avner, 2011)
- autosomal dominant polycystic kidney disease: enlarged cystic kidneys, pattern of inheritance, liver/pancreatic cysts, cerebral aneurysms (Torres and Harris, 2009)
- medullary cystic kidney disease: autosomal dominant, older age of onset of ESRF, only extrarenal feature is gout (Hildebrandt and Otto, 2000) (see Chapters 316 and 318)
- cystic dysplastic kidneys: developmental abnormalities, usually detected *in utero* on antenatal ultrasound/at birth, tend to be grouped with other congenital abnormalities of the kidney and urinary tract (CAKUT) (Winyard and Chitty 2008)
- karyomegalic interstitial nephritis (KIN): autosomal recessive, mutations in *FAN1*, histologically identical to NPHP except karyomegaly, links ESRF to ineffective DNA damage repair (Zhou et al., 2012).

Management

There is no specific treatment for NPHP and management should focus on the optimization of blood pressure and pre-dialysis care to enable timely delivery of renal replacement therapy. Renal transplantation is feasible in patients with NPHP and there is no recurrence of cystic kidney disease following transplantation (Steele et al., 1980). In fact, in a North American paediatric cohort of renal transplant recipients, graft survival was improved in recipients with NPHP compared to recipients with other primary renal diagnoses (Hamiwka et al., 2008).

Following diagnosis genetic counselling should be offered, including to other members of the family. Prenatal testing is possible particularly for infantile NPHP as imaging may reveal cystic kidney disease and associated structural central nervous system anomalies. Preimplantation testing may facilitate a genetic diagnosis.

Awareness of associated extrarenal manifestations is essential to facilitate appropriate investigation performed regularly. Cerebral imaging using magnetic resonance imaging (defines pathognomic changes in JS or MKS) and review by a neurologist may be required. Referral for ophthalmological review is recommended following diagnosis of NPHP and retinal examinations should be performed at least annually. Liver function tests should be performed at least annually and if there is concern of liver disease, ultrasound imaging should be requested.

There is hope for targeted, likely combination therapies for NPHP in the future. Several pharmaceuticals including vasopressin receptor antagonists (Gattone et al., 2003) and cyclin-dependent kinase inhibitors (roscovitine) (Bukanov et al., 2006) have been tested in mouse models of NPHP and shown to reduce cyst growth. The mammalian target of rapamycin (mTOR) inhibitor, rapamycin, has been shown to reduce the development of pro-nephric cysts in zebrafish models of NPHP (Tobin and Beales, 2008). Additionally, zebrafish models of NPHP and related cystic kidney diseases are being utilized to facilitate drug development and enable high-throughput drug screening (Norris and Grimes, 2012).

Clinical trials are ongoing in humans with related cystic kidney diseases; however, this is currently restricted to adults or at least teenagers. Further clear understanding of the molecular pathogenesis of NPHP and related disorders is considered essential prior to initiating clinical trials in children.

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CHAPTER 318

Autosomal dominant interstitial kidney disease including medullary cystic disease

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Introduction

The terminology around these diseases has shifted as underlying genetic causes have been discovered. An umbrella heading of autosomal dominant interstitial kidney disease (ADIKD) is now preferred. Medullary cystic kidney disease (MCKD) was previously the umbrella term, but the diseases included affect the cortex as well as the medulla, and often are not cystic. Here it is classified by genetic type first where possible.

MUC1-associated ADIKD, MCKD1

Clinically this disorder presents with slowly progressive renal failure, with additional family members affected in an autosomal dominant pattern. The age of onset is between the third and fifth decades of life but may vary between families and within families.

The rate of decline of renal function is not precisely known, but end-stage renal failure (ESRF) typically occurs by 50 years of age (Stavrou et al., 2002; Kiser et al., 2004). Gout may be associated with medullary cystic kidney disease (MCKD) type 1, but is not a prominent feature compared to MCKD type 2. The urine sediment is usually bland; if proteinuria is present it is typically subnephrotic range. Imaging of the renal tract may identify cysts, typically cortical or corticomedullary in location, but these are not essential for the diagnosis. Renal biopsy findings reveal a focal global sclerosis of glomeruli and tubular atrophy with interstitial fibrosis (Kiser et al., 2004). Families with MCKD type 1 patients have demonstrated linkage to a disease locus on chromosome 1q21 (Christodoulou et al., 1998; Parvari et al., 2001; Stavrou et al., 2002; Wolf et al., 2006). This disease locus contained numerous genes, but the exact causative gene within the region defied molecular geneticists and nephrologists for > 10 years until recently, a gene that was missed by massively parallel sequencing approaches was identified. In a landmark study, Kirby et al. detail how, in six families linked to the MCKD type 1 locus, they found mutations (cytosine insertions) in one copy of the large variable-number tandem repeat (VNTR) sequence of the *MUC1* gene (Kirby et al., 2013).

The clinical presentation in these families was remarkably similar. Urinalysis demonstrated minimal proteinuria, renal biopsies revealed tubulointerstitial fibrosis, and renal imaging showed cortical cysts (rather than medullary) in a few cases. More typical renal ultrasound scan findings included small echogenic kidneys. The age of renal failure in these families ranged from 25 to 79 years.

The *MUC1* gene encodes a mucoprotein called mucin 1, which is widely expressed on the surface of epithelial cells. The insertion mutations seen in MCKD type 1 are predicted to cause a frameshift which disrupts the cleavage module and causes retention of the molecule in the loop of Henle, distal convoluted tubule, and the collecting ducts (Kirby et al., 2013). Developmental studies have shown that *MUC1* is expressed in the human kidney during nephrogenesis including in the cap mesenchymal cells undergoing mesenchymal to epithelia transition, the ureteric bud tips and the collecting tubules, as well as in the renal vesicles, comma bodies, and S-shaped bodies (Fanni et al., 2012).

Kirby et al., in addition to the six index families, screened an additional 21 unsolved families with MCKD for *MUC1* mutations. Over 60% of these had a *MUC1* mutation, suggesting that *MUC1* mutation screening will help yield a positive molecular diagnosis when used appropriately (Kirby et al., 2013).

UMOD-associated ADIKD, MCKD2

The clinical presentation of MCKD type 2 resembles that of MCKD type 1, with insidious loss of renal function over time and a bland urine (Scolari et al., 1999). A key feature, however, is the incidence of gout which occurs in around half of cases and may commence early, during the second decade of life. The gout is typically out of keeping with the degree of renal dysfunction and its presence in females provides a diagnostic clue. ESRF occurs typically between the fourth and sixth decades of life, somewhat earlier than MCKD type 1, but again intra- and interfamilial variability is seen. Occasionally the disease may present in children < 10 years of age (Wolf et al., 2007). Renal ultrasound may reveal small kidneys and occasional medullary cysts (Dahan et al., 2003). Histologically, there is a diffuse tubulointerstitial fibrosis and tubular atrophy (Dahan et al., 2003) but uric acid crystals are not seen (Puig et al., 1993).

Mutations in the gene *UMOD,* which encodes the urinary mucoprotein uromodulin underlie MCKD type 2 (Hart et al., 2002; Wolf et al., 2003). Uromodulin was formerly known as Tamm–Horsfall protein (Tamm and Horsfall, 1950). The expression pattern of UMOD is specific to the thick ascending limbs of the loop of Henle (Bachmann et al., 1990). Like mucin-1, uromodulin is a

glycosylated protein which is expressed on the apical surface of epithelial cells and is released into the urine following proteolytic cleavage (Santambrogio et al., 2008). The biological function of uromodulin is fascinating (Rampoldi et al., 2011) and has been implicated to have roles in the regulation of fluid and electrolyte balance (Renigunta et al., 2011). *Umod* knockout mice studies suggest a role for uromodulin in the defence against bacterial urinary tract infections (Bates et al., 2004, Mo et al., 2004) and *in vitro* data suggests it has a role in innate immunity of the kidney (Rampoldi et al., 2011).

Mutations in *UMOD* may also account for an early-onset phenotype known as familial juvenile hyperuricaemic nephropathy (FJHN). This phenotype was first described in 1960 in a family with early-onset gout, hyperuricaemia, and renal disease (Duncan and Dixon, 1960). UMOD mutations may also cause features of glomerulocystic kidney disease, with marked dilatation of Bowman's space (Rampoldi et al., 2003). Together MCKD type 2 and FJHN are referred to as uromodulin-associated kidney disease (UAKD) (Hart et al., 2002). The hyperuricaemia is a result of a reduced fractional excretion of uric acid, and predisposes to early gout. There may also be a mild urinary concentrating defect (Dahan et al., 2003).

There are no specific treatments for UAKD, although raised serum urate can be treated with allopurinol (Bleyer et al., 2011) or febuxostat. It is unknown whether lowering serum uric acid in this way impacts upon the progression of chronic kidney disease. No randomized trials addressing this question have been published. Mutational analysis of the *UMOD* gene is widely available and mutations in *UMOD* account for around one-third of cases of all MCKD(Hildebrandt et al., 2006).

REN-associated ADIKD

Patients typically present between 20 and 30 years of age with gout and chronic kidney disease. The urine is typically bland with an absence of proteinuria age and ESRF occurs usually after 40 years of age (Zivna et al., 2009; Bleyer et al., 2010).

Key distinguishing features in REN-associated kidney disease include childhood anaemia. This complication is secondary to a defective renin–angiotensin pathway leading to a hypoproliferative anaemia with low erythropoietin levels and may occur as early as 12 months of age. The anaemia due to reduced levels of angiotensin resolves during adolescence, but may recur with the onset of chronic kidney disease. Secondary to low renin levels, the blood pressure may be low and there may be an associated hyperkalaemia. The hyperuricaemia may be explained by a hypothesis where renin deficiency leads to relative aldosterone deficiency which results in a fluid-depleted state leading to increased proximal reabsorption of uric acid and a reduced fractional excretion of urate (Zivna et al., 2009).

REN mutations are rare. Zivna et al. reported two families with early-onset anaemia, hyperuricaemia, and progressive renal failure (Zivna et al., 2009). Two novel mutations were identified in the signal sequence of *REN* leading to either a reduction or complete absence of prorenin and renin biosynthesis and secretion (Zivna et al., 2009). A third family with an autosomal dominant pattern of anaemia, polyuria, hyperuricaemia, and chronic kidney disease has been shown to have *REN* mutations. Functional analysis of the mutation demonstrated accumulation of non-glycosylated preprorenin in the cytoplasm leading to ultrastructural damage of the kidney (Bleyer et al., 2010). In a screen of 39 families with hyperuricaemia and chronic kidney disease (in whom mutations in *UMOD* and hepatocyte nuclear factor-1B (*HNF1B*) had been excluded) just one family with a novel *REN* mutation was identified (Beck et al., 2011). This confirms its rarity even in a well-selected cohort, although the exact incidence of *REN* mutations is not known. Treatment is supportive care of the chronic kidney disease, management of the anaemia with erythropoietin, and control of hyperuricaemia with xanthine oxidase inhibitors and other agents.

Differential diagnosis of medullary cystic kidney disease

Mutations in *HNF1B* (also known as transcription factor 2 (*TCF2*)) encoding the transcription factor homeobox transcription factor HNF1B can have a diverse range of renal phenotypes including cystic kidney disease, hyperuricaemia, and gout (Bingham et al., 2000, 2001, 2002). The pattern of inheritance of this disorder (also known as renal cysts and diabetes syndrome, Chapter 315) is autosomal dominant and the presence of cystic kidneys (not always apparent), gout, and a bland urine in such a patient can mimic *UMOD*-associated disease (see Chapter 315). A familial pattern of diabetes, congenital abnormalities of the kidney and urinary tract, and hypomagnesaemia would all point towards a diagnosis of *HNF1B* mutation.

In addition, hypomorphic phenotypes of autosomal dominant polycystic kidney disease (ADPKD) may also mimic ADIKD/MCKD with only very minimal cystic change within the kidney (Rossetti et al., 2009). An autosomal dominant pattern of disease and the presence of gout may lead to diagnostic confusion in such cases and a molecular genetic diagnosis should be sought where there is diagnostic uncertainty (see Chapter 306).

For almost all of these disorders a molecular genetic diagnosis is now possible, if not yet routinely available for all of them. The absence of medullary cysts does not preclude any of these diagnoses. The presence of cysts is not diagnostic either, but together with progressive renal failure, gout, and a positive family history of ESRF that isn't typical of ADPKD should help identify patients and their at-risk family members.

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CHAPTER 319

Oral-facial-digital type 1 syndrome

Brunella Franco

Introduction

Oral-facial-digital type 1 syndrome (OFD1; OFDI; OMIM #311200) is a rare developmental disorder inherited as an X-linked dominant trait and represents a rare syndromic form of inherited renal cystic disease. Embryonic lethality in affected hemizygous males is usually reported in the first and second trimesters of pregnancy. The condition was originally described by Papillon-Leage and Psaume in 1954 and better defined in 1962 (Papillon-Leage and Psaume, 1954; Gorlin and Psaume, 1962) and has an incidence estimated between 1/50,000 and 1/150,000 live births (Gorlin et al., 2001). OFD1 belongs to the heterogenous group of oral-facial-digital syndromes which comprises several different forms (Gurrieri et al., 2007).

The clinical spectrum

The clinical spectrum for this disease includes malformation of the face, oral cavity, and digits with a high degree of phenotypic variability, even within the same family, possibly due to X-inactivation. Renal involvement (see below) is an important feature.

Craniofacial abnormalities are observed in > 87% of cases and include abnormal hair/alopecia, facial dysmorphisms, and facial milia clefts of lip and palate. Oral signs are present in > 96% of cases and are represented by oral frenula, tongue and teeth abnormalities, and alveolar ridge clefting.

Skeletal involvement is reported in about 88% of cases and include brachydactyly, clinodactyly, and syndactyly and rarely polydactyly. Upper limbs are usually more affected than lower limbs.

Central nervous system involvement is present in about 50% of cases.

Additional signs include hearing impairment and cysts in other organs (pancreas, ovary, liver). Fig. 319.1 depicts some of the typical findings observed in OFD1 cases.

Renal involvement in oral-facial-digital type 1

Renal cystic disease is commonly observed in OFD1 patients and is present in > 60% of adult patient (Prattichizzo et al., 2008; Saal et al., 2010). Renal cysts are usually observed in the second and third decades of life with few reports of occurrence in the first decade of life and examples of patients in which the renal involvement completely dominates the clinical course of the disease (Coll et al., 1997; Feather et al., 1997a). Histological examinations of renal tissues demonstrate a predominantly glomerulocystic kidney disease with a small population of tubular cysts (Feather et al., 1997a; Saal et al., 2010). The cystic kidneys in OFD1 are usually of normal size or moderately increased. In addition, in contrast to what is observed in autosomal dominant polycystic kidney disease, renal cysts do not alter the contour of the kidneys.

Molecular studies

The locus for OFD1 was mapped to the Xp22 region in 1997 (Feather et al., 1997b) and sequence analysis demonstrated mutations in the *CXORF5* transcript, subsequently named *OFD1* (Ferrante et al., 2001). To date, > 100 different mutations in classical OFD1 cases have been reported (Chetty-John et al., 2010; Macca and Franco, 2009; Diz et al., 2011, Bisschoff et al., 2013; Del Giudice et al., 2014, and references therein). Interestingly, recent studies demonstrated that mutations in the OFD1 transcript can also be associated to X-linked recessive disorders such as Joubert syndrome (JBTS10), a syndromic form of mental retardation comprising macrocephaly, ciliary dysfunction, and retinitis pigmentosa. Different studies have failed to identify convincing genotype–phenotype correlation.

Animal models and functional studies

Ubiquitous inactivation of the Ofd1 transcript in the mouse resulted in embryonic male lethality and perinatal lethality in females. Affected females displayed craniofacial and limb abnormalities including a severe cleft palate, which is the likely cause of the observed lethality. Although the mutant kidneys had normal external morphology, a highly penetrant polycystic kidney disease of glomerular origin was observed in all cases examined. Immunofluorescence analysis and ultrastructural studies demonstrated dysfunction of primary cilia of cells that lined the cyst (Fig. 319.2). Ultrastructural analysis showed dysfunction of nodal cilia (Ferrante et al., 2006). Conditional mutants with limb- and kidney-specific Ofd1 inactivation in the mouse contributed insights into the functional role of Ofd1 (Zullo et al., 2010; Bimonte et al., 2011). Ofd1 disruption in zebrafish supports the role of this transcript in primary cilia function and in the pathogenesis of renal cystic disease (Ferrante et al., 2009). *In vitro* studies demonstrated that OFD1 is a component of centriolar satellites and controls centriole length.

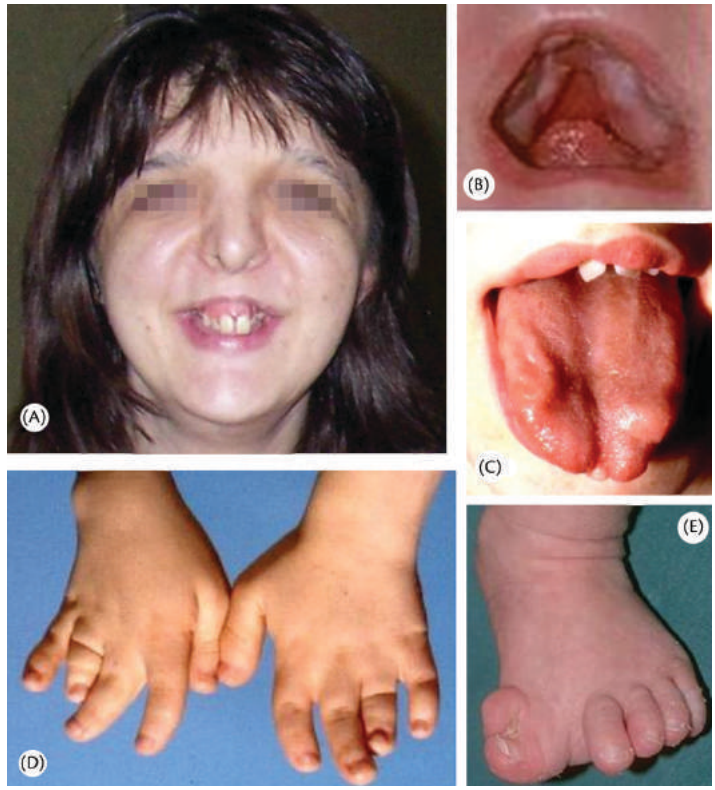

Fig. 319.1 Examples of the oral-facial-digital findings observed in OFD1 patients. (A) Facial dysmorphisms. (B) Cleft palate. (C) Bifid and lobulated tongue. Limb abnormalities are also a frequent finding and include brachydactyly and clinodactyly (D) and duplication of the allux (E).

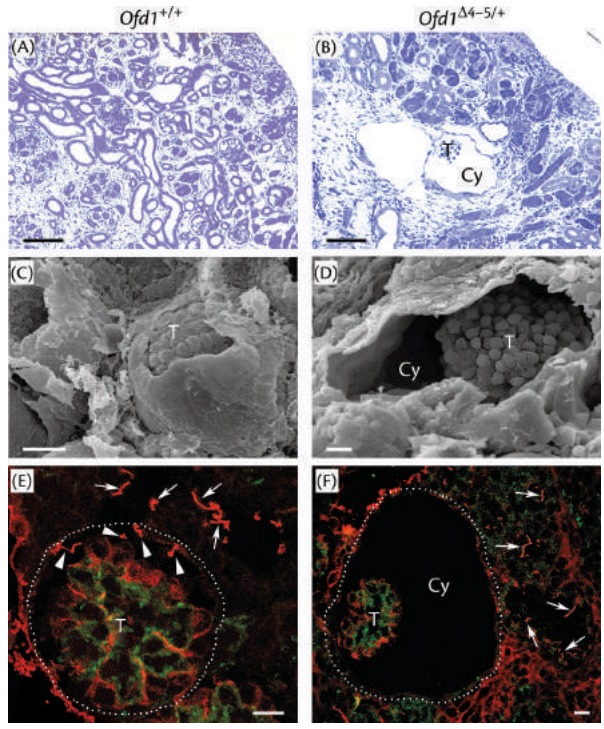

Fig. 319.2 Ofd1 null mutants display cystic kidneys. (A, B) Immunohistochemical studies indicate the presence of glomerular cyst (Cy) in mutant animals (B). T = tuft. Scale bar = 100 µm. (C, D) Scanning electron micrographs showing normal glomeruli in the wt (left) and a cystic glomerulus in the mutant (right). Scale bar = 10 µm. (E, F) Paraffin embedded sections were immunostained with anti-acetylated tubulin (red) to mark ciliary axonemes, and with ZO1 (green) to mark the apical surface of cells. Arrowheads indicate primary cilia in the wt glomerulus (E), arrows indicate primary cilia in the tubuli of wt (E) and mutant (F); no cilia are observed on the surface of epithelial cells lining the cyst in the mutant (F). Scale bar = 10 µm.

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CHAPTER 320

The molecular basis of glomerular basement membrane disorders

Rachel Lennon and Neil Turner

Introduction

The glomerulus is comprised of unique cellular and extracellular matrix (ECM) components, which together enable this unit to function as a highly sophisticated filter. There are four predominant cell types: parietal epithelial cells of Bowman's capsule, capillary endothelial cells, podocytes, and mesangial cells and these are all supported by specialized ECM compartments. ECM creates a microenvironment for cells and influences cell functions such as adhesion, migration, differentiation and proliferation. Glomerular ECM compartments include the glomerular basement membrane (GBM) separating endothelial cells from podocytes, the mesangial matrix which embeds and supports the mesangial cells, and the basement membrane associated with Bowman's capsule (Figs 320.1 and 320.2).

Basement membranes are condensed sheets of ECM proteins which associate with epithelial and endothelial cell layers throughout the body. The GBM has typical features of a basement membrane comprising an assembly of structural ECM proteins including collagen IV, laminin, nidogens, and heparan sulphate proteoglycans; however, specific isoforms of these core proteins are required for intact barrier function. In addition to structural ECM components, ECM regulators such as proteases are required to maintain and remodel the GBM and as with other basement membranes, the GBM serves as a reservoir for secreted growth factors, which can independently direct glomerular cell function.

ECM proteins within the GBM interact with each other and also with cell surface adhesion receptors. These include integrins and dystroglycan, which anchor cells to their underlying ECM (Fig. 320.3). Adhesion receptor binding to GBM ligands leads to receptor activation and the subsequent assembly of an intracellular focal complex. This matures into a focal adhesion protein complex, which has direct links to the cytoskeleton. The signalling pathways initiated by ligand interaction with adhesion receptors can influence diverse cellular functions including migration and differentiation and the synthesis of new ECM components. The interactions between GBM proteins, adhesion receptors, and the cytoskeleton are key to normal barrier function and therefore defects of components within this pathway can have overlapping phenotypes.

During glomerular development, there are two distinct basement membranes produced by endothelial cells and podocytes. As these cell layers come together, the basement membrane coalesces to form a single structure. This appears as a composite structure comprising the lamina rara interna, the lamina densa, and lamina rara externa following fixation for electron microscopy (EM). By contrast, in ultrastructural imaging of frozen sections, the GBM appears as a continuous structure suggesting that the composite appearance is an artefact of EM fixation (Chan et al., 1993). The mature human GBM has a width of between 300 and 350 nm, which compares to 100–200 nm widths of basement membranes in other tissues.

Functionally, the glomerular filtration barrier allows selective filtration with water and small solutes passing freely through the barrier and macromolecules and cells being maintained within the circulation. This selective permeability is based on molecule size and electrostatic charge with evidence for the latter arising from early studies incorporating the use of charged and neutral dextran molecules (Brenner et al., 1978). The GBM was considered to contribute to the charge barrier, although this hypothesis has not been supported by genetic deletion of charge components within the GBM. More recently, investigation has turned to the endothelial sugar coat or glycocalyx to examine its role in maintaining the charge barrier (Friden et al., 2011).

The composition and function of the GBM is critical for the maintenance of filtration barrier integrity. This has been highlighted by human disorders caused by genetic mutations in core components including collagen IV and laminin. In addition, basic science research has built understanding about the role of basement membrane-associated proteins in maintaining filtration barrier function.

Collagen IV

There are six collagen IV α subunits, each encoded by a distinct gene. *COL4A1* and *COL4A2* are located on chromosome 13, *COL4A3* and *COL4A4* appear on chromosome 2, and *COL4A5* and *COL4A6* are on the X chromosome. Three α chains combine to form the collagen IV triple helix (Fig. 320.4) and following secretion into the extracellular space, these protomers assemble to create distinct networks of collagen IV.

Three different protomers have been identified, with the compositions 112, 345, and 556 (in this terminology, 112 indicates a protomer made up of two α1 and one α2 chains, and so on). 112 and 345 protomers form discrete networks by associating with others of the same composition. However 556 protomers appear to associate with 112 protomers in a common network (Borza et al., 2002); although the more correct terminology may therefore be '556–112,' we have used 556 to indicate this network.

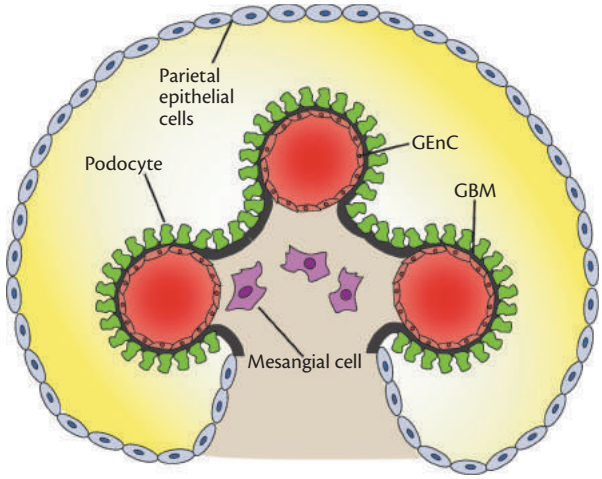

Fig. 320.1 Cross-section through a normal glomerulus indicating cellular and extracellular matrix compartments. GBM = glomerular basement membrane; GEnC = glomerular endothelial cell.
Figure kindly produced by Michael Randles, University of Manchester, UK.

The protomers associate in a head-to-head arrangement with apposition of the non-collagenous (NC1) domains to create globular hexamers that can be released from basement membranes by digestion with bacterial collagenase. Further crosslinking of the network is thought to enhance tensile properties and thus provide a strong structural support for the overlying cells. The absence of one chain within the protomer results in failure of network assembly, therefore mutations in *COL4A5* result in deficient or absent 345 network in the GBM (see Chapter 321).

The localization of the collagen IV networks is distinct within the glomerulus and this is likely to represent the specific function these networks support. In the mature glomerulus, the 112 network is predominant in the mesangium (Fig. 320.5), whereas 345 is found in the GBM and both 112 and 556 are evident in the basement membrane of Bowman's capsule. During glomerular development the pattern is different and the GBM comprises a predominant 112 network until the capillary loop stage of development when there is an isoform switch to the 345 network (Harvey et al., 1998). The cellular origins of collagen IV have been investigated and whilst endothelial cells, podocytes, and mesangial cells are capable of synthesizing the 112 network, it appears only podocytes produce the 345 network (Abrahamson et al., 2009). This network is thought to be more able to withstand hydrostatic pressures and more resistant to proteolysis, and therefore maintains structural support within the filtration barrier. The recent discovery of a new chemical bond joining the NC1 domains of collagen IV protomers is thought to be an evolutionary development creating a greater capacity for collagen IV networks to withhold mechanical forces (Vanacore et al., 2009). In the absence of the 345 network, for example during kidney development and in Alport syndrome, the 112 network predominates in the GBM (Kashtan and Kim, 1992).

Alport syndrome

The importance of collagen IV in the GBM was highlighted by the discovery that mutations in *COL4A5* cause X-linked Alport syndrome (Barker et al., 1990) (see Chapter 321). This hereditary nephritis has since been associated with mutations in *COL4A3* and *COL4A4*. The 112 (IV) network is clearly sufficient for glomerular development and early function, but patients with Alport syndrome develop significant glomerular barrier dysfunction from the second decade onwards.

Goodpasture disease

In anti-GBM (Goodpasture) disease (Chapter 71), individuals develop autoantibodies against the carboxy-terminal NC1 domain of one of the tissue-specific type IV collagen chains, α3(IV). Renal presentation is typically with a rapidly progressive glomerulonephritis. A similar phenomenon is occasionally observed in patients with Alport syndrome following renal transplantation (Chapter 75). Two to five per cent of patients with Alport syndrome develop post-transplant anti-GBM glomerulonephritis (Jais et al., 2000). The precise molecular targets in the two conditions are subtly different. In spontaneous anti-GBM disease (Goodpasture disease)

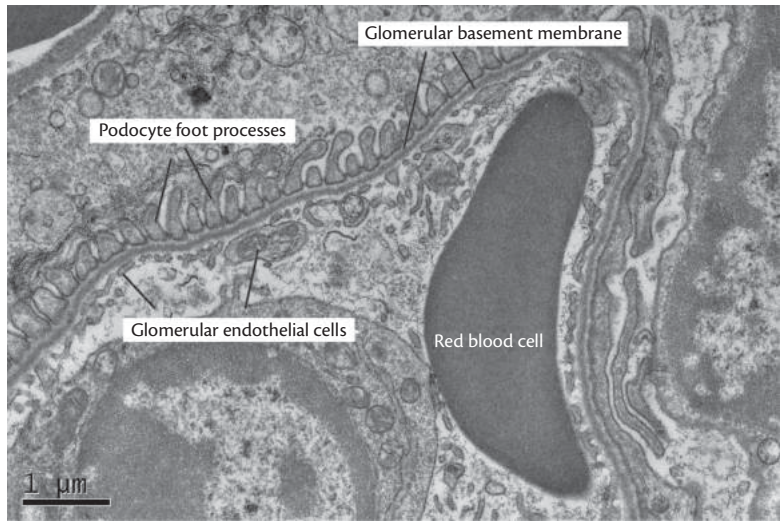

Fig. 320.2 Electron micrograph of a mouse glomerulus showing the components of the cellular components of the glomerular filtration barrier and their close association with the GBM.

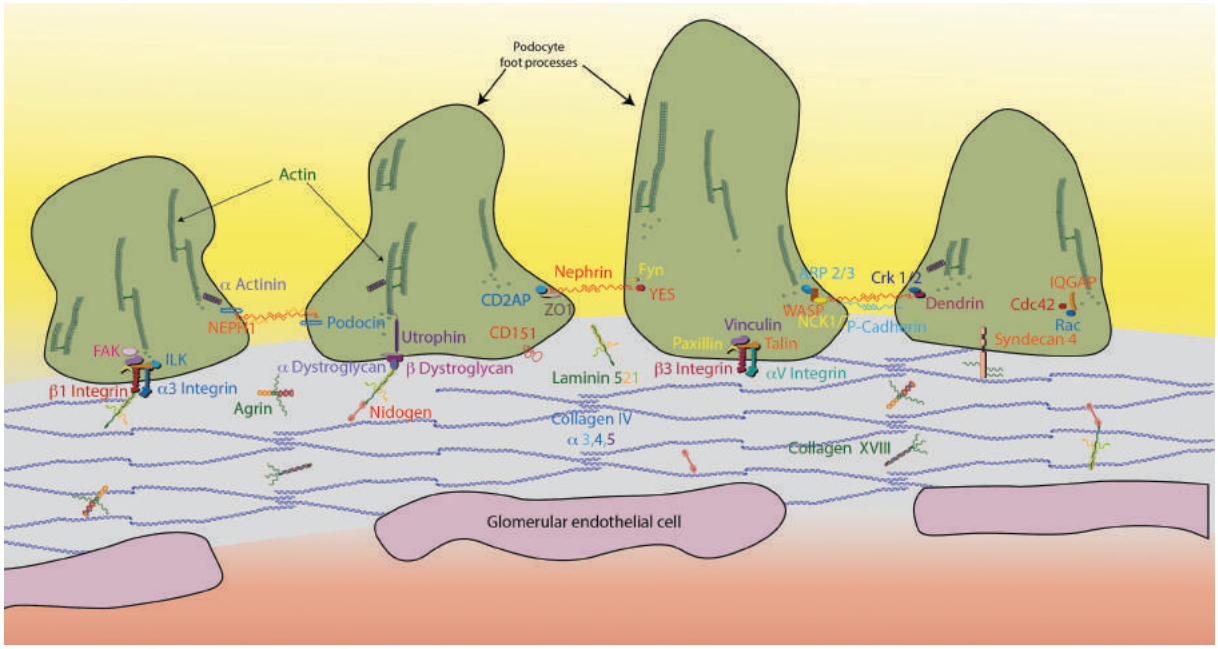

Fig. 320.3 The glomerular filtration barrier demonstrating the interaction between GBM components and adjacent glomerular cells.
Figure kindly produced by Michael Randles, University of Manchester, UK.

the major target is an epitope in α3(IV)NC1. In Alport anti-GBM disease, the epitopes are also in NC1 domains, but is typically in the chain affected by the mutation, so usually in α5(IV)NC1 (see Chapter 72).

Thin basement membrane nephropathy

Thin basement membrane nephropathy (see Chapter 325) has an association with mutations in *COL4A3* and *COL4A4* (Rana et al., 2005), accounting for up to 40% of cases. Individuals are heterozygote for these mutations and in the absence of proteinuria or hypertension, this condition has been termed benign familial haematuria. Given what we know about X-linked Alport carriers (see Chapter 322), it is not surprising that patients with heterozygote mutations in autosomal collagen IV genes may also have adverse outcomes (Voskarides et al., 2008; Temme et al., 2012). It is unclear whether modifying genes are contributing to progression of the renal phenotype in individuals who develop severe phenotypes.

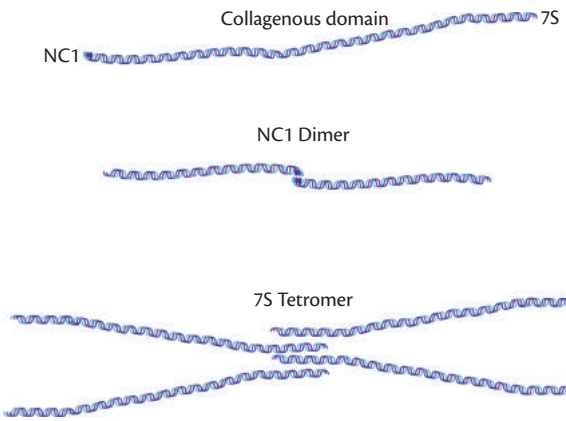

Fig. 320.4 Collagen IV α3α4α5 chains can interact through their NC1 domains to form a dimer (centre) or through their N-terminal 7S domains to form a tetramer. Through complex interactions, these molecules can interact further to form higher-order supramolecular organization and three-dimensional networks. The networks are further enforced by supramolecular twisting and lateral associations of their collagenous domains.

HANAC syndrome

Human mutations in the α1 chain of collagen IV have also been described. These mutations result in multisystem pathology in which renal disease is usually a minor manifestation. Hereditary angiopathy, nephropathy, aneurysms and muscle cramps comprise HANAC syndrome, which exhibits phenotypic variability (Plaisier et al., 2007). The nephropathy associated with the syndrome includes haematuria, which can be microscopic and macroscopic, and in addition renal cysts are described, together with abnormalities in the tubular basement membrane, Bowman's capsule, and peritubular capillaries. However, the GBM in HANAC syndrome is typically described as normal. Inheritance is generally autosomal dominant and haematuria may be the result of defective tubular and peritubular capillary basement membranes resulting in leakage of red blood cells. The mutations associated with HANAC syndrome all localize to a critical region of the gene, which is required for cell adhesion receptor binding.

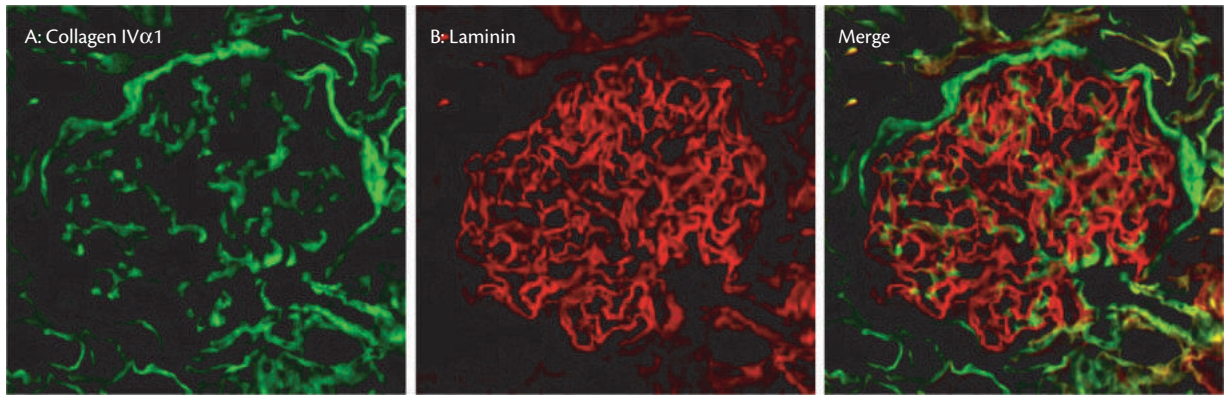

Fig. 320.5 Collagen IVα1 is expressed in the mesangial matrix (MM) compartment and in Bowman's capsule (A). Laminin is expressed within the capillary walls in the GBM (B). The merged images outline the GBM and MM compartments.
Collagen IV α1 chain specific antibody was kindly provided by B. Hudson, Vanderbilt Medical Center, Nashville, TN, USA.

Laminin

Laminins are glycoproteins, which are secreted as α, β, γ heterotrimers and provide a template within the ECM for the attachment of other matrix proteins (McKee et al., 2007). Five α chains, four β chains, and three γ gamma chains combine to create 15 distinct laminin isoforms which have tissue-specific expression. The nomenclature for laminins is taken from the chain number therefore laminin α5β2γ1 is termed laminin-521 (Aumailley et al., 2005). The assembled chains form a cross shape (Fig. 320.6) and the globular domain of the protein is the binding site for adhesion receptors including integrins and dystroglycan. Whilst other ECM proteins, including collagen IV are dispensable for the initial formation of basement membranes, laminin polymerization in the extracellular space is required to initiate basement membrane formation.

In the mature GBM, laminin-521 is the predominant isoform; however, during development there is a sequence of laminin expression. Laminin-111 is expressed initially and this is replaced with laminin-511. As with collagen IV, there is an isoform switch at the capillary loop stage of glomerular development and at this stage laminin-521 is predominantly expressed. Both podocytes and endothelial cells have been shown to contribute to the synthesis of this laminin isoform (Steenhard et al., 2011).

Much of our understanding about laminin in the GBM has come from elegant genetic studies in mice (Miner, 2012). These demonstrated that the global absence of laminin α5 caused abnormal glomerular capillary development, indicating the importance of this chain for the creation of the GBM. Furthermore, reduced expression of laminin α5 was associated with proteinuria, haematuria, and polycystic kidneys (Shannon et al., 2006) and the deletion of laminin α5 in podocytes alone resulted in a phenotype range from proteinuria to nephrotic syndrome (Goldberg et al., 2010). In additional studies, absence of laminin β2 led to early-onset nephrotic syndrome (Noakes et al., 1995) and the later development of podocyte foot process fusion, suggesting a role of laminin β2 in the maintenance of podocyte slit diaphragms. In this mouse model, overexpression of laminin β1 improved the phenotype, suggesting that excess β1 could compensate for the absence of the β2 laminin chain.

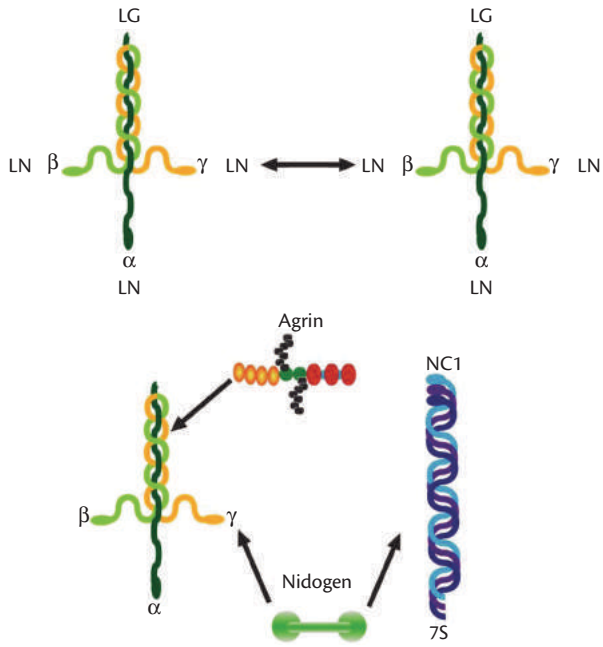

Fig. 320.6 Laminin interactions. Top panel shows a laminin–laminin interaction. Bottom panel shows interactions between laminin and other ECM proteins including collagen IV (right hand side). 7S = non-collagenous N terminal domain. LG = globular C terminal domain; LN = globular N terminal domain; NC1 = non-collagenous C terminal domain.
Image kindly generated by Michael Randless, University of Manchester, UK.

Pierson syndrome

Human mutations in laminin β2 have also been described and result in Pierson syndrome (Zenker et al., 2004). The syndrome is characterized by neonatal nephrotic syndrome, eye defects including microcoria, and abnormalities of neuromuscular junctions. In addition to the severe phenotypes originally described, milder phenotypes have since been associated with *LAMB2* mutations (Hasselbacher et al., 2006).

Nidogens

Nidogen-1 and nidogen-2, also known as entactins, are both expressed in kidney ECM compartments, including the GBM. The

nidogens are highly conserved linker molecules and they bind to both collagen IV and laminin isoforms (Fig. 320.6). Their role in linking ECM proteins was thought to be critical for the formation of the GBM; however, mouse models deficient in either isoform have normal glomerular phenotypes with intact GBMs. The double-nidogen knockout mouse has perinatal lethality and whilst basement membranes develop, the absence of both nidogens is critical for the late stages of lung and cardiac development (Bader et al., 2005). A small proportion of these mice had unilateral or bilateral renal aplasia suggesting overall that the function of nidogens in the kidney can be compensated by other mechanisms. To date, human nidogen mutations have not been associated with defects in the GBM although the induction of renal injury in nidogen-2 knockout mice showed a significant deterioration in renal function compared to controls (Amann et al., 2009). This suggests that the role of nidogens may be more important in protection from glomerular injury.

Heparan sulphate proteoglycans

The GBM contains a number of heparan sulphate proteoglycans (HSPGs) consisting of a core protein with heparan sulphate and sugar side chains. These proteins have a strong anionic charge (McCarthy and Wassenhove-McCarthy, 2012) and this property was considered to be an important contributor to barrier integrity.

Perlecan and agrin

Agrin is a ubiquitous HSPG and its role in glomerular filtration was investigated using mouse genetics (Harvey et al., 2007). The agrin gene was deleted specifically in podocytes and the mice did not develop proteinuria. The same was true with the perlecan podocyte-specific knockout and also the double agrin–perlecan knockout (Goldberg et al., 2009). In the latter, there were no abnormalities in the glomeruli or GBM, however the anionic charge density was reduced. This was also examined using a different approach by deleting the enzyme Ext1 from podocytes. Ext1 is required for sugar chain assembly and when this was deleted from podocytes there was no overt phenotype but there were ultrastructural abnormalities in the GBM and the development of late podocyte changes (Chen et al., 2008). To date, neither perlecan nor agrin mutations have been described in human disease.

Collagen XVIII (+ endostatin)

This collagen is a HSPG and is widely expressed in the kidney within tubular and glomerular ECM compartments. C-terminal cleavage of the protein releases endostatin, which has anti-angiogenic properties. Collagen XVIII is required for the normal development of the retinal basement membrane (Fukai et al., 2002) and more recently it has been shown to be necessary for normal podocyte morphology and the maintenance of glomerular mechanical properties (Kinnunen et al., 2011). Collagen XVIII α1-deficient mice are more susceptible to anti-GBM disease (Hamano et al., 2010) suggesting that this collagen has a protective role in barrier function. An association with human glomerular disease and collagen XVIII mutations has yet to be described.

Fibronectin

Fibronectin is a high-molecular-weight glycoprotein, which has key roles in cell adhesion, differentiation, and migration. It is present as a circulating protein in plasma and within tissue where protein dimers assemble to form networks. Fibronectin is expressed in the normal glomerulus where it is predominantly localized in the mesangium. It is not considered to be a component of the mature GBM although it is detected in this compartment during development. Mutations in *FN1* have been associated with human glomerular disease where fibronectin deposits are found in the glomerulus (Castelletti et al., 2008). Individuals exhibit proteinuria, haematuria, and progression to renal failure between the second and sixth decades.

Transcription factors

A number of transcription factors are key for normal glomerular development and several directly regulate the expression of ECM proteins (Chugh et al., 2007). *LMX1B* is a LIM homeodomain transcription factor and it is mutated in nail patella syndrome (NPS) (see Chapter 326). This transcription factor has a LIM domain at the N-terminus, which enables protein interactions. The central homeodomain binds to DNA and mutations are typically found in this region. The *LMX1B* knockout mouse had reduced expression of *COL4A3* and *COL4A4* in addition to the genes encoding slit-diaphragm proteins podocin and CD2AP. This suggested that *LMX1B* directly regulates the expression of the key glomerular proteins; however, these finding were not confirmed in human patients with NPS (Heidet et al., 2003).

Nail-patella syndrome

This multisystem disorder (see Chapter 326) includes dystrophic nails, absence or hypoplasia of the patella, and nephropathy. The latter can range from mild proteinuria to nephrotic syndrome and 30% of patients with nephropathy will progress to renal failure. Heterozygous mutations in the transcription factor *LMX1B* have been associated with this syndrome (Dreyer et al., 1998) and inheritance is autosomal dominant. The GBM has irregular thickening and accumulation of type III collagen (Heidet et al., 2003). This feature has also been described in collagen III glomerulopathy, which can also be familial and presents with proteinuria and progression to renal failure within 10 years. In the latter, collagen III deposits are found in the mesangial matrix and in the subendothelial capillary walls.

Adhesion receptors

Cells attach to their associated ECM via adhesion receptors, which are activated upon ligand binding. The activated receptor then initiates a cascade of signalling events which direct cell behaviour. Adhesion receptors have ligand and tissue specificity and within the glomerulus the predominant adhesion receptors are integrins and dystroglycan. These receptors enable cell adhesion to collagen IV, laminin, and HSPGs. The close interaction between ECM ligand and adhesion receptors indicates that these molecules are likely to be key in maintaining the integrity of the filtration barrier.

Integrins are heterodimeric cell surface receptors comprised of α and β subunits. Mouse genetic studies have highlighted the importance of the laminin receptor integrin α3β1 with the α3 knockout mouse, which develops lung and kidney defects (Kreidberg et al., 1996). Podocyte specific deletion of the α3 subunit also resulted in

a glomerular phenotype with nephrotic syndrome and subsequent renal failure (Sachs et al., 2006). More recently, human integrin α3 homozygous mutations have been associated with basement membrane abnormalities in kidney, lung, and skin (Has et al., 2012). In the reported case series, clinical presentation combined congenital nephrotic syndrome, epidermolysis bulosa, and interstitial lung disease.

In further mouse studies, homozygous deletion of integrin β1 led to embryonic lethality (Fassler and Meyer, 1995), which may explain the absence of known human mutations in this integrin subunit. However, the podocyte-specific deletion of β1 demonstrated the importance of this integrin in glomerular function (Pozzi et al., 2008). The mice developed early renal failure and podocyte effacement was seen followed by podocyte apoptosis and degeneration of the glomerular capillaries and mesangium, thought to be secondary to concomitant loss of podocyte-derived growth factors.

The tetraspannin CD151 closely associates with integrin α3β1 and podocyte-specific deletion of this molecule reduced glomerular resistance to hypertension-related injury in a susceptible mouse genetic background (Sachs et al., 2012). Furthermore, a human frameshift mutation has been described in *CD151* resulting in focal thickening and irregularity of the GBM. This phenotype also combined hearing loss and skin defects (Karamatic Crew et al., 2004).

The adhesion receptor dystroglycan binds agrin, perlecan, and laminin and the role of this receptor has also been investigated in mouse models. The podocyte-specific deletion of this protein did not result in a renal phenotype (Jarad et al., 2011). However, interruption of receptor glycosylation was associated with altered podocyte architecture suggesting that this receptor has a minor role in maintaining barrier integrity. Human mutations have not been associated with abnormalities of the GBM.

Cytoskeletal proteins

Cells adhere to the GBM via adhesion receptors, which in turn connect to the cytoskeleton of cells. These connections enable tension to build between cells and the underlying ECM network and force transduction can influence cell behaviour. In support of this hypothesis, defects in cytoskeletal components have been associated with GBM phenotypes.

MYH9 encodes non-muscle myosin heavy chain IIA, which combines with additional subunits to form a class II myosin. Myosins are motor proteins which hydrolyse ATP to enable movement along actin fibres. Within the glomerulus, *MYH9* is expressed in podocytes, mesangial cells, arteriolar and peritubular capillaries, and mutations have been associated with hereditary nephritis. In addition population genetic screening initially proposed an association with *MYH9* polymorphisms and the development of kidney disease in African American individuals. However this association has subsequently been attributed to genetic variants in the adjacent *APOL1* gene, which are thought to have evolved in response to pressure from an infectious pathogen in West Africa (see Chapter 341; and Rosset et al., 2011).

Although mutations of MYH9 do cause renal disease (see Chapter 342), and have occasionally been associated with GBM abnormalities, this does not seem to be a primary feature of MYH9-associated renal disease.

Table 320.1 Genotype and phenotype correlations in glomerular basement membrane disorders

Gene	Renal phenotype	Extrarenal phenotype
COL4A3	Haematuria, proteinuria, chronic kidney disease (CKD) with homozygous mutations	Ocular defects, sensorineural hearing loss
COL4A4	Haematuria, proteinuria CKD with homozygous mutations	Ocular defects, sensorineural hearing loss
COL4A5	Haematuria, proteinuria, CKD in males	Ocular defects, sensorineural hearing loss
COL4A1	Haematuria (microscopic/macroscopic), cortical renal cysts	Angiopathy, aneurysms, muscle cramps
LAMB2	Congenital nephrotic syndrome	Microcoria, neurological defects
LMX1B	Proteinuria to nephrotic syndrome, CKD	Dystrophic nails, absent/dysplastic patellae
ITGA3	Congenital nephrotic syndrome	Epidermolysis bullosa, interstitial lung disease
CD151	Haematuria, nephrotic syndrome, CKD	Bullous skin lesions and sensorineural hearing defects

Summary

There have been significant advances in our understanding of GBM disorders over the past two decades, assisted by advances in genomic and basic science research. With the increasing application of global approaches to investigate the GBM (Lennon et al., 2014), it is likely that the catalogue of molecular diagnoses will increase and allow closer genotype and phenotype correlation (Table 320.1). In turn this will impact on prognosis counselling and personalized therapy for patients with disorders of the GBM.

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CHAPTER 321

Alport syndrome: overview

Laurence Heidet, Bertrand Knebelmann, and Marie Claire Gubler

Alport syndrome is an inherited renal disorder characterized by early haematuria, progressing to proteinuria, sensorineural hearing loss, and progressive renal failure typically in the third or fourth decade but with wide variation. It is responsible for about 1% of end-stage renal failure (ESRF). Over 80% of cases are X-linked and young men are most affected, but female heterozygous carriers of the abnormal gene are also at significantly increased risk of ESRF in their lifetime. Those affected by the autosomal recessive variant are phenotypically very similar. It is caused by mutations in tissue-specific isoforms of basement membrane (type IV) collagen encoded by *COL4A5* (X chromosome), *COL4A3*, and *COL4A4* (chromosome 2).

Collagen IV is the major component of all basement membranes. Its molecular structure and the biochemical composition of the glomerular basement membrane are described in Chapter 320. Fig. 321.1 illustrates how its six isoforms are expressed in human kidney.

A firm diagnosis is based on the clinical picture plus either identifying the characteristic ultrastructural changes of the GBM seen by electron microscopy, or (and increasingly) by identification of a mutation (see Chapter 323). Light microscopy examination of renal biopsies does not show any specific changes. Immunofluorescence studies of the type IV collagen antigens can sometimes be useful.

Angiotensin-converting enzyme inhibitors probably slow the progression of Alport syndrome substantially, and should be prescribed at full or maximum tolerated dose to all males affected by X-linked disease, and to all individuals with autosomal recessive disease, and to all carriers who have proteinuria (see Chapter 324).

Hearing impairment commonly develops during high school years and can be a significant burden but rarely leads to total loss of hearing. With the use of hearing aids, ability to communicate is usually excellent. These features contrast to some of the other causes of hearing impairment with kidney disease (see Chapter 170).

Patients with Alport syndrome generally do well on dialysis or after renal transplantation. The rare complication of Alport anti-GBM disease (see Chapter 75) is seen only in a very small minority (< 5%).

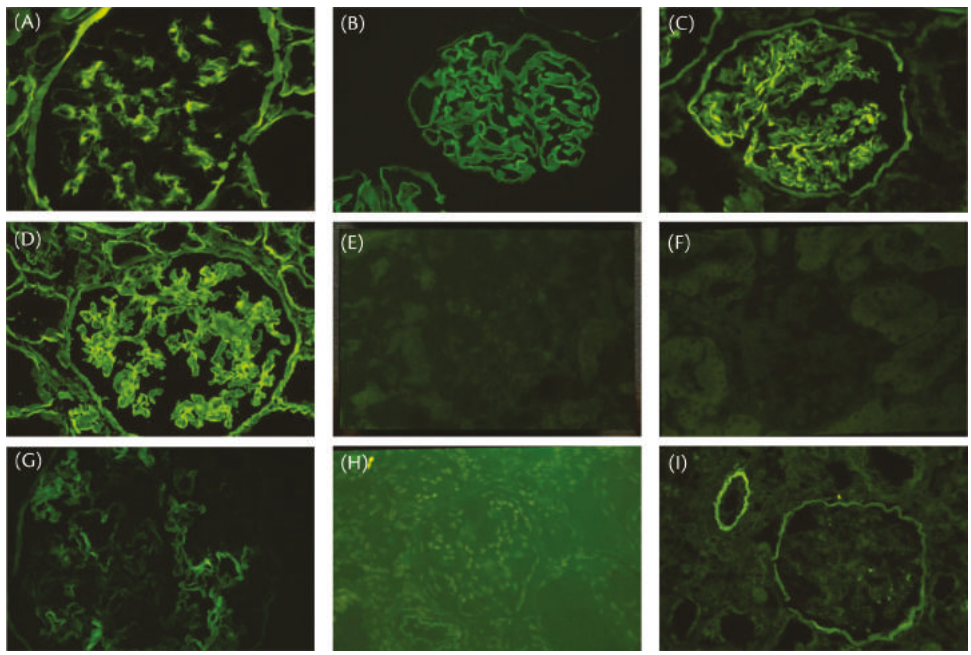

Fig. 321.1 Distribution of type IV collagen chains in kidney basement membranes. Normal kidney: (A) Anti-α2(IV). (B) Anti-α3(IV). (C) Anti-α5(IV). Male patient with X-linked Alport syndrome: (D) Anti-α1(IV). (E) Anti-α3(IV). (F) Anti-α5(IV). Female patient with X-linked Alport syndrome: (G) Anti-α(5IV). Patient with autosomal recessive Alport syndrome: (H) Anti-α3(IV). (I) Anti-α5(IV).

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CHAPTER 322

Alport syndrome: clinical features

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Introduction

Alport syndrome is characterized by the familial occurrence of progressive haematuric nephropathy with ultrastructural changes of the glomerular basement membrane (GBM) and sensorineural hearing loss. The family reported by Dr A. Cecil Alport (Alport, 1927) had been studied since the beginning of the twentieth century. Alport clearly noted that 'males develop nephritis and deafness and do not as a rule survive' whereas 'females have deafness and haematuria, and live to old age'. This statement has subsequently been largely confirmed: X-linked dominant inheritance is the most frequent mode of inheritance in this disorder (80–85% of the families).

Alport syndrome is reported to be the cause of approximately 0.6% of end-stage renal failure (ESRF) in Europe (Gretz et al., 1987), 1% in the United Kingdom (unpublished data from UK Renal Registry, 2012), 1.1% in India (Chugh et al., 1993) and 0.3% in adults and 2.3% in children in the United States, including African American families (United States Renal Data System, 2004). This proportion is probably underestimated since Alport syndrome is underdiagnosed in small kindreds or in sporadic cases, particularly if the characteristic hearing defect is not present at the time the index patient presents.

Classic X-linked Alport syndrome

Renal disease

Alport syndrome may be detected in infancy or childhood. All affected males have microscopic haematuria, and 50–60% exhibit gross haematuria, often before the age of 5 years (Gubler et al., 1981; Pajari et al., 1996). Recurrence (often following upper respiratory tract infection) is frequent during the first years of life. Gross haematuria does not generally recur after the age of 15. In early childhood, proteinuria may be absent, intermittent, or permanent and mild; its incidence and abundance increase progressively with age. Approximately 40% of patients, mainly boys, develop moderate nephrotic syndrome with or without hypertension, after the age of 10 years, a marker of poor renal prognosis.

The renal disease is often first discovered in adults. In them, gross haematuria is rare and proteinuria and microhaematuria, with hypertension and/or renal failure, are the presenting abnormalities. The slower the rate of progression, the rarer the nephrotic syndrome.

The rate of progression to renal failure is heterogeneous and depends on sex and genetic factors (Jais et al., 2000, 2003; Bekheirnia et al., 2010; Demosthenous et al., 2012). All affected males progress to renal failure. ESRF is rarely reached in males before 10 years of age. The median age of ESRF has risen by several years in the last two decades. According to Jais et al. (possibly a study biased towards more severely affected individuals), the median age at ESRF was 20 years and 90% of male patients developed ESRF before 40 years (Jais et al., 2000). A more recent study based on multiple ESRF registries (Temme et al., 2012a) showed a median age of ESRF of 26.6 years in 1990–1994 rising to 33.7 years in 2005–2009.

Over 90% of female carriers of X-linked (COL4A5) Alport syndrome have intermittent or persistent microhaematuria, and most do not progress to renal failure. Proteinuria is found in some women, and this is prognostically important (Temme et al., 2012b). Progression to ESRF occurs in only 12% of females before the age of 40. The youngest age at ESRF reported is 19 years. However, > 30% develop ESRF after the age of 60 (Jais et al., 2003; Rheault, 2012). Anyway, the rate of progression is slower than in males. Heavy proteinuria indicates poor prognosis, and the finding of FSGS lesions on renal biopsy, and of thick and split GBM on electron microscopy, have been associated with poor renal outcomes (Grünfeld et al., 1985). Random inactivation of one X chromosome in each cell of the body leading to variability in expression of X-Alport syndrome in females is usually postulated to account for some variations in severity on X-linked diseases (Guo et al., 1995; Rheault, 2012). However, skewed X-inactivation in non-renal accessible tissues (skin, lymphocytes) does not predict the severity of renal disease (Rheault, 2012).

Intrafamilial resemblance with regard to age at ESRF in males has been emphasized (Grünfeld, 1985), but large intrafamilial variability can be observed. In such a family with a proven mutation in the *COL4A5* gene, ESRF in affected males was reached between 30 and 70 years of age (Knebelmann et al., 1992). It seems that missense mutations, often involving glycine residues, are more often associated with such a variability than large deletions and nonsense mutations (Jais et al., 2000).

The predictive value of extrarenal signs in a given patient is controversial. Correlation between progression of hearing loss and renal failure is the rule. Early detection of eye defects appear associated with a more rapid progression to renal failure (Perrin et al., 1980; Tan et al., 2010).

Hearing defect

High-tone sensorineural hearing loss is bilateral and may lead to clinically evident deafness. In its early stage, however, it can only be detected by audiometry, and audiogram should be performed in any patient with suspected hereditary nephritis because subclinical hearing impairment, involving the non-conversational range (high frequencies), may otherwise be missed. This hearing defect is usually not detected before late childhood in boys with X-linked Alport syndrome. It may be progressive in children, necessitating the use of hearing aid at high school ages. The differential diagnosis of hearing loss with renal disease is discussed in Chapter 323.

Mutations in the *COL4A5* gene have occasionally been identified in families with progressive hereditary nephritis without hearing defect. Contrary to Dr Alport's observation in 1927 (above), hearing loss in carriers (e.g. female heterozygotes for a *COL4A5* mutation) is inconsistent.

Ocular abnormalities

Ocular defects involving the lens and retina occur in 15–40% of patients with Alport syndrome. Bilateral anterior lenticonus is specific: all cases investigated were found to have evidence of nephritis (Nielsen, 1977; Govan, 1983). Anterior lenticonus is characterized by a conical protrusion of the anterior part of the lens into the chamber, leading to visual disturbance (Nielsen, 1978). Diagnosis is based on slit-lamp examination, which reveals a lens that looks like 'an oil droplet in water'. Lens opacities are rather frequently found but are non-specific.

Retinal flecks in the macula and mid periphery have been detected by systematic examination (Perrin et al., 1980; Govan, 1983). These flecks are round and symmetrical, have a pale yellowish colour, and appear to be located in the innermost layer of the retina. Fluorescein angiography of the macular region is normal. Electroretinograms and electro-oculograms are normal (Perrin et al., 1980; Govan, 1983) and visual acuity is not altered. Retinal changes are more frequent than lens abnormalities (Perrin et al., 1980; Shaw et al, 2007). Their finding is highly suggestive of Alport syndrome and should encourage further family investigations.

A group in Thailand described a particular but unspecific lesion of the cornea, called posterior polymorphic dystrophy, in 11 of their 17 Alport patients (Teekhasaenee et al., 1991). Furthermore, recurrent corneal epithelial erosions seem to be abnormally frequent but can be overlooked (Burke et al., 1991; Rhys et al., 1997).

Aortic abnormalities

Significant aortic diseases including dissection and aneurysm have been reported in a few male patients with a severe form of Alport syndrome leading to end-stage kidney disease between 10 and 22 years of age. Screening for aortic abnormalities may be indicated in these families (Kashtan et al., 2010).

Autosomal recessive Alport syndrome

In 10–15% of the families, features highly suggestive of autosomal recessive inheritance have been reported (Feingold et al., 1985): (a) appearance of the disease in the family after a consanguineous marriage, (b) equally severe disease in males and females, or more specifically, (c) severe renal disease in females leading to ESRF before 20 years of age.

Both progression and extrarenal features are similar to those found in X-linked classic Alport syndrome (Boye et al., 1998; Heidet et al., 2001; Wang et al., 2014). The GBM ultrastructural lesions are not distinguishable from those of the X-linked form. The distribution of α(IV) chains is abnormal in most patients (but few cases are documented): the α3, α4, and α5 chains are co-absent in the GBM but the α5 chain is normally present in a series of basement membranes, including Bowman's capsule, collecting duct, and epidermal basement membranes in which it participates to the formation of the 556 network (Fig. 322.1) (Gubler et al., 1995). Thus, immunohistochemical analysis of kidney biopsy can be of great value to establish not only the diagnosis of Alport syndrome but also its mode of inheritance. However, in autosomal recessive cases as in X-linked Alport syndrome, the expression of type IV collagen chains may be normal.

Heterozygous carriers of a *COL4A3* or *COL4A4* gene mutation usually present with no symptoms or intermittent microscopic haematuria. However, depending on the type of mutation and maybe also on other genetic defects or environmental factors, they sometimes display more severe symptoms including proteinuria and late renal failure (Heidet et al., 2001; Longo et al., 2002) (see Chapter 325).

Autosomal dominant Alport syndrome

Autosomal dominant forms of Alport syndrome, characterized by male-to-male transmission, was regarded as very rare. However, several families were reported with identification of

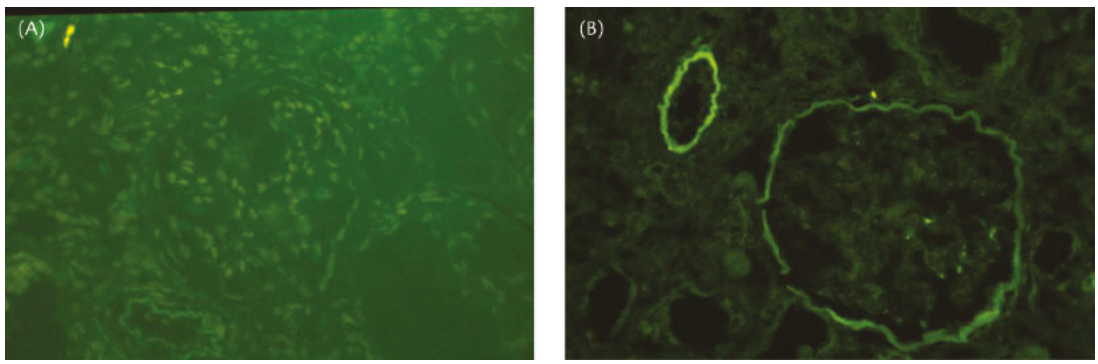

Fig. 322.1 Distribution of type IV collagen chains in kidney basement membranes. Patient with autosomal recessive Alport syndrome: (A) Anti-α3(IV). (B) Anti-α5(IV).

heterozygous mutations in *COL4A3* or *COL4A4* (Van der Loop et al., 2000; Pescucci et al., 2004; Marcocci et al., 2009). Recently, next-generation sequencing of *COL4* genes clearly showed that this mode of transmission is more frequent than previously thought (Fallerini et al., 2014; Moriniere et al., 2014).

Its manifestations are generally milder than the X-linked form with inconstant and late progression to ESRF between 40 and 80 years. Late occurring hearing loss is sometime present. Eye defects have never been reported. EM studies showed thin GBM or the association of thick and split lesions. Normal or reduced expression of the α3, α4, and α5 chains of type IV collagen has been described.

This is discussed further in Chapter 323.

MYH9-associated disease (Fechtner or Epstein syndromes, see Chapter 342) are not true Alport variants but, as causes deafness with renal failure and is inherited in an autosomal dominant pattern. It should be considered in the differential diagnosis.

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CHAPTER 323

Alport syndrome: diagnosis

Laurence Heidet, Bertrand Knebelmann, and Marie Claire Gubler

Outline

The two characteristic clinical elements of Alport syndrome are inherited progressive haematuric nephritis and sensorineural hearing loss. However, genetic deafness (including genetic nerve deafness) is not rare in the general population and sensorineural hearing defect may be associated with various types of renal disease, in addition to Alport syndrome (Table 323.1) (see Chapter 170).

Positive diagnosis of Alport syndrome is based on the following criteria: a positive family history of progressive haematuric nephritis (i.e. leading to renal failure), sensorineural hearing loss, specific eye defects, characteristic ultrastructural changes of the glomerular basement membrane (GBM), and defect in GBM antigenicity (Flinter et al., 1988; Jais et al., 2000). Identification of the mutation, co-segregating with the disease in the kindred, is now the strongest diagnostic criteria (Table 323.2). Of note, among 195 families with a proven *COL4A5* mutation in a European study, 14 (7.5%) had only one and 58 (30%) only two diagnostic criteria (Jais et al., 2000). Sensorineural hearing loss should only be considered as a feature suggestive, but not pathognomonic, of Alport syndrome (Pirson, 1999).

Pathology

Light microscopy shows normal or nearly normal renal tissue in young children. With increasing age, increased mesangial matrix, areas of segmental proliferation, thickening of capillary walls, and focal segmental glomerulosclerosis (FSGS) develop. Tubulointerstitial changes appear early. Interstitial foam cells (lipid-loaded macrophages) have been considered as suggestive of Alport syndrome, but these cells may be seen in most types of glomerular disease with persistent heavy proteinuria. Conventional immunofluorescent studies are generally negative or show non-specific deposits.

Electron microscopy reveals the most characteristic lesions (Fig. 323.1). Without it, erroneous diagnoses (including FSGS) may be made. The GBM is irregularly thickened with splitting and splintering of the lamina densa, thus delimiting clear zones containing small granulations. The external aspect of the GBM is irregularly festooned and bordered by hypertrophied podocytes. This ultrastructural lesion, when diffuse and associated with negative conventional immunofluorescence, is highly suggestive of Alport syndrome (Hinglais et al., 1972; Spear and Slusser, 1972; Churg and Sherman, 1973). The diagnostic value of electron microscopy has been repeatedly demonstrated in patients with no positive family history (Hinglais et al., 1972; Yoshikawa et al., 1987).

GBM thickening is usually diffuse in adults whereas it is segmental in children, associated with thinning and occasional ruptures of the GBM. Furthermore, diffuse thinning may be the only GBM lesion in patients with progressive nephritis (Fig. 323.1D) (Gubler et al., 1993; Heidet and Gubler, 2009). Heterozygous females express the GBM defect in a mosaic pattern. Thus sampling errors may sometimes make it difficult to form a firm histological diagnosis in these women.

Exceptional electron microscopic studies of ocular and cochlear basement membranes have been reported. The anterior lens capsules obtained at the time of surgery were thinner than in controls, with vertical dehiscences (Streeten et al., 1987; Junk et al., 2000; Ohkubo et al., 2003) and the Bruch's membrane from a donated Alport eye was also thinned (Savige et al., 2010). Abnormalities of the basement membrane of cells of the organ of Corti and dysmorphogenesis of the organ of Corti were observed in the four cases studied (Merchant et al., 2004).

Immunohistochemical analysis of type IV collagen

Kidney biopsy

Analysis of kidney biopsies from patients with X-linked Alport syndrome, using monoclonal antibodies against the α5 chain of type IV collagen, shows a lack of fixation along the GBM (i.e. a defect in GBM antigenicity) in most, but not all, male patients (Fig. 323.2A–C). In female patients from the same families, binding is either normal or discontinuous, with regions of normal reactivity interspersed with gaps of unreactive basement membrane, suggesting random inactivation of the X chromosome in an X-linked disease (Fig 323.2D). The defect in α5(IV) expression is found in approximately 75% of families (Gubler et al., 1993; Yoshioka et al., 1994). It is associated with the lack of GBM binding of α3(IV) and α4(IV) antibodies (Heidet et al., 2009).

This indicates that many *COL4A5* mutations prevent the assembly of the 345 network in the GBM so that the less stable embryonic 112 network continues to be expressed (see Chapter 320). These immunochemical anomalies have diagnostic value in X-linked Alport syndrome, however, they are observed predominantly when thick and split rather than thin GBM is the main ultrastructural lesion, and they are correlated with severe renal disease. Approximately 25% of the male patients have normal GBM antigenicity.

Table 323.1 Inherited conditions with hearing loss and renal disease

Diseases	Gene	OMIM #
Major diseases confused with Alport		
Alport syndrome (Chapter 321)	*COL4A3/4/5*	301050, 203780
BOR syndrome (branchio-oto-renal syndrome, Chapter 170)	*EYA1, SIX1, SIX5*	113650
MYH9-associated disease (Chapter 342)	*MYH9*	160775
Mitochondrial cytopathies (Chapter 340)		
Less likely to be		
Tubular disorders		
Renal distal tubular acidosis with deafness (Chapter 36)	*ATP6VB1, ATP6VB4*	267300
Bartter syndrome type IVa (Chapter 31)	*BSND, ClC-Kb*	602522
SESAME (EAST) syndrome (Chapter 31)	*KCNJ10*	612780
Variable or unknown		
Wolfram (DIDMOAD) syndrome	Various	
Developmental defects		
HDR syndrome (hypoparathyroidism deafness renal dysplasia)	*GATA3*	146255
Townes–Brocks syndrome	*SALL1*	107480
Ciliopathies		
Bardet–Biedl syndrome (Chapter 314)	*BBS1–BBS16*	209900
Alström syndrome (Chapter 314)	*ALMS1*	203800
Muckle–Wells syndrome	*NLRP3*	191900
Fabry disease (Chapter 335)	*AGAL*	301500
Nephropathy, epidermolysis bullosa and deafness	*CD151* (tetraspanin)	609057
Refsum disease	*PHYX, PEX7*	266500
Cockayne syndrome	*ERCC8*	216400

See also Chapter 170.

Table 323.2 Molecular genetics of Alport syndrome

Clinical presentation	Defective α(IV) chain	Mutant gene	Chromosomal location
X-linked disease with or without sensorineural hearing loss	α5	*COL4A5*	Xq22
X-linked disease with diffuse leiomyomatosis	α5 and α6	*COL4A5* and *COL4A6*	Xq22
Autosomal recessive disease and Autosomal dominant disease	α3 or α4	*COL4A3* *COL4A4*	2q35–q37 2q35–q37

Skin biopsy

As shown in Table 323.3, α5(IV), through the 556 network, is normally present in the epidermal basement membrane (EBM) whereas α3 and α4 are not. In 75% of male patients with X-linked Alport syndrome, α5(IV) is absent in the EBM (Fig. 323.3B), as in the GBM. In female heterozygotes, a mosaic pattern of α5 expression is found (Fig. 323.3C). This patttern can, however, be very heterogeneous, and normal expression cannot rule out a carrier status. It has also been suggested that the extent of defective α5 chain expression in the skin was correlated with progression of renal disease in heterozygous women (Nakanishi et al., 1998), a finding not confirmed by a further study (Massella et al., 2003). In contrast, α5 is present in EBM in autosomal recessive Alport syndrome (Table 323.3) since α3 and α4 are not required for its integration. Thus absence of α5 in the skin is diagnostic of Alport syndrome but also of its X-linked mode of inheritance. However normal expression of α5 in the skin (as in the kidney) of a patient suspected of Alport syndrome does not exclude the diagnosis (Patay-Mariaud de Serre et al., 2007; Wang et al., 2012). The first of these sources also illustrates how demanding and error-prone studies in skin are. Their place in diagnostics is small.

Molecular diagnostics

X-linked disease

The gene responsible for the classical X-linked Alport syndrome is *COL4A5* located at Xq22 (Barker et al., 1990). Mutations in *COL4A5* had been found in > 600 families throughout the world by 2010 (Crockett et al., 2010). Each family appears to have its own 'private' mutation, making mutation-finding laborious before next-generation sequencing protocols. The gene mutation frequency is estimated to be 1/5000 to 1/10,000.

Major rearrangements, mainly deletions, are detected in 5–16% of the kindreds (Antignac et al., 1994; Renieri et al., 1995; Lemmink et al., 1997; Jais et al., 2000; Gross et al., 2002; Bekheirnia et al., 2010). These deletions are located throughout the gene with no hot spot. Most deletions are associated with early progression to end-stage renal failure (ESRF), before the age of 20 in males, with hearing loss and ocular lesions in > 50% of the patients.

Small mutations in *COL4A5*, missense, nonsense, splice site, small deletions/insertions of a few base pairs, are the most common lesions. Frameshift mutations and stop codons observed in 40–45% of families are generally associated with early progression to ESRF, hearing defect, and ocular changes (Jais et al., 2000; Bekheirnia et al., 2010). In this group of patients, mutations located towards the 5′ end of the gene have been found to be associated with earlier age at ESRF, higher incidence of ocular changes, and hearing impairment (Bekheirnia et al., 2010).

Substitutions for glycine residues in the collagenous domain are the most frequent type of missense mutations, detected in 25–35% of families. They interfere with the normal folding of the α5 chain into triple helices with other α(IV) chains. The rate of progression of the renal disease is globally slower in male patients with such mutations than in patients with truncating mutations, with a probability of having ESRF at 30 years of 50%. However, because of its variation between and within families, the precise prognosis in a given patient is unpredictable. The prognostic significance of the position of the glycine

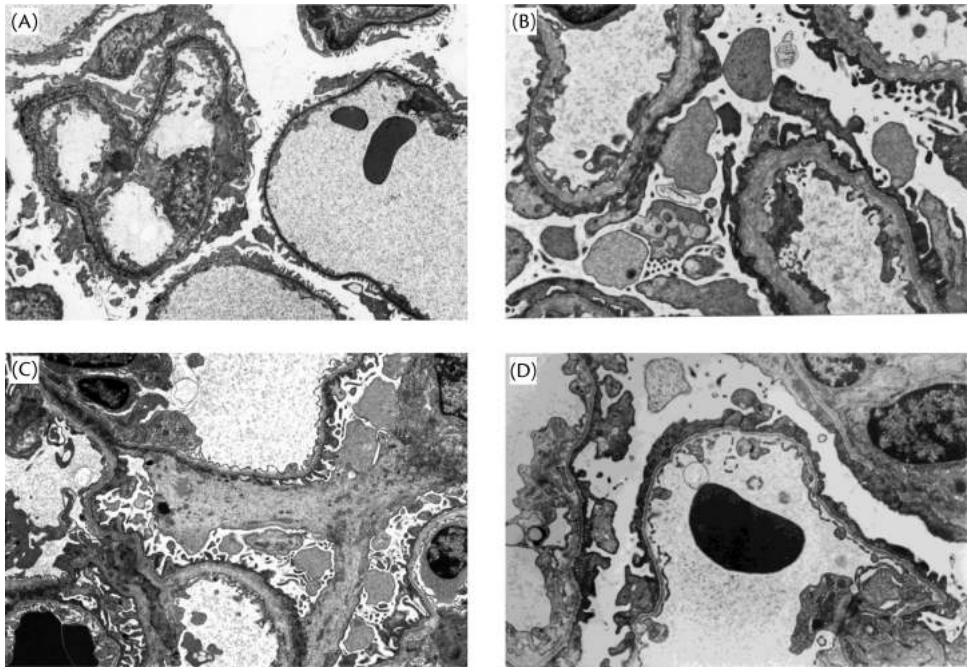

Fig. 323.1 Renal electron microscopy of Alport syndrome patients. (A) Marked irregularity in the GBM thickness with thick and split GBM segments contrasting with very thin ones (silver methenamine × 2700). (B) Thickening and irregular contours of the GBM and splitting(?) of the lamina densa (uranyl acetate and lead citrate × 5000). (C) Irregular thickening of the GBM, podocyte hypertrophy, and effacement of foot processes along the thin GBM segments (uranyl acetate and lead citrate × 3600). (D) Thin GBM with diffuse effacement of foot processes (uranyl acetate and lead citrate × 4800).

substitution, towards the 5′ or the 3′ end of the gene, is debated (Gross et al., 2002; Bekheirnia et al., 2010, Demosthenous et al., 2012).

Sporadic cases of Alport syndrome without positive family history could represent *de novo* mutations. Such mutations have been demonstrated by molecular genetic studies (Knebelmann et al., 1996; Lemmink et al., 1997). Their incidence has been estimated at up to 12% (Jais et al., 2000). Germline mosaicism may mimic *de novo* mutation. In such cases, the mother's somatic cells, such as leucocytes, contain two copies of the normal gene and therefore her phenotype is normal. However, she can transmit the disease through her mutated gametes with an unpredictable frequency. Similarly germline mosaicism in males may be responsible for mutations occurring apparently *de novo* in their daughters.

Autosomal recessive disease

Molecular studies have confirmed autosomal recessive inheritance: mutations have been identified in the *COL4A3* or *COL4A4* gene in several families (Mochizuki et al., 1994; Lemnink et al. 1994; Boye et al., 1998; Heidet et al., 2001; Longo et al., 2002; Zhang et al., 2012). They can be nonsense, frameshift, splicing, or missense mutations,

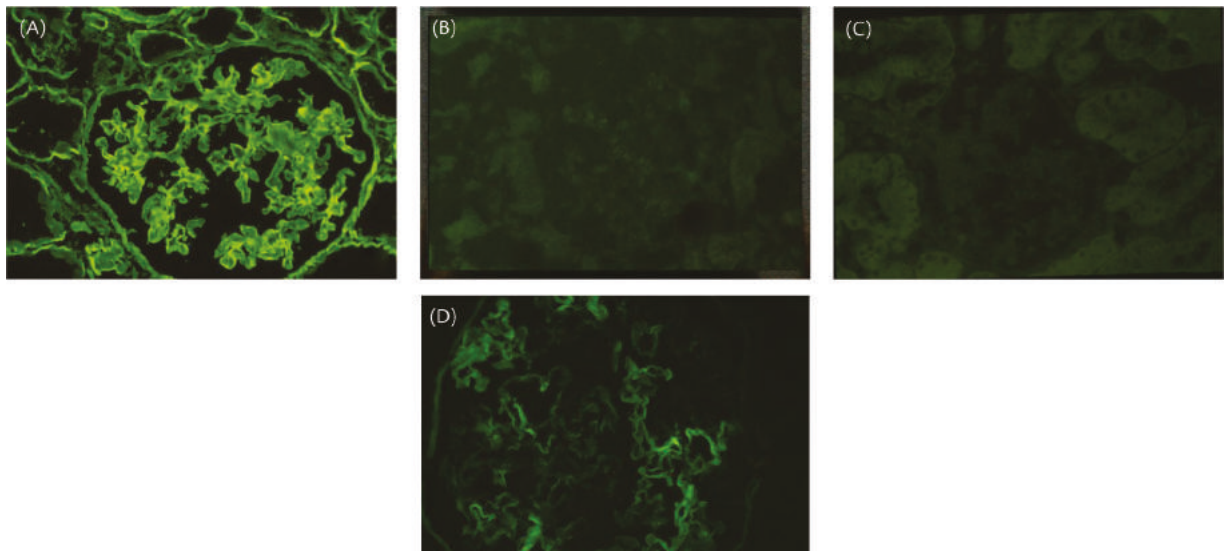

Fig. 323.2 Distribution of type IV collagen chains in kidney basement membranes. Male patient with X-linked Alport syndrome: (A) Anti-α1(IV). (B) Anti-α3(IV). (C) Anti-α5(IV). Female patient with X-linked Alport syndrome: (D) Anti-α5(IV).

Table 323.3 Tissue distribution of type IV collagen chains in basement membranes

Type IV collagen Disease	GBM	Bowman's capsule	Collecting duct basement membrane	Epidermal basement membrane
Normal α3/α4 (IV) α5(IV)	+ +	+/– +	– +	– +
X-linked Alport in males α3/α4 (IV) α5(IV)	– –	– –	– –	– –
X-linked Alport in females α3/α4 (IV) α5(IV)	Mosaic Mosaic			– Mosaic
Autosomal recessive Alport syndrome α3/α4(IV) α5(IV)	– –	– +	– +	– +

the latter frequently affecting glycine residues, as in the *COL4A5* gene. Affected patients are homozygotes, or compound heterozygotes having two different mutations in the two alleles of *COL4A3* or *COL4A4*.

Autosomal dominant disease

Molecular studies have identified different types of heterozygote mutations in either *COL4A3* or *COL4A4* associated with ADAS. The boundary between these (usually milder) phenotypes and the disease occurring in heterozygous 'carriers' of more typical disease is blurred.

Genetic counselling

The prerequisite for genetic counselling is to identify the inherited nature of the kidney disease. Genetic diagnosis is first based on the family history. Careful establishment of the family pedigree is therefore a crucial step. The clinical difficulties have been illustrated above and in Table 323.1 (also see Chapter 322).

The second step is to identify the mode of inheritance. In X-linked Alport syndrome, affected (hemizygous) males transmit the mutant gene to all daughters but not to sons. Affected (heterozygous) females carry a 50% risk to transmit the disease to their offspring, whatever their sex. In the autosomal recessive form, both parents are heterozygous, and the risk of transmission is 25%, whatever the sex. Molecular genetics can be very helpful in counselling: by direct identification of the mutation or by linkage analysis using highly polymorphic repetitive sequences within or close to the gene.

Genetic counselling should be considered a partnership between an at-risk individual and a counsellor (see Chapter 301). It should be shared by a clinical geneticist and a nephrologist. Indeed, both are able to offer information on the natural history of the disease, options for presymptomatic testing and support, and the at-risk subject or patient makes the decision. Presymptomatic testing using molecular techniques should focus on females who desire it, before they become pregnant. It should be kept in mind that it may have serious drawbacks if the results are misused by third parties, namely other family members, employers, or insurance companies.

Prenatal diagnosis (see Chapter 302) can be considered in the X-linked and autosomal recessive forms. The question may arise whether it is an acceptable option given the partially treatable nature of the condition. This issue has to be discussed between the carrier female and the geneticist.

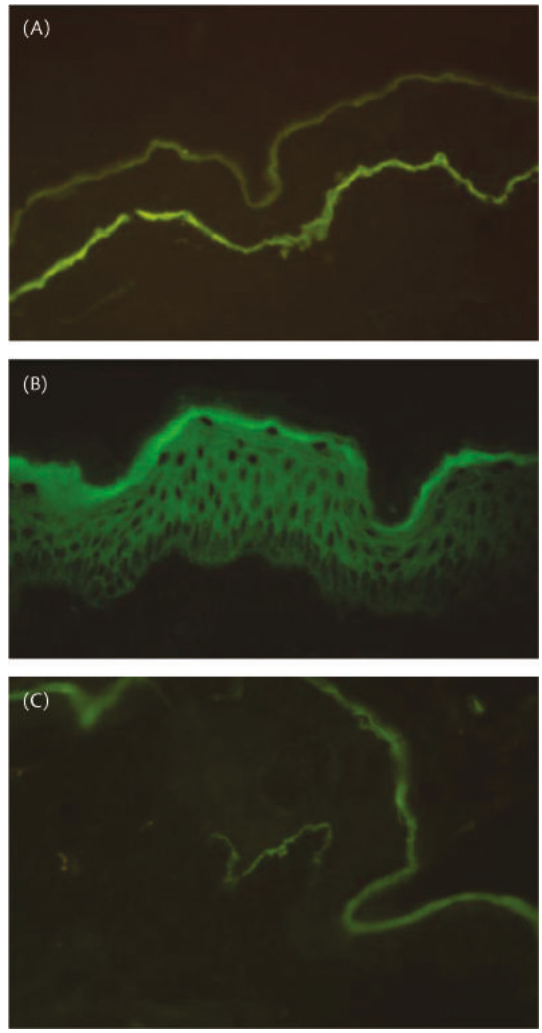

Fig. 323.3 Distribution of type IV collagen α5 chain in dermo-epidermal basement membrane. (A) Control skin. (B) Absence of α5 (IV) expression in skin of a male affected with X-linked Alport syndrome. (C) Segmental expression of the chain in an affected female. Note the non-specific labelling of the stratum corneum with the anti-α5 antibody.

Differential diagnosis

Haematuria

Macroscopic haematuria in infancy and early childhood is a characteristic feature of Alport syndrome with an important differential diagnosis. It occurs in a large proportion of X-linked males and autosomal recessive disease, but also in some X-linked carriers.

Gross haematuria, often recurrent, may be the revealing symptom of Wilms tumour in young children, of stone disease, or urologic abnormalities, all causes that have to be excluded by imaging.

The incidence of asymptomatic haematuria in the paediatric population ranges from 0.5% to 2.0% (Trachtman et al., 1984; and see Chapter 46). It may be an incidental finding, but if persistent the diagnosis of Alport syndrome should be considered. Urine testing of first-degree relatives is an essential part of the initial investigation. Even in the absence of extrarenal signs of the Alport series, and of positive family history, persistence of microscopic haematuria over a period of 6 months, episodes of gross haematuria, and occurrence of microalbuminuria/proteinuria are indications for renal biopsy with immunofluorescence and electron microscopy examination.

Trachman et al. (1984) found renal lesions, mainly thinning of the GBM, in 40% of children and young adults with isolated microhaematuria. Renal biopsy was more often abnormal in children and young adults with microscopic and gross haematuria; ultrastructural lesions (mainly thickening) and immunoglobulin A (IgA) nephropathy were found with similar frequencies. Clinicopathological correlations showed that boys with Alport syndrome almost invariably had persistent haematuria, whereas intermittent haematuria was a pointer to IgA nephropathy.

Similarly in a series of 322 children with persistent haematuria for > 6 months, biopsies were classified as IgA nephropathy in 78 patients (24%), Alport syndrome in 86 (26%), and thin basement membrane in 50 (15%). The biopsies in 48 (15%) patients showed normal glomeruli (Piqueras et al., 1998).

These studies demonstrated the importance of renal biopsy in the setting of persistent haematuria in young people, but they also posed the difficult question or the significance of the thin GBM: Alport syndrome or familial benign haematuria? (see also Chapter 325).

In adults > 40 years of age, microscopic haematuria has more alternative explanations (Chapter 46), but it can be a sign of carrying a mutation in *COL4A3, -4*, or *-5*.

Nephritis, deafness, and macrothrombocytopenia (Epstein syndrome)

This is not an Alport variant or basement membrane disorder, but can be confused with it (see Chapter 342; Knebelmann et al., 2001). The conditions are caused by mutations in the non-muscle myosin gene *MYH9*.

Rare Alport variants

X-linked diffuse oesophageal leiomyomatosis and Alport syndrome (ATS-DL, OMIM #308940)

Diffuse leiomyomatosis is a rare tumoural condition characterized by smooth muscle cell proliferation. Sporadic and inherited cases have been described. The association of Alport syndrome with leiomyomatosis involving the oesophagus, the tracheobronchial tree, and the genital tract in females (with vulvar and clitoral enlargement) was first described by Garcia-Torrès (reviewed in Garcia-Torrès and Orozco, 1993) and has been reported in several families. Oesophageal involvement, sometimes limited to the lower third of the oesophagus, is responsible for dysphagia, usually from childhood. In most cases, surgical treatment is required. Respiratory symptoms may also reveal leiomyomatosis. Tracheobronchial leiomyoma should be sought by endoscopy because of the risk of sudden death (Cochat et al., 1988).

The association is observed in juvenile-type Alport syndrome, with severe renal disease in males, and hearing loss in most cases. Another peculiar feature is the high incidence of bilateral congenital cataract, an eye abnormality not commonly found in classical Alport syndrome. Leiomyomatosis in female patients is as severe as in males whereas renal involvement is milder, often limited to microscopic haematuria, indicating that the leiomyomatosis element is dominantly inherited, whereas the nephropathy is of the familiar X-linked pattern.

In all patients studied, large deletions have been observed, removing the 5′ end of *COL4A5* and the two first exons of *COL4A6*, with the intergenic region thought to bear regulatory sequences (Zhou et al., 1993; Heidet et al., 1995; Uliana et al., 2011). The α5(IV) and α6(IV) chains are absent in the oesophageal smooth muscle cell basement membranes, whereas a COL4A6 transcript is expressed by the tumour cells. The mechanism leading to smooth muscle cell proliferation is not yet elucidated. However, it is clear that the absence of the 556 network is not responsible for smooth muscle cell proliferation because Alport syndrome patients with *COL4A5/COL4A6* deletions extending further into *COL4A6* display no such tumours (Heidet et al., 1995). In addition, in a canine model of Alport syndrome linked to *COL4A5* deletion, the absence of α5(IV) and α6(IV) chains in the bladder smooth muscle cell basement membrane has been demonstrated and is not associated with the occurrence of tumour (Zheng et al., 1999).

X-linked Alport syndrome with mental retardation (OMIM #300194)

The association of Alport syndrome with intellectual disability, midface hypoplasia, and facial hypoplasia has been reported in four families (Meloni et al., 2002; Rodriguez et al., 2010). Similar to ATS-DL, it is a contiguous gene syndrome due to a large deletion in Xq22.3 extending beyond *COL4A5* in the telomeric direction, and includes the *ACSL4* gene.

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CHAPTER 324

Alport syndrome: management

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Outline

General management

Alport syndrome patients must be regularly followed with blood pressure measurement. Glomerular filtration rate as well as micro- or macroproteinuria must be measured regularly. Audiograms must be regularly checked and a hearing aid must be proposed as early as after a loss of 35 dB. Lens alterations must be detected and treated. Children must lead a normal life, and receive usual vaccinations. Choices for professional life must take into account the risk of deafness and the progression towards end-stage renal disease (ESRD) in boys.

ACE inhibitors

There is no treatment proved to be efficient by controlled trials, but, retrospective observational data have shown that angiotensin-converting enzyme inhibitor therapy may delay renal failure in Alport patients with proteinuria (Gross et al., 2012). These data need to be confirmed by controlled studies, which are currently ongoing (Kashtan et al., 2012). Renin–angiotensin system blockers are routinely prescribed to Alport patients with proteinuria in many centres. Whether they should be started as early as the microalbuminuria stage remains to be established.

Other current drugs

Ciclosporin was found to delay progression of renal failure in humans and dogs in initial studies (Callis et al., 1999; Chen et al., 2003). However, this favourable action has not been observed by Massella et al. (2010), and ciclosporin has also been found to be rapidly associated with nephrotoxicity (Charbit et al., 2007), thereby precluding its long-term use.

Kidney donation from heterozygous relatives in Alport families

This practical issue is often raised. In X-linked disease, clinical detection of heterozygous females can be difficult, but definite diagnosis may be provided by molecular genetics. As indicated in Chapter 323, if most female carriers have a non-progressive renal disease, approximately 30% of them develop late ESRD.

The question now is whether non-proteinuric carrier females should be excluded or accepted for kidney donation. Few data are available on the long-term effect of uninephrectomy in such women. However, it seems reasonable to accept as a potential donor non-proteinuric (meaning also without microalbuminuria) female carriers with normal renal function and normal blood pressure, after the age of 45 years or if further pregnancy is not possible. Kidney donation should be considered with caution; information has to be provided on the long-term risks of renal disease after donation (Sessa et al., 1995; Kashtan, 2009; Gross et al., 2009; Niaudet, 2010). Potential living related kidney donors for patients with autosomal recessive Alport syndrome should be carefully evaluated for the presence of microhaematuria and microalbuminuria. Individuals with isolated microhaematuria might be eligible to donate but, again, they should be advised about the uncertainty of the long-term renal function (Niaudet, 2010).

Anti-glomerular basement membrane disease after renal transplantation

Most Alport patients who have undergone kidney transplantation have achieved satisfactory long-term results (Peten et al., 1990; Gobel et al., 1992; Byrne et al., 2002; Temme et al., 2012). Approximately 15% of the patients develop silent immunoglobulin G linear deposits along the glomerular basement membrane (GBM) of the grafted kidney, in the absence of detectable circulating anti-GBM antibodies, whereas such fixation is found in only 1–2% of other renal transplanted patients (Quérin et al., 1986; Byrne et al., 2002). A small number, maybe 2.5%, of Alport patients develop pathogenic levels of alloantibodies against the GBM of the renal allograft and anti-GBM nephritis (Ding et al., 1994; Kalluri et al., 1994; Turner and Rees, 1996; Jais et al., 2002; Kashtan, 2006). Its onset occurs mainly in the first year following transplantation. It leads to severe crescentic glomerulonephritis and subsequent graft loss in nearly 90% of the cases. If it occurs once, subsequent transplantion is risky as anti-GBM glomerulonephritis recurs, usually more aggressively in most but not all retransplanted patients (Browne et al., 2004). Alport anti-GBM disease is described further in Chapter 75.

Why is this alloimmunization such a rare event, and can it be predicted in X-linked forms? In the European study (Jais et al., 2000), only three of the 118 transplanted male patients with identified *COL4A5* mutations developed post-transplant anti-GBM glomerulonephritis. All three had a large deletion. The risk for these patients of developing this complication is 15%, which represents a sixfold increase compared to the total Alport syndrome population. However, 16 other patients with large *COL4A5* rearrangements and 32 with mutations expected to produce a truncated protein lacking the NC1 domain, did not develop anti-GBM

glomerulonephritis in the graft, showing that other factors must contribute to alloimmunization.

See also Chapter 75 on Alport post-transplant anti-GBM disease, and Chapters 71–74 on primary anti-GBM disease.

Possible future therapies

Several animal models of X-linked or autosomal recessive Alport syndrome have been observed in dogs, that mimic most of the clinical and immunohistological features of human Alport syndrome (reviewed by Kashtan, 2011). Along with knockout mouse models, they are useful to understand the processes leading to the development of progressive renal lesions and to test new therapeutic approaches (Ninichuk et al., 2005; Zeisberg et al., 2006; Koepke et al., 2007; Gross and Kashtan, 2009; Lebleu et al., 2009).

Therapies such as antiprotease, chemokine receptor blockers, and statin or stem cell delivery have been suggested from initial studies in animal models. Blocking simultaneously at least MMP2, MMP3, and MMP9 in *Col4a3*$^{-/-}$ mice delays the progression of the disease if treatment is given before development of GBM injury and occurrence of proteinuria in a C57BL6 genetic background (Zeisberg et al., 2006). In addition, either an MMP12 inhibitor or CCR2 receptor antagonist attenuates the GBM thickening in *Col4a3*$^{-/-}$ mice (Rao et al., 2006). It was also shown that chemokine receptor-1 blockade as well as statin treatment improves survival and renal lesions in Alport mice (Ninichuk et al., 2005).

Bone marrow transplantation of *Col4a3*$^{-/-}$ mice shows recruitment of bone marrow cells as future podocytes and mesangial cells, (very) partial restoration of the expression of the α3-α4-α5(IV) network, and clinical and histologic improvement (Floege et al., 2006; Prodromidi et al., 2006; Sugimoto et al., 2006). However, another study suggested that irradiation alone, which preceded bone marrow transplantation, may improve the survival of *Col4a3*$^{-/-}$ mice, through as yet unidentified mechanisms (Katayama et al., 2008). More recently, Sedrakyan et al. (2012) have shown that injection of amniotic fluid stem cells delays progression of renal fibrosis in Alport mice.

A recent study suggests that anti miR21 oligonucleotides prevent renal disease progression in mice (Gomez et al., 2014).

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CHAPTER 325

Thin glomerular basement membrane nephropathy and other collagenopathies

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Thin glomerular basement membranes and 'benign familial haematuria'

These are discussed together because of the significant overlap between them.

Benign familial haematuria (BFH) is defined by the familial occurrence of isolated persistent microscopic haematuria, without hearing defect and progression to renal failure in the proband and in his/her kindred. To confirm the benign character of the haematuric disease, additional criteria are necessary, including absence of gross haematuria, absence of proteinuria, and presence of adult male patients with isolated microhaematuria in the kindred. Familial investigation using simple tests may be rewarding (Blumenthal et al., 1988). Inheritance is compatible with autosomal dominant transmission, but apparently sporadic cases have been observed (Piel et al., 1982; Yoshikawa et al., 1984; Gauthier et al., 1989; Schröder et al., 1990).

Renal biopsy in families with these features shows various non-specific and minor glomerular changes in approximately half of the cases, and thinning of the glomerular basement membrane (GBM), as initially reported by Rogers et al. (1973) in the other half (Tina et al., 1982; Yoshikawa et al., 1984). Because of this last finding, thin GBM has been regarded as a marker of a benign disease, but it has to be pointed out that the lesion—thin GBM—is not specific and may be observed at an early stage of typical Alport syndrome progressing to end-stage kidney disease, especially in renal biopsies from young children. It is also characteristic of carrier status for Alport syndrome, and as described in clinical features of Alport syndrome (Chapters 322 and 323), some of whom do develop end-stage renal failure.

A thin GBM may also be seen in conjunction with other renal diagnoses. It is not known whether there are causative relationships, or whether this might be a coincidence of two conditions.

Great caution should be exercised in establishing the diagnosis of BFH, especially in young children; thinning of the GBM may be prominent, or even isolated, in patients with true Alport syndrome, and thickening and lamination excluding the diagnosis if present, may be limited to rare segments of the capillary wall (Gubler et al., 1981). The clinical diagnosis may also be difficult in kindreds where only adult women are affected within a kindred. It should be kept in mind that in families with X-linked Alport syndrome, carrier females may present only with microhaematuria.

It has been shown that some patients with BFH are heterozygous for a mutation in *COL4A3* or *COLA4* (Badenas et al., 2002). However, cases unlinked to this locus have been reported (Piccini et al., 1999) and a *COL4A5* mutation has been reported in a family with BFH (Kaneko et al. 2010). And, as indicated previously, mutations in *COL4A3* or *COL4A4* are associated with autosomal recessive and dominant Alport syndrome (Lemmink et al., 1994; Boye et al., 1998; Heidet et al., 2001).

Thin basement membrane nephropathy

The term thin basement membrane nephropathy (TBMN) has been coined to describe diffuse thinning of the GBM, but its definition is controversial. Normal GBM thickness varies with age and sex. In neonates, the mean thickness is about 100–150 nm, then it increases progressively to reach 200 nm at 1 year, 300 nm by 10 years, and between 320 and 395 nm in adults (according to the technique used) with mild sex differences. A GBM of 200 nm or less is generally accepted as 'thin' and between 200 and 250 is considered borderline.

As described above, TBMN does not correspond to a clear clinical entity and is not a guarantee of a benign disease (Gubler et al., 1990). It has been observed in patients with microhaematuria, either in isolation or associated with gross haematuria or substantial proteinuria, in patients with familial or non-familial disease, and in patients who progressed or not to renal failure (Dische et al., 1985; Tiebosch et al., 1989). Patients with typical Alport syndrome or carriers of the same mutations may present with thin GBM as the only lesion on electron microscopy.

Collagen type IV (α3-α4) nephropathy

Thus it appears now that heterozygote mutations affecting only one allele of *COL4A3* or *COL4A4* may be associated with diverse phenotypes from no symptoms at all or isolated microscopic

haematuria as seen in heterozygote carriers of autosomal recessive Alport syndrome to a more severe disease with possible progression to end-stage kidney disease in autosomal dominant Alport syndrome. Moreover as indicated below, *COL4A3* or *COL4A4* mutations in the heterozygous state are also involved in BFH. These conditions might be described under a heading of 'collagen type IV (α3-α4) nephropathy' (after Gubler, 2008; Torra et al. 2004).

HANAC syndrome

Hereditary angiopathy, nephropathy, aneurysms, and cramps syndrome is very rare and is associated with certain minor mutations in *COL4A1*, one of the COL4 isoforms that is universally expressed. The main phenotype is not renal but it is associated with haematuria probably of tubular origin. It is outlined in Chapter 320. The GBM is normal and lesions may be seen in tubular and vascular basement membranes

Nail patella syndrome

Nail patella syndrome is associated with gene mutations in the transcription factor *LMX1B* and further described in Chapter 326. Severe renal disease is rare but some patients are proteinuric and some do reach end-stage renal failure. The GBM has characteristic inclusions and it is reported that quantities of collagen IV 345 may be reduced (see Chapter 326).

Further reading

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CHAPTER 326

Nail patella syndrome

Laurence Heidet and Marie Claire Gubler

Introduction

Nail patella syndrome (NPS) or hereditary onycho-osteodysplasia (HOOD syndrome) (OMIM #161200) is an autosomal dominant disease first described by Little (1897), who reported on a family of 18 members in whom nails and patellae were absent. Since then, > 500 cases have been reported. The clinical spectrum of this pleiotropic disorder has been completed with the description of additional skeletal anomalies, of muscular, ocular, and possible hearing impairment, and moreover with the recognition of renal involvement as a feature of the disease (Carbonara and Alpert, 1964; Beals and Eckhardt, 1969).

The responsible gene was located on chromosome 9q34 by linkage to the ABO group locus and identified some 40 years later (Vollrath et al., 1998; Dreyer et al., 1998). This gene, *LMX1B*, encodes a LIM-homeodomain transcription factor that plays a fundamental role during development (Vogel et al., 1995; Chen et al., 1998). At this time, > 140 distinct mutations have been reported.

The disease is rare and its precise incidence, not well known, has been estimated to be 1/50,000.

Clinical expression of the disease

Dysmorphic features

Dysmorphic features including nail abnormalities and skeletal deformities are bilateral symmetrical, and characteristic (Carbonara and Alpert, 1964; Meyrier et al., 1990; Bongers et al., 2002).

Nail involvement is nearly constantly observed in 92–98% of patients, and present at birth. Lesions vary from total aplasia to hemi-anonychia, hypoplasia, longitudinal ridging, or splitting. The more discrete lesion, the triangular appearance of the lunulae, is pathognomonic. Lesions predominate on the hand, especially on the thumb and index finger. Toenails are less frequently involved.

Patella aplasia, or more frequently hypoplasia, is observed in 85–92% of patients. It may be asymptomatic or responsible for knee pain and disability, recurrent patella dislocation, and finally femoropatellar arthrosis. Hypoplasia of the femoral condyles may be associated.

Another frequent skeletal abnormality is elbow dysplasia described in 40–92% of patients leading to limitation of elbow movements and recurrent subluxation.

Iliac horns of various sizes, discovered by pelvic radiography in 70–80% of patients, are asymptomatic. Foot deformities, talipes equinovarus or equinovalgus, are also frequent in NPS patients.

Other extrarenal symptoms

Glaucoma, primary open-angle glaucoma, and ocular hypertension (Lichter et al., 1997; Vollrath et al., 1998) are detected in 30% of patients aged between 40 and 50 years and they are treatable, a reason for systematic ophthalmologic screening of NPS patients (Bongers et al., 2002; Sweeney et al., 2003). Various other ocular changes have been observed, including Lester's sign, consisting of abnormal pigmentation of the iris. The significance of gastrointestinal, neurological, and vasomotor symptoms observed in some patients remains to be evaluated. Mild bilateral sensorineural hearing impairment of the presbycusis type is frequently detected in NPS patients (Bongers et al., 2002).

Renal involvement

Renal involvement is not constant in patients with NPS. It occurs in approximately 40% of patients (Meyrier et al., 1990; Sweeney et al., 2003; Bongers et al., 2005), belonging to families with or without renal involvement (Looij et al. 1988). According to the comprehensive review of Lemley (2009), two patterns of renal involvement may be distinguished. In the majority of patients, renal disease is asymptomatic, mild proteinuria and/or haematuria are detected by systematic urinalysis and remain stable over years. However, age-related decline in creatinine clearance was greater than in controls. On the other hand, a small group of patients (5–10%) develop abundant proteinuria and nephrotic syndrome in childhood or early adulthood and progress often rapidly to end-stage renal disease. This severe evolution may affect the different members of a given family (Lee et al., 2009) or only one among others (Meyrier et al., 1990).

Pathology

Renal glomerular changes on light microscopy are not specific and are correlated with the severity of clinical findings: minimal glomerular changes in early specimens, segmental glomerular basement membrane (GBM) thickening, than more or less extensive focal and segmental glomerular sclerosis. Non-specific immunoglobulin M and C3 deposits are seen within segmental lesions. The characteristic lesion of the disease has been identified by electron microscopy. It consists of irregular GBM thickening enclosing electron lucent areas giving the GBM a 'moth-eaten appearance' alternating with normal GBM segments (del Pozo and Lapp, 1970). Using phosphotungstic or tannic acid staining, fibrillar material with the characteristic striation of interstitial collagen is observed within areas of rarefaction (Fig. 326.1) (Ben Bassat et al., 1971). It has been shown to be type III collagen (Heidet

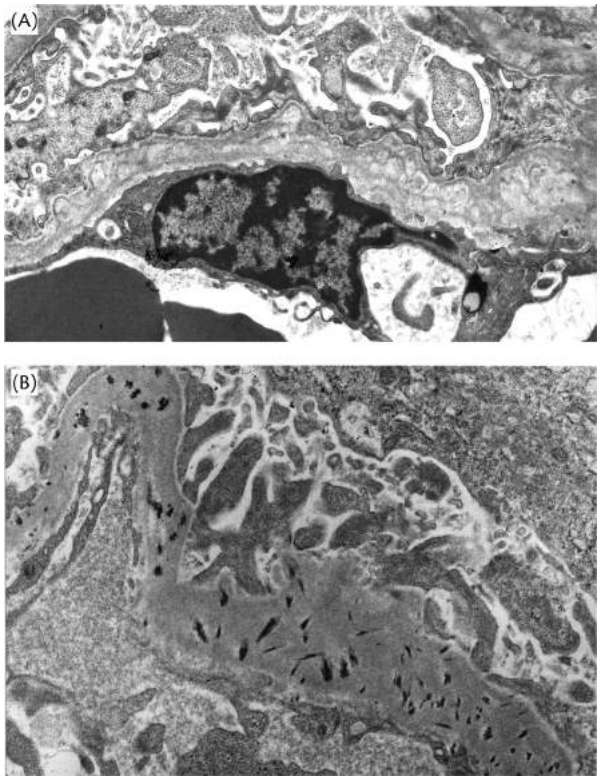

Fig. 326.1 GBM changes in nail patella syndrome. (A) Moth-eaten appearance of the GBM (uranyl acetate, lead citrate × 11,000). (B) Presence of dense fibrillar material within the thickness of the GBM detected by phosphotungstic staining (× 14000).

et al., 2003). The lesion appears to be constant, being observed in patients with or without renal symptoms (Hoyer et al., 1972; Bennett et al., 1973; Morita et al., 1973; Taguchi et al., 1988). Its extension is not correlated with the patient's age or the severity of clinical findings.

Genetics

Heterozygous mutations in *LMX1B* are responsible for NPS. This gene encodes a transcription factor involved in the dorsoventral patterning of limb tissue as shown by studies of limb development in chick embryos and analysis of *Lmx1b* knockout mice (Vogel et al., 1995; Chen et al., 1998). It is also strongly expressed in the kidneys, especially in the podocytes. *Lmx1b* knockout mice develop a severe glomerular disease leading to death on the day of birth (Chen et al., 1998; Suleiman et al., 2007). LMX1B has been shown to regulate the expression of the podocyte genes, *COL4A4* and *NPHS2*, that are no longer expressed in *Lmx1b* conventional knockout mice (reviewed in McIntosh et al., 2005; Witzgall, 2008), a possible explanation for their glomerular disease. However, this defect was not observed in NPS patients with heterozygous *LMX1B* mutation or in constitutive podocyte-specific *Lmx1b* knockout mice (Heidet et al., 2003; Suleiman et al., 2007).

All types of mutations distributed all over the gene have been identified in NPS patients and no correlation with the severity of NPS extrarenal symptoms has been reported. However, familial aggregation of clinically relevant nephropathy was noted, frequently associated, according to Bongers et al. (2005), with mutations located in the homeodomain of the gene, a segregation not observed by Lemley (2009). The role of modifying genes interacting with *LMX1B* and/or expressed in the podocyte could explain the variable severity of renal and extrarenal symptoms among patients and perhaps the more frequent occurrence of severe renal disease in some families (McIntosh et al., 2005; Suleiman et al., 2007; Lee et al., 2009).

Management

Regular screening for proteinuria is indicated in NPS patients with the aim of detecting the rare individuals with progressive renal disease. In this situation, renal biopsy may be indicated because other glomerulopathies may be superimposed to NPS lesions. No specific treatment is available but angiotensin-converting enzyme inhibition could be indicated in patients with increasing proteinuria (Lemley, 2009). No special complication has been reported after kidney transplantation.

Diagnosis

Diagnosis of NPS is based on the presence of the dysmorphic tetrad affecting the nails, patellae, elbows, and iliac bones and confirmed by the identification of the mutation. However, the diagnosis is frequently overlooked and delayed because of the rarity of the disease.

The presence of bundles of fibrillar collagen irregularly distributed within the thickness of the GBM is pathognomonic for the disease. However, another disease, collagen type III glomerulopathy, also named collagenofibrotic glomerulopathy or primary glomerular fibrosis, is also characterized by the presence of type III collagen in the extracellular matrix of the glomerulus (Salcedo, 1984; Ikeda et al., 1990; Imbasciati et al., 1991; Gubler et al., 1993; Vogt et al., 1995; Tamura et al., 1996; Duggal et al., 2012). The morphological pattern is quite different from the one in NPS, however: the fibrillar collagen is present within the mesangial matrix and the subendothelial aspect of the GBM adjacent to the normal lamina densa, leading to a diffuse and often massive increase in the mesangial matrix and generalized thickening of the capillary walls clearly visible by light microscopy. Moreover none of the patients exhibit the characteristic dysmorphic tetrad. This very rare pathologic entity has been observed in adults, mostly in Japan or in India, and in children presenting with early and progressive glomerular disease. Family cases suggesting an autosomal recessive inheritance have been reported in paediatric series (Salcedo, 1984; Gubler et al., 1993; Tamura et al., 1996).

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CHAPTER 327

Molecular basis of nephrotic syndrome

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Introduction

Molecular genetics have revealed the podocyte as the central player in the control of glomerular filtration of protein. More specifically the cell–cell junction between adjacent podocyte foot processes, the slit diaphragm, has been revealed to be made up of a sophisticated multiprotein complex which dynamically controls foot process architecture via signalling to the actin cytoskeleton. Key genes have been identified from the study of inherited nephrotic syndromes, such that it is now possible to identify genetic causes underlying a proportion of nephrotic syndromes presenting at any age, but particularly in childhood.

Nephrotic syndrome is described in Chapter 52. It is the most common presentation of glomerular disease in childhood with an incidence in an unselected cohort of children approximately 2/100,000.

The nature of the condition and the proportion of familial forms have led to much work on the genetics of nephrotic syndrome with a resultant explosion in the knowledge of genes involved. This in turn has helped identify the underlying cellular mechanisms of the glomerular filtration barrier (GFB), principally of the podocyte, whose structure and function are key to maintaining the slit diaphragm membrane.

Podocyte physiology

The three layers of the GFB (Fig. 327.1) consist of the fenestrated capillary endothelial cells, the glomerular basement membrane, and the highly specialized epithelial cell—the podocyte. The podocyte's interdigitating foot processes connect to form a slit diaphragm membrane which is a dynamic structure controlling the ultrafiltration of molecules by its signalling to the cytoskeleton of the podocyte. Through landmark genetic advances, the biology of the slit diaphragm has been partially unveiled.

Nephrotic syndrome type 1 (*NPHS1*) coding for nephrin was the first gene identified in hereditary SRNS in 1998, and these gene mutations can present with the worst phenotype. This transmembrane protein is a putative member of the immunoglobulin superfamily which appears to be at the heart of the working slit diaphragm, integral to the podocyte functioning. An extracellular component links to NEPH1 and homodimerizes nephrin molecules to form the zipper-like structure of the slit diaphragm and the intracellular component associates with lipid rafts and appears key in regulating signalling pathways in the podocyte (Kestila et al., 1998; Welsh and Saleem, 2010). *NPHS2* coding for podocin, described in 2000 (Boute et al., 2000), is a member of the stomatin protein family and is essential in recruiting nephrin (and CD2AP) to these lipid rafts and enhancing nephrin signalling (Boute et al., 2000).

Further important actin-related proteins include CD2AP and α-actinin-4. Mice with a homozygous knockout of CD2AP were developed to investigate T-cell function (it is also present in T-cell membranes) but surprisingly were noted to die *in utero* of renal failure with histology comparable to diffuse mesangial sclerosis. Heterozygous mutations resulted in mice developing changes consistent with FSGS at 9 months of age (Shih et al., 1999). CD2AP has been subsequently found to interact with nephrin and podocin at the slit diaphragm whilst also communicating directly with F-actin within the actin cytoskeleton (Kim et al., 2003). Interestingly, human mutations have not been as clear cut with histological changes of FSGS (Gigante et al., 2009). α-Actinin 4 is an actin-binding protein involved in the maintenance of the actin cytoskeleton, and mutations have been described in adult-onset autosomal dominant FSGS (Kaplan et al., 2000), with distinct ultrastructural characteristics on biopsy which may aid in histological diagnosis (Hendersonet al., 2009). TRPC6 is a cation channel which localizes in the podocyte proximal to the slit diaphragm and interacts with nephrin and podocin. It has also been described as causing an autosomal dominant, late-onset FSGS (Winn et al., 1999). Interestingly, some of the mutations so far described are gain of function, suggesting an influx of calcium into the podocyte cell disrupting signalling (Reiser et al., 2005).

There are further proteins that have been identified whose roles in regulating the podocyte slit diaphragm are less clear, and may affect other cellular functions. The Wilms tumour suppressor gene, *WT1* (see Chapter 329), is a transcription factor located in the nucleus of podocytes. Although originally described for its role in the development of Wilms tumours it has subsequently been described as important in infantile- and childhood-onset nephrotic syndrome often with other clinical features (Haber et al., 1990; Aucella et al., 2006). Mutations have been described in phospholipase C epsilon 1 (*PLCE1*), which belongs to the phospholipase C family involved in intracellular signalling, although its role in the podocyte is not fully clarified (Hinkes et al., 2006).

Phenotype

A list of genes and the proteins they code for that are recognized as causative in nephrotic syndrome are listed in Table 327.1.

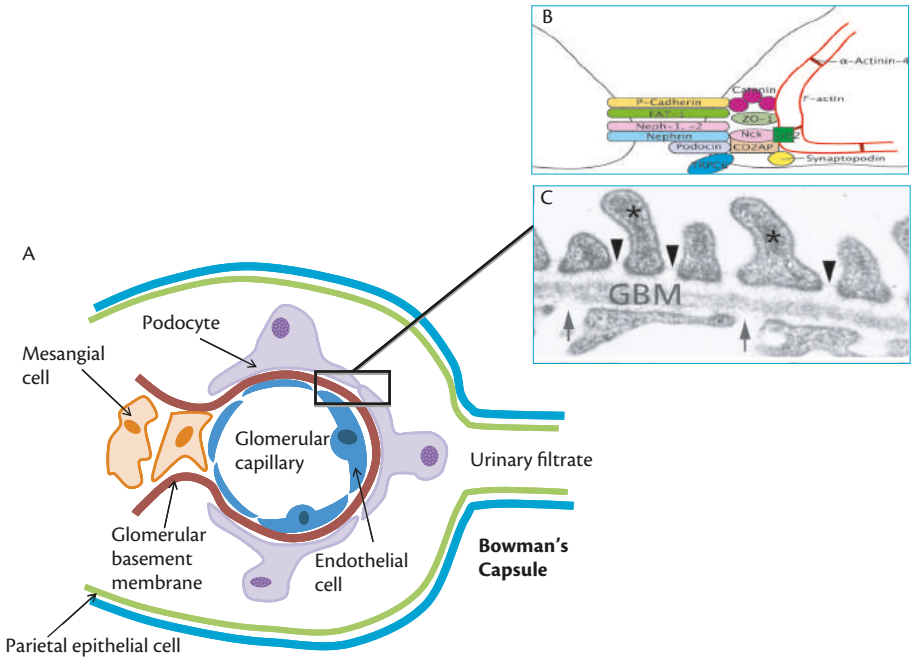

Fig. 327.1 A: Schematic diagram of a glomerular capillary, and its constituent cells. B: Expanded view of the slit diaphragm protein complex, with some key slit diaphragm molecules and their links to the actin cytoskeleton demonstrated. C: Electron microscopy image of the filtration barrier; thin arrows indicate endothelial cell fenestrations, thick arrows indicate the podocyte slit diaphragm.

Congenital and infantile nephrotic syndrome

Of the nephrotic syndromes that present in the first year of life, the congenital (0–3 months) and infantile (3–12 months) forms are heterogeneous, but it is in these forms that gene mutations are most common. In a large European cohort of 89 children from 80 families, 66.3% of patients presenting within the first year of life were found to carry a mutation in one of four genes: *NPHS1, NPHS2, WT1*, and laminin beta 2 (*LAMB2*). Of these, *NPHS1* mutations were found exclusively in the cohort presenting within 3 months (except for one with a heterozygous mutation) and *NPHS2* mutations predominated in those aged > 4 months (Hinkes et al., 2007).

NPHS1

Nephrin, encoded by *NPHS1*, is probably the most important component of the slit diaphragm and is responsible for the autosomal recessive condition, congenital nephrotic syndrome of the Finnish type. Most common in Finland (incidence of 1/10,000), it often presents *in utero* with fetal growth retardation, polyhydramnios, and massive proteinuria at birth. There is a high mortality. Infants often require daily albumin infusions and parenteral nutrition until they can undergo renal transplantation, if possible.

Patients with nephrin mutations display the earliest and most severe clinical phenotype, compared with mutations in other genes, indicating an essential role for this protein in intact filtration. Commonest mutations in Finland are the Fin major (a frameshift mutation in exon 2 resulting in a truncated protein) and Fin minor (a nonsense mutation in exon 26 also resulting in a truncated protein) (Kestila et al., 1998). Many different mutations have been discovered throughout this gene, causing an almost invariably severe clinical phenotype, with onset of life-threatening proteinuria *in utero* or within the first 3 months of life (Patrakka et al., 2000). Disruption of function of almost any domain of the molecule has severe consequences.

As well as acting as an adhesion molecule, nephrin is a regulator of podocyte intracellular signalling. Effects include stimulation of members of the mitogen-activated protein (MAP) kinase family, and activation of the phosphoinositide 3-OH kinase/protein kinase B pathway (Huber et al., 2003).

This signalling role leads to regulation the actin cytoskeleton (the dynamic scaffold for podocyte foot processes), for example, through an interaction with the Nck subfamily of adaptor proteins (Jones et al., 2006; Verma et al., 2006). The Nck adaptor proteins contain one SH2 domain, which can bind phosphorylated tyrosine, and three SH3 domains which through binding to proline rich motifs in downstream effectors, such as N-WASP and p21-activated kinase (PAK) serine/threonine kinase, can regulate actin dynamics.

NPHS2

Podocin, encoded by *NPHS2*, was identified in 2000 in familial childhood FSGS. It was originally thought to cause FSGS in older children (Boute et al., 2000), but in the European cohort of infants presenting in the first year of life described above, 39% of those with congenital nephrotic syndrome and 35% of those presenting from 4 to 12 months had mutations in *NPHS2* (Hinkes et al., 2007). It accounted for 56% of mutations found in the whole cohort. In the original description, one particular mutation (R138Q) was found in 1/3 of all patients with a mutation (Boute et al., 2000). Similarly, in the early years study, this mutation was common. Of the 30 families, 12 were homozygous for this mutation and eight were compound heterozygous (Kestila et al., 1998). The incidence of *NPHS2* mutations in African American children with FSGS appears much lower. This group has a higher incidence of FSGS overall with a poorer outcome (Hinkes et al., 2008).

Table 327.1 Detailing the common mutations associated with *NPHS1*, *NPHS2*, and *WT1* and the resultant phenotype

Function	Protein	Gene	Syndrome	Mode of inheritance	Penetrance	Histology
Slit diaphragm protein	Nephrin	*NPHS1*	Congenital nephrotic syndrome	AR	Complete	Microcystic dilatation of tubules and progressive mesangial sclerosis
			SRNS	AR	Complete	MPGN, MCD, FSGS
	Podocin	*NPHS2*	Congenital nephrotic syndrome	AR	complete	
			SRNS	AR	Complete (although R229Q mutation has variable penetrance)	FSGS
	PLCE1	*NPHS3*	DMS	AR	probably incomplete	DMS
			SRNS	AR	probably incomplete	FSGS
	CD2AP	*CD2AP*	SRNS	AD/AR	probably incomplete	FSGS
SD ion channel	TRPC6	*TRPC6*	SRNS	AD	Complete	FSGS
Developmental	WT1	*WT1*	Denys–Drash syndrome	AD	Complete	DMS
			Frasier	AD	Complete	FSGS
			Isolated SRNS	AR	Complete	DMS, FSGS
Actin regulating	α-Actinin 4	*ACTN4*	Adult onset SRNS	AD	Complete	FSGS
	NMMHC-A	*MYH9*	Increased propensity to FSGS		Incomplete	FSGS
	Inverted formin 2	*INF2*	SRNS	AD	Complete	FSGS
Glomerular basement membrane	Laminin β2	*LAMB2*	Pierson's syndrome	AR	Complete	DMS, FSGS
Others	tRNA-LEU	*MTTL1*	Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke like episodes (MELAS)	Mat	Incomplete	FSGS
	Parahydroxybenzoate-polyprenyltransferase	*COQ2*	CoQ10 deficiency	AR	Incomplete	Collapsing glomerulopathy
	Prenyl diphosphate synthase subunit 2	*PDSS2*	CoQ10 deficiency/Leigh syndrome	AR	Incomplete	FSGS
	SMARCA like protein	*SMARCL1*	Schimke immuno-osseus dysplasia	AR	Complete	FSGS
	Lysosomal integral membrane protein type 2	*SCARB2*	Action myoclonus-renal failure syndrome	AR	Complete	
	LIM homeobox transcription factor 1β	*LMX1B*	Nail patella syndrome	AD	Incomplete	FSGS
	Zinc metallo-proteinase STE24	*ZMPSTE24*	Mandibuloacral dysplasia	AR		
	?	*GMS1*	Galloway–Mowat syndrome	AR	Complete	DMS, FSGS
	Phosphomannomutase 2	*PMM2*	Congenital defects of glycosylation	AR		Collapsing glomerulopathy
	β-1, 4-Mannosyltransferase	*ALG1*	Congenital defects of glycosylation	AR		FSGS

AD = autosomal dominant, AR = autosomal recessive, Mat = maternal (mitochondrial); DMS = diffuse mesangial sclerosis; FSGS = focal segmental glomerulosclerosis; MCD = minimal change disease; MPGN = membranoproliferative glomerulonephritis.

Podocin also appears to also be involved in the intracellular trafficking of nephrin since mutations in this protein result in the mislocalization of nephrin to the endoplasmic reticulum instead of the plasma membrane (Huber et al., 2003). Proper localization of nephrin within these specialized lipid microdomains at the plasma membrane is known to be essential for correct slit diaphragm signalling (Schermer and Benzing, 2009).

This is supported by the report of a patient with a digenic inheritance of *NPHS1* and *NPHS2* mutations, which resulted in a triallelic hit, and appeared to modify the phenotype from Finnish type, congenital nephrotic syndrome to one of congenital FSGS. The authors proposed an overlap in the *NPHS1*/*NPHS2* mutation spectrum.

WT1

WT1 (see also Chaper 329) is a podocyte transcription factor that was identified in 1990. It was originally associated with Wilms tumours and the WAGR (Wilms tumour, Aniridia, Genito-urinary malformation and Mental retardation) collection. Again, originally thought to represent a small cohort of patients, there are now several phenotypes identified. A *WT1* mutation in the zinc finger (DNA binding) region causes the Denys–Drash syndrome, which comprises diffuse mesangial sclerosis (DMS) and extrarenal manifestations (pseudohermaphroditism and Wilms tumour), though occasionally it can present as an isolated form, with just the renal phenotype (Niaudet and Gubler, 2006). The renal phenotype is usually severe and early onset with non-responsiveness to immunosuppression.

NPHS3 (PLCE1)

Hinkes et al. (2006) described truncating mutations in *PLCE1*, leading to isolated (i.e. non-syndromic) DMS and missense non-truncating mutations leading to FSGS. Increasingly recognized as a mutation resulting in isolated DMS with rapid progression to end-stage renal failure and a poor outcome generally, there are features which differ from the other hereditary forms of nephrotic syndrome (Gbadegesin et al., 2008). The initial study described two out of 14 children reported who responded to immunosuppression (one on steroids, one on ciclosporin). They both presented within the first year of life and both had siblings affected who had progressed to end stage rapidly. The authors promoted the idea that a *PLCE1* mutation in the absence of mutations in any of the other known genes warranted a trial of immunosuppression. Interestingly, a further report of a family with DMS and a recognized *PLCE1* truncating mutation identified an affected sibling pair whose father had the same homozygous mutation but was not symptomatic. Thus, the penetrance of the disease can be highly variable, and other gene modifiers are likely to play a part. Hinkes (2008) postulated that for those children who responded to therapy, the treatment activated another PLC family member to take over. The protein coded for appears to be important as a secondary messenger at the slit diaphragm, and may play a role in ion channel regulation.

Childhood-onset steroid-resistant nephrotic syndrome

The predominant mutation found in children presenting over the age of 1 year, is in *NPHS2*, with a significantly smaller proportion having *WT1* or *NPHS1* mutations.

NPHS2

In a cohort of 430 patients from 404 families (aged 0–21) screened for podocin mutations, the R138Q mutation was again the most common found, present in 57% of those families with two mutations. There is also a genotype–phenotype correlation with homozygous mutations or compound heterozygous with a truncating mutation having a mean age of onset of 1.7 years compared to heterozygous R138Q and a missense mutation with a mean age of onset of 5.9 years. Homozygous mutations in the R229Q mutation are likely to represent a polymorphism, although reported in two patients with childhood-onset SRNS (Hinkes et al., 2008). Podocin exists as a dimer or heterodimer, and now it has been discovered that R229Q is only pathogenic when in combination with very specific NPHS2 variants on the second allele (Tory et al., 2014). This is because the R229Q variant causes a structural change to alter heterodimerization with a trans variant in exon 7 or 8 (C-terminal), and hence mislocalize the R229Q podocin protein in the podocyte.

NPHS1

In a European cohort with childhood-onset SRNS in whom mutational analysis for *NPHS2* had been negative, 11 children were found to have *NPHS1* mutations. Of these, nine had sporadic disease and two were siblings, and age of onset of proteinuria ranged from infancy to 8 years (Philippe et al., 2008). Furthermore, in a similar study of 97 patients of all ages from 87 families, 13 patients were identified with *NPHS1* homozygous or compound heterozygous mutations. Of these, eight presented within the first 3 months of life (five displaying the histology of congenital nephrotic syndrome, one DMS, and two FSGS). Four presented during early childhood and one during adulthood (Santin et al., 2009). The latter cases displayed FSGS on their histology. The numbers are small but show the diversity of phenotype associated with nephrin mutations when it is looked for.

WT1

Mutations that cause abnormal splicing of *WT1* result in FSGS with a variable onset from infancy to adolescence, and also cause pseudohermaphroditism (female phenotype, male genotype) (see also Chaper 329). Interestingly, several large-scale studies looking at prevalence in cohorts of children with sporadic SRNS have shown an overall prevalence of *WT1* mutations of approximately 6% but in females with no other phenotypic features up to 12% (Ruf et al., 2004; Aucella et al., 2006; Mucha et al., 2006). Typically, the disease is severe with rapid progression to end stage with no response to therapy. However, there are now case reports of possible response to intensive, prolonged, ciclosporin therapy (Gellermann et al., 2010).

Adult-onset steroid-resistant nephrotic syndrome

Whereas childhood-onset genetic SRNS is usually autosomal recessive inheritance, genes accounting for predominantly juvenile- and adult-onset SRNS are usually autosomal dominant. Of the genes most commonly implicated, recent studies have found 12% overall (non-familial) cases had pathogenic *NPHS2* mutations (Santin et al., 2011), 6% had *TRPC6* mutations (Santin

et al., 2009), and 4% of familial cases had *ACTN4* mutations (Weins et al., 2005)

ACTN4

The commonly recognized, although still rare, genes involved in SRNS with adult onset include *ACTN4* which codes for one of the four alpha-actinins in humans. It is an actin-binding and cross-linking protein integral to the podocyte cytoskeletal structure. Originally identified in 1998 in two pedigrees with an autosomal dominant FSGS, the original case reports suggest missense mutations causing an adolescent or young adult onset with slow progression to end-stage renal failure in later life (Kaplan et al., 2000). However, there are now case reports describing mutations resulting in childhood-onset disease (Weins et al., 2005; Choi et al., 2008).

TRPC6

Similarly, *TRPC6* was first described in a large pedigree in New Zealand of British ancestry, again causing an autosomal dominant FSGS (Winn et al., 2005). Youngest onset was 17 years of age although generally presentation was in the third or fourth decade. Unlike the *ACTN4* mutations, the disease progresses rapidly to end-stage renal failure within 10 years. The original pedigree had a missense mutation which causes a gain of function, resulting in enhanced calcium entry into the cell. More mutations have since been described of which several cause a gain of function; interestingly, the most recent case reports include two with childhood onset (Heeringa et al., 2009; Santin et al., 2009). The first appears to cause the largest increase in calcium influx and the most aggressive phenotype with a childhood onset (Heeringa et al., 2009). The second describes a milder phenotype which is partially responsive to therapy (Santin et al., 2009).

CD2AP

Given the animal findings of *CD2AP* mutations, there was an expectation that there would be a significant cohort of patients with mutations. However, there have been only a very limited number of case reports and phenotype is variable (Kim et al., 2003; Lowik et al., 2007; Gigante et al., 2009). The first description was in adults with a heterozygous mutation in exon 7, but the next was a homozygous mutation in exon 18 in a child with infantile onset and rapid progression to end stage (Kim et al., 2003; Lowik et al., 2007). A further study in Italy described three more mutations with age of onset ranging from 2 to 23 years (Gigante et al., 2009). Interestingly, a young sibling of the patient with adult onset shared the same mutation but had no symptoms and a mother of one affected child shared the same mutation but was again asymptomatic, but the first sibling may have been too young to express a phenotype and in the second case, a *NPHS1* polymorphism was identified in the affected child (but not the mother) which, the author's postulate, may have had a modifying role. Importantly all these affected patients present a histology of FSGS, rather than DMS which is the phenotype of the mouse *Cd2ap* knockout. It remains possible that patients with isolated DMS will be found with CD2AP mutations.

NPHS1 and NPHS2

As previously mentioned, there is one case in the literature of a patient with adult-onset FSGS in whom compound heterozygous nephrin mutations (one mild and one non-sense) were identified, but this combination has also been described in an infant with nephrotic syndrome (Santin et al., 2009).

To a greater degree, mutations in podocin have been recognized as causative in adult-onset FSGS. In a large cohort of adults and children with familial and sporadic nephrotic syndrome, the R229Q mutation was compound heterozygous with a known pathogenic mutation in 36 cases from 27 families with a median age of onset of 19 years. Those cases with a homozygous R229Q mutation showed a variable penetrance (Machuca et al., 2009).

Other genes

More recently, *INF2* (inverted formin 2) has been described. It is a member of the formin family of actin-regulating proteins that can accelerate actin polymerization. Mutations were described in 11 families with an autosomal dominant FSGS with variable age of onset (11–72 years) (Brown et al., 2010). In autosomal dominant SRNS, this has been found to have a high prevalence of 17%, with a median age of presentation of 27.0 years (Boyer et al., 2011). Disease mechanism is not yet known, although INF2 is known to interact with IQGAP (a nephrin interactor), so dysfunction or dysregulation of the actin cytoskeleton via slit diaphragm signalling appears likely.

Importantly, the predilection in African American adults for idiopathic FSGS and that associated with HIV can be explained in large part by variants of the *APOL1* gene (see Chapter 341).

Further genes are being discovered month by month, and a fascinating area of dual- (or greater) gene contribution to phenotypes is developing.

Mitochondrial and other rare syndromes

Mitochondrial cytopathies

Mitochondrial cytopathies (see Chapter 340) are a rare cause of nephrotic syndrome. However, due to the coexistence of mutated and wild type mitochondrial DNA and the apparent non-uniformity of distribution, the phenotype can be varied.

Mitochondrial diseases are discussed in detail in Chapter 341, but mutations that cause MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke like episodes) and CoQ10 (coenzyme Q10) deficiency, can also cause FSGS in isolation (Yamagata et al., 2002; Diomedi-Camassei et al., 2007). In the former, the renal phenotype ranges from mild to severe and progression to end-stage renal failure. In the latter, two genes are recognized: *COQ2* encodes a protein involved in the biosynthetic pathway—and may cause isolated nephropathy (collapsing glomerulopathy or crescentic glomerulosclerosis) or with an encephalopathy; *PDSS2* codes for the first enzyme in the pathway and has been described in a case of severe Leigh's syndrome with seizures, hypotonia, cortical blindness, and nephrosis (Lopez et al., 2006; Diomedi-Camassei et al., 2007). There is a case report of early administration of CoQ10 restoring renal function in an infant newly diagnosed as CoQ10 deficient with a *COQ2* mutation (Montini et al., 2008).

Syndromic nephrotic syndrome

Mutations in the *LAMB2* gene (see Chapter 320) are responsible for Pierson syndrome (microcoria and congenital nephrotic syndrome) and *LAMB2* is one of the four common genes involved in

early-onset nephrotic syndrome described above (Hinkes et al., 2007). There appears to be a genotype—phenotype correlation with ocular changes either absent or minor in those with missense or non-truncating mutations compared to the microcoria associated with truncating mutations (Hasselbacher etal., 2006).

Alport syndrome (see Chapter 322) may present with nephrotic syndrome on occasion.

Schimke immuno-osseous dysplasia is an autosomal recessive condition characterized by spondyloepiphyseal dysplasia, T-cell immunodeficiency, and FSGS. The affected gene, *SMARCAL1*, codes for an actin-dependent regulator of chromatin involved in DNA remodelling after replication and the nephrosis can be severe progressing to end-stage renal failure (Elizondo et al., 2009; Baradaran-Heravi et al., 2013).

Another autosomal recessive condition, action myoclonus-renal failure syndrome is characterized by progressive myoclonus epilepsy and renal failure due to FSGS. It is caused by mutations in the *SCARB2* gene which codes for lysosomal integral membrane protein type 2 and can be considered a lysosomal storage disorder (Berkovic et al., 2008).

Nail patella syndrome (see Chapter 326), caused by mutations in *LMX1B*, (a transcription factor involved in podocyte maturation) describes an autosomal dominant disorder with dysplastic nails, hypoplastic or absent patella, and variable nephropathies including FSGS (Sweeney et al., 2003).

Genetic screening

In childhood-onset SRNS, the commonest mutations to date are found in genes encoding nephrin, podocin, and WT1 (Table 327.2), and these should be screened for in order to direct clinical management and to give genetic advice. In adults with FSGS, particularly if there is a family history, mutations in *NPHS2, ACTN4, INF2*, or *TRPC6* should be sought, the latter three being autosomal dominant.

With recent advances in sequencing technologies, the advent of next-generation sequencing will shortly allow us to screen for all known genes in a single test (McCarthy et al., 2013). This has the potential to make early clinical decisions regarding immunosuppression therapy, and it will be important to continue to monitor genotype–phenotype correlations to help guide specific management.

Advancing the biology

Despite the important aspects of genetic diagnosis, antenatal screening, and immunosuppressive management for patients identified with monogenic disorders, the greatest burden of nephrotic disease lies in the idiopathic group. Therefore a crucial aspect of gaining knowledge from the affected genes is to use this information to understand the biology of the GFB. In this regard, advances in the knowledge of podocyte biology have escalated hugely in the last 10 years, with the promise of identifying specific targeted therapies in the near future. These have been based predominantly on the study of the molecules identified from genetic studies in humans, and how their behaviour might change in disease models. For example, exposure of podocytes to plasma from patients with SRNS relapse causes cytoplasmic relocalization of nephrin, podocin, and CD2AP (Coward et al., 2005), and also causes enhanced activity of TRPC6 in podocytes (unpublished data). All of these responses are nephrin dependent, emphasizing the key role of this protein in directing podocyte behaviour (Welsh and Saleem, 2010). Further insights into signalling and actin-regulating pathways activated by disease plasma will elucidate unique mechanisms which may be therapeutically manipulated, as well as providing a tool to explore plasma fractions which contain disease causing activity.

Table 327.2 Detailing the common mutations associated with *NPHS1, NPHS2,* and *WT1* and the resultant phenotype

Gene/protein (locus and number of exons)	Common Mutation	Associated Phenotype	Age of onset	Reference
NPHS1/nephrin (19q13.1, 29 exons)	Fin-major (a two- nucleotide deletion in exon 2 resulting in a frameshift mutation & truncated protein)	Congenital nephrotic syndrome of the Finnish type (severe nephrosis)	Prenatally	Kestila et al. (1998)
	Fin-minor (R1109X—a nonsense mutation in exon 26)	Congenital nephrotic syndrome of the Finnish type (severe nephrosis)	Early postnatal period	
NPHS2/podocin (1q25–q31, 8 exons)	R138Q—a missense mutation in exon 3	A homozygous mutation or compound heterozygous with a truncating mutation results in early-onset FSGS	Median age 1.7 years	
		A compound heterozygous mutation with a missense mutation results in FSGS	Median age 5.9 years	Hinkes et al. (2007)
	R229Q— a missense mutation in exon 5	Variable penetrance in the homozygous form but heterozygous with another known mutation results in FSGS	Late adolescence/ adulthood	Machuca et al. (2006)
WT1/Wilms tumour (1q13, 10 exons)	Missense mutations in exon 8 & 9	Diffuse mesangial sclerosis ± Denys Drash syndrome	Within first year of life	
	Spice site mutations at IVS9 in exon 9	Frasier syndrome or isolated FSGS	Variable throughout childhood	Aucella et al. (2006)

FSGS = focal segmental glomerulosclerosis.

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CHAPTER 328

Molecular basis of renal tumour syndromes

Thomas Connor and Patrick H. Maxwell

Introduction

Kidney cancer is not a single disease, but comprises a number of histological subtypes. The Heidelberg classification system recognizes five subtypes of malignant renal parenchymal neoplasm (Table 328.1). Cancers of the renal parenchyma are also known as renal cell carcinomas (RCCs). Tumours in the renal pelvis consist mainly of transitional cell carcinomas (TCCs).

Much of our understanding of the molecular basis of renal cancer has come from the study of a number of familial renal cancer syndromes (Table 328.1). Inherited RCC is characterized by an earlier age at diagnosis than in sporadic cases, and it is often multicentric or bilateral. Although only 2–3% of all cases of RCC are familial (Maher, 2011), their identification is important so that they can receive surveillance for further tumours, and to allow screening of their relatives (Ricketts et al., 2008). The presence of characteristic tumours or extrarenal signs may point to a diagnosis of a specific RCC susceptibility syndrome.

Table 328.1 illustrates the seven genes that have been implicated in familial renal carcinoma. These genes are all implicated in two interrelated metabolic pathways. The most common cause of inherited RCC is von Hippel–Lindau (VHL) disease, which is caused by mutations in the *VHL* tumour-suppressor gene and which is discussed in Chapter 332. *VHL*, fumarate hydratase (*FH*), and succinate dehydrogenase subunit B (*SDHB*) gene products all impact on the cellular response to changes in oxygen tension which is discussed in more detail in Chapter 331 but summarised in Fig. 328.1. *FLCN, MET, TSC1*, and *TSC2* are all implicated in the activity of the energy-sensing pathway that is centred on the mammalian target of rapamycin (mTOR) (Fig. 328.2).

Birt–Hogg–Dubé syndrome

Birt–Hogg–Dubé (BHD) syndrome (OMIM #135150) is an autosomal dominant condition characterized by cutaneous fibrofolliculomas, pulmonary cysts, spontaneous pneumothorax, and renal cancer (Menko et al., 2009). In 2001, a BHD-associated gene locus was localized to chromosome 17p11.2, and subsequently truncating germline mutations were identified in a novel gene, the *FLCN* (*BHD*) gene (Nickerson et al., 2002).

BHD is probably under-diagnosed because of the wide variation in clinical presentation. Skin lesions (follicular hamartomas) are the most common manifestation, affecting 75% of patients and usually appearing in the third decade as whitish papules on the face and neck. Similarly, 80% of adult BHD patients have multiple lung cysts on computed tomography, but the lung parenchyma generally appears normal and lung function is usually unaffected (Toro et al., 2008). The main problem associated with these cysts is a 50-fold increased risk of pneumothorax, with 24% prevalence of pneumothorax and a median age of 38 years (Toro et al., 2007, 2008). In some families, cystic lung disease or pneumothorax can be the only manifestation of BHD.

A quarter of patients with BHD develop RCC, which presents at an early age (mean age at diagnosis 50 years) (Toro et al., 2008; Menko et al., 2009). Chromophobe RCC and mixed chromophobe and oncocytic tumours are typical for BHD, although other histological subtypes can occur, including clear cell and papillary RCC. Somatic second hit mutations have been identified in BHD-associated renal tumours, consistent with a tumour-suppressor function; however, these were not seen in the skin tumours. BHD has also been reported in association with a range of tumours other than RCC; however, a causal relationship has not yet been proven (Toro et al., 2008).

The *FLCN* gene codes for a protein of unknown function called folliculin (Nickerson et al., 2002). A germline mutation in *FLCN* was found in 84% of BHD families, and a further 4.3% of inherited RCC probands without evidence of a syndromic cause, were found to have pathogenic *FLCN* mutations (Menko et al., 2009). To date, most reported *FLCN* mutations are truncating (European Birt-Hogg-Dube Consortium, 2011). There is a mutation hot spot in a hypermutable 8-cytosine tract in exon 11 (Nickerson et al., 2002). No clear genotype–phenotype correlations have been described.

The energy-sensing mTOR pathway has been implicated in the pathogenesis of several hereditary hamartoma syndromes, including BHD (Menko et al., 2009). FLCN has been shown to interact with 5′ AMP-activated protein kinase (AMPK) via two FLCN-interacting proteins, FNIP1 and FNIP2. BHD knockout mice, exhibit renal cysts and tumours, and show activation of mTOR complex (mTORC)-1 and -2 (Chen et al., 2008). Treatment with mTOR inhibitors reduces the renal pathology (Chen et al., 2008). There is an overlap between the clinical features of BHD and the tuberous sclerosis complex (see below and Chapter 330); however, the precise role of folliculin in the mTOR pathway requires further elucidation. Moreover it seems likely that folliculin has other important functions, such as modulating transforming growth factor beta signalling.

Table 328.1 Classification of renal cell tumours

Subtype	Percentage	Syndrome	Mutation
Clear cell	75–80%	Von Hippel–Lindau disease	*VHL* (loss of function)
		Familial paraganglioma	*SDHB* (loss of function)
Papillary type I	10–15% (types 1 and 2)	Hereditary papillary renal carcinoma	*MET* (activating)
Papillary type II		Hereditary leiomyomatosis and renal cancer	*FH* (loss of function)
Chromophobe +/– oncocytic	5%	Birt–Hogg–Dubé syndrome	*FLCN* (loss of function)
Angiomyolipoma	< 1%	Tuberous sclerosis complex	*TSC1* and *TSC2* (loss of function)
Collecting duct	< 1%	—	—

Hereditary papillary renal carcinoma

Hereditary papillary renal carcinoma (HPRC) is a dominantly inherited familial cancer syndrome characterized by a predisposition to develop multiple, bilateral papillary renal tumours (OMIM #605074). Linkage analysis in 1997 showed that this condition was caused by activating mutations in the c-*MET* proto-oncogene on 7q34 (Schmidt et al., 1997). The renal tumours in HPRC have a distinct histological appearance, characterized as type 1 papillary, and have a better prognosis than type 2 papillary RCC (Maher, 2011). Ninety-five per cent of sporadic tumours with this appearance show trisomy of chromosome 7, which includes both the *MET* and hepatocyte growth factor (*HGF*) genes.

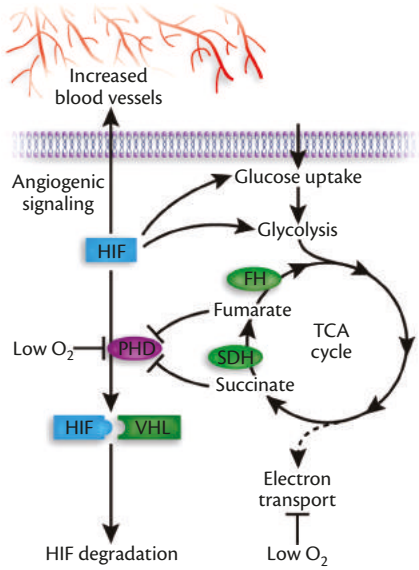

Fig. 328.1 The oxygen-sensing pathway and kidney cancer. In normal cells, the transcription factor hypoxia-inducible factor (HIF) is targeted for degradation in a manner dependent on prolyl hydroxylase (PHD) and von Hippel–Lindau protein (VHL) protein. When oxygen levels fall, PHD is inhibited and HIF is stabilized. Fumarate hydratase (FH) and succinate hydrogenase (SDH) are enzymes of the tricarboxylic acid (TCA) cycle. Loss-of-function mutations in FH and SDH lead to inherited cancer syndromes. Recent work shows that these mutations lead to increased levels of fumarate and succinate, which inhibit PHD and stabilize HIF. Activation of HIF changes gene expression, causing increases in angiogenesis, glucose uptake, and glycolysis.

MET encodes the cell surface receptor for HGF, which is involved in motility, proliferation, survival, and morphogenesis (Peruzzi and Bottaro, 2006). Germline mutations in HPRC affect the intracellular tyrosine kinase domain, analogous to mutations in another tyrosine kinase, *RET*, that causes familial medullary thyroid cancer and type 2 multiple endocrine neoplasia (Schmidt et al., 1997). Interestingly, somatic mutations in *MET* have since been found in several other cancers. However, these mutations are located in other regions of the molecule, in particular adjacent to the transmembrane domain (Peruzzi and Bottaro, 2006).

Activating mutations with the tyrosine kinase domain leads to increased signalling through the phosphatidylinositol 3-kinase (PI3K). One effect of this is the activation of the AMPK–mTOR nutrient and energy sensing pathway.

HPRC is very rare (approximate incidence 1/10 million), but identification of germline *MET* mutations allows precise diagnosis and targeted surveillance of mutation carriers. Moreover, such molecular insights have facilitated the use of targeted oncological therapies. A number of strategies have been devised to target the MET pathway, including antibodies that antagonize the MET receptor–ligand interaction, antibodies targeting the MET receptor itself, kinase inhibitors targeting MET, and agents that disrupt intracellular signalling pathways downstream of MET (mTOR inhibitors) (Linehan et al., 2010b). Such agents may be effective in both hereditary and sporadic papillary tumours, although it is not clear whether they would be active against tumours arising from mutations in genes downstream of this pathway (Linehan et al., 2010b).

Hereditary leiomyomatosis and renal cancer

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an autosomal dominant disorder characterized by smooth-muscle tumours of the skin and uterus and/or renal cancer (OMIM #605839). Only a minority of affected individuals develop RCC, which is of the type II papillary or collecting duct subtype. HLRCC tumours differ from other types of hereditary renal cancer in that they often present as solitary lesions with a propensity to metastasize early to regional and distant lymph nodes. By contrast, in VHL disease or BHD syndrome they can be managed conservatively until they reach 3 cm in diameter (Toro et al., 2003).

Germline mutations in the fumarate hydratase (*FH*) gene have been detected in > 90% of individuals with HLRCC (Tomlinson et al., 2002; Toro et al., 2003; Wei et al., 2006). Interestingly, homozygous or compound heterozygous mutations in *FH* cause a separate condition, autosomal recessive fumarate hydratase deficiency (FHD), characterized by neurological impairment and encephalopathy. Mutations in families with leiomyomatosis occur towards the N-terminus, whereas those associate with FHD are more C-terminal. FH is a key component of the tricarboxylic acid cycle (Krebs cycle) and catalyses the conversion of fumarate to malate. Loss of FH leads to increased levels of fumarate

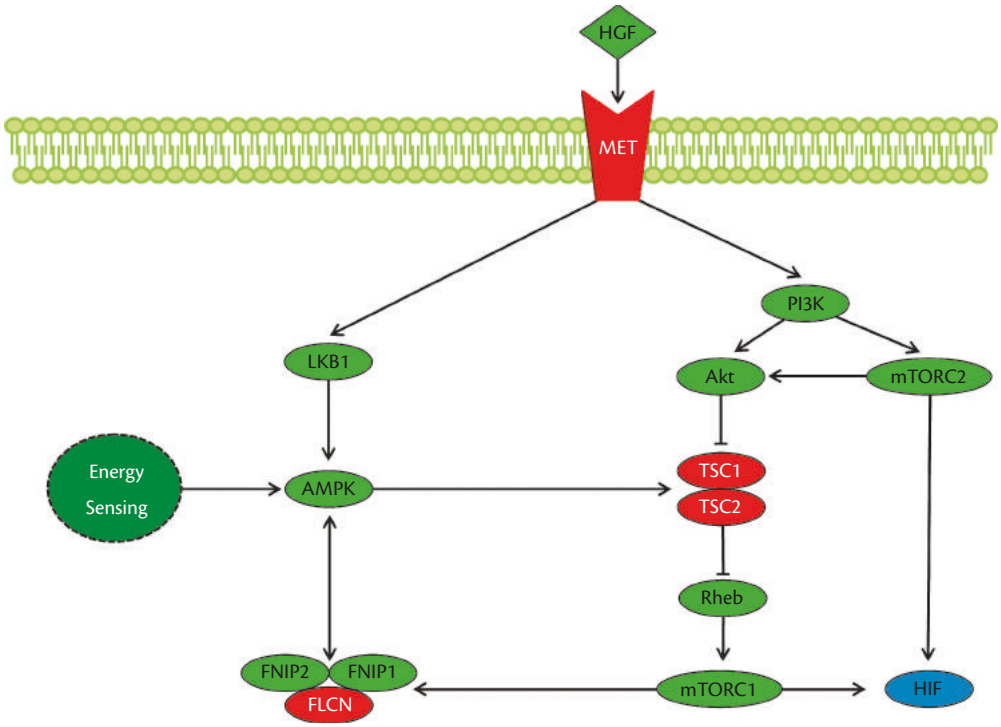

Fig. 328.2 The mTOR energy-sensing pathway and kidney cancer. Activation of MET induces LKB1 phosphorylation via the Raf pathway, uncoupling AMPK energy sensing control of mTOR. The FLCN–FNIP1–FNIP2 complex binds AMPK, the primary energy sensor in the cell, and FLCN is phosphorylated by a rapamycin-sensitive kinase (e.g. mTORC1). TSC1–TSC2 is phosphorylated by the LKB1–AMPK cascade and helps mediate the cell's response to energy and nutrient sensing. Stimulation of mTORC2 via PI3K affects translational control of HIF. Akt = proto-oncogene c-Akt; AMPK = 5′-AMP-activated protein kinase; FNIP = folliculin interacting protein; LKB1 = serine–threonine protein kinase 11; mTOR = serine–threonine protein kinase mammalian target of rapamycin; PI3K = phosphatidylinositol 3-kinase.
Adapted from Linehan, W. M., Bratslavsky, G., Pinto, P. A., *et al.* (2010a). Molecular diagnosis and therapy of kidney cancer. *Annu Rev Med*, 61, 329–43.

that inhibit the activity of hypoxia-inducible factor (HIF) prolyl hydroxylases (O'Flaherty et al., 2010). This leads to stabilization of HIF and transcriptional upregulation of HIF target genes (see Chapter 331).

FH-deficient kidney cancer cell lines are glucose dependent and show impaired oxidative phosphorylation (Sudarshan et al., 2009). This dependence on glycolysis may make these tumours more susceptible to the anti-angiogenic therapies that have been used to target conventional CCRCC (Linehan et al., 2010b).

Note that diffuse leiomyomatosis associated with Alport Syndrome (OMIM #308940, see Chapter 323) is a distinct condition not associated with renal cell cancer.

Succinate dehydrogenase

Germline mutations in three of the four subunits of succinate dehydrogenase (*SDHB, SDHC*, and *SDHD*) have been associated with familial head and neck paragangliomas (HNPGL) and sporadic and familial phaeochromocytoma (Baysal et al., 2000; Astuti et al., 2001). Subsequently, early onset renal tumours were found to develop in individuals with germline *SDHB* mutations (Vanharanta et al., 2004). A variety of histological subtypes of RCC may be associated with *SDHB* mutations (and less frequently *SDHD*), and the lifetime risk of RCC in *SDHB* mutation carriers was estimated to be about 15% (Ricketts et al., 2010).

In pre-clinical models, increased succinate has been shown to inhibit HIF prolyl hydroxylase and affect HIF stability in a manner analogous to FH-deficient tumours (Isaacs et al., 2005). Inactivation of SDH would be expected to severely impair oxidative phosphorylation and lead to glucose-dependence of SDH-deficient tumours (Linehan et al., 2010b).

Tuberous sclerosis complex

Tuberous sclerosis complex (TSC) (see Chapter 330) is a multisystem disorder characterized by skin lesions (e.g. facial angiofibromas (adenoma sebaceum), hypopigmented macules and periungual fibromas), central nervous system complications (epilepsy, learning disability, and giant cell astrocytomas), and cardiac lesions (rhabdomyomas) (Crino et al., 2006). The most frequent renal lesions are multiple angiomyolipomas and cysts, but early-onset RCCs have been reported (Rosser et al., 2006). TSC has a highly variable phenotype, and two-thirds of cases result from sporadic genetic mutations.

Molecular genetic studies have identified at least two loci for TSC, *TSC1* and *TSC2*. *TSC1* encodes for the protein hamartin, on 9q37. *TSC2* is contiguous with the polycystin-1 gene on 16p13.3, and encodes for the protein tuberin. Hamartin and tuberin form a heterodimer that interacts with Rheb, a Ras-family GTPase, preventing it from activating mTOR signalling (via mTORC1) (Inoki et al., 2005). Thus, mutations at the *TSC1* and *TSC2* loci result in a loss of inhibition of the mTOR nutrient and energy-sensing pathway, similar to the activating mutations in *MET* discussed earlier. Moreover, lack of TSC1/2 inhibition also increases HIF accumulation via increased *HIF* mRNA translation by activated mTORC1.

Sirolimus has been shown to cause regression of renal angiomyolipomas in patients with TSC (Dabora et al., 2011; Davies et al., 2011). Sirolimus forms a complex with FK binding protein and inhibits mTORC1 signalling. The result of these and current trials provides the rationale for a molecular therapeutic approach for the treatment of renal tumours associated with the TSC1/TSC2 pathway.

Other causes of familial renal cell carcinoma

A number of other inherited disorders may be associated with renal tumours. Wilms tumour, or nephroblastoma (see Chapter 173), is the most common type of renal cancer in childhood. Mutations of the *WT1* gene on chromosome 11p13 are observed in approximately 20% of Wilms tumours and are discussed in Chapter 329. At least half of the Wilms tumours with mutations in *WT1* also carry mutations in *CTNNB1*, the gene encoding the proto-oncogene beta-catenin (Maiti et al., 2000). A further 30% of Wilms tumours show inactivation of a gene on the X chromosome, *WTX* (Rivera et al., 2007), but most cases do not have mutations in any of these genes (Ruteshouser and Huff, 2004).

Another rare cause of familial RCC is hereditary translocation of chromosome 3. The first to be described was a t(3;8)(p14;q24) translocation, and a further 11 have since been described (Woodward et al., 2010). In some cases, characterization of the translocation breakpoints has identified candidate tumour suppressor genes, but this is not true in all cases. When routine karyotype analysis identifies a chromosome 3 translocation in the context of familial RCC this is likely to be the cause (Maher, 2011). However, the risk of developing RCC for translocation carriers in the absence of a family history has not been defined.

The hyperparathyroidism–jaw tumour (HPT–JT) syndrome (OMIM #145001) is an autosomal dominant, multiple neoplasia syndrome primarily characterized by hyperparathyroidism due to parathyroid tumours (Wassif et al., 1999; Carpten et al., 2002). Kidney lesions occurring in HPT–JT include bilateral cysts, renal hamartomas or Wilms tumour. HPT-JT syndrome is due to mutations in the tumour suppressor gene, *HRPT2* or parafibromin (Carpten et al., 2002).

Lynch syndrome (hereditary non-polyposis colorectal cancer, OMIM #120435) is caused by mutations in DNA mismatch repair genes, including *MSH2, MLH1*, and *MSH6*. Upper urinary tract (UUT) cancer is the third most common cancer in Lynch syndrome (5%), after colorectal cancer (63%) and endometrial cancer (9%) (Roupret et al., 2008; Crockett et al., 2011). The most common renal tumours are transitional cell carcinomas of the renal pelvis and ureter. These are uncommon in the general population, and up to 6% of the lifetime risk for all UUT cancer is attributable to Lynch syndrome. Individuals with Lynch syndrome develop UUT cancer at a younger age (62 vs 70 years), and males and females are affected equally.

Further reading

BHD Foundation website: <http://www.bhdsyndrome.org/>

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CHAPTER 329

WT1 and its disorders

Nick Hastie and Eve Miller-Hodges

The *WT1* gene

The Wilms tumour suppressor gene, *WT1*, was first identified on chromosome 11p13 in 1990 (Call et al., 1990; Haber et al., 1990). Inactivating mutations of *WT1* account for 15–20% of cases of Wilms tumour, a developmental kidney cancer (see Chapter 173), in which *WT1* behaves as a classical tumour suppressor gene. These tumours are the archetypal example of aberrant development leading to tumour development, and Wilms tumour was one of the original childhood cancers on which Alfred Knudson based his 'two-hit' hypothesis for tumour suppressor genes (Knudson and Strong, 1972).

The *WT1* gene encodes a complex 55 kDa protein containing four C-terminal zinc-finger domains, characteristic of transcription factors. A number of differing roles for *WT1* have been identified (Hohenstein and Hastie, 2006):

1. Tumour suppressor gene (see Chapter 173)
2. Oncogene
3. Transcription factor and regulator of the epithelial-to-mesenchymal balance
4. RNA metabolism and splicing.

A combination of alternative exons, start sites, splice sites, and RNA editing can theoretically give rise to 36 different isoforms (Hohenstein and Hastie, 2006). These are incompletely understood. The most well-studied isoform variations occur due to alternative splicing events of exon 5 (± exon 5) and of three amino acids (KTS) between zinc fingers 3 and 4 (± KTS).

The KTS isoforms are conserved throughout vertebrate evolution. The –KTS isoform appears to act as a transcriptional regulator, with a number of transcriptional targets identified, including renin, podocalyxin, *PAX2* and *WNT4*, amongst others (Roberts, 2005; Essafi et al., 2011). The +KTS isoform is thought to play a post-transcriptional role, and binds the *IGF2* transcript, although no further endogenous RNA targets have been identified (Caricasole et al., 1996).

Genetically engineered mice which can only express the +KTS isoform develop severe genitourinary developmental abnormalities, with small kidneys containing only few glomeruli, and streak gonads. In contrast, mice unable to express the +KTS isoform (mirroring human Frasier syndrome) develop albuminuria secondary to focal segmental glomerulosclerosis (FSGS) and die of renal failure at a few months of age, similar to the human disease (Hammes et al., 2001). This demonstrates distinct roles for these isoforms, yet also implies a degree of redundancy as neither of these phenotypes is as severe as the *Wt1* knockout model (Hastie, 2001).

WT1 in development

Wt1 is essential for normal mouse embryonic development. The *Wt1*-null mouse was found to be embryonic lethal at the mid gestation stage, due to abnormal development of the heart and coronary vessels. There was complete absence of kidneys and gonads, abnormal heart and liver development, and diaphragmatic hernia (Kreidberg et al., 1993). Given this early embryonic lethality, the role of Wt1 in later developmental processes has been more difficult to study.

WT1 in kidney development

The mammalian kidney forms via a complex reciprocal interaction between the ureteric bud, and metanephric mesenchyme, with nephron formation occurring via a process of mesenchymal-to-epithelial transition (MET) (Dressler, 2009). Kidney development is described in detail in Chapter 343. A low level of WT1 expression is found in the intermediate mesoderm, from which the early metanephric kidney will form. WT1 expression then increases in the induced mesenchyme and becomes concentrated in the developing renal vesicle, until eventually it is restricted to the podocyte, where it continues to be expressed through adult life. WT1 expression has also been identified in the juxtaglomerular apparatus (Steege et al., 2008) and in parietal epithelial cells (Bariety et al., 2006). Studies using the *Wt1*-null mouse have demonstrated apoptosis of early renal precursors in *Wt1* knockout animals, so the kidneys fail to form. Therefore investigating the role of Wt1 in later renal development has relied mainly upon *ex vivo* organ culture approaches which demonstrate that this nephron MET fails to occur in the absence of Wt1 (Davies et al., 2004). This has recently been confirmed *in vivo*, where conditional knockout of *Wt1* in the renal mesenchyme led to reduced formation of mature epithelialized structures (Essafi et al., 2011) (Fig. 329.1).

Wilms tumours (see Chapter 173) are thought to arise from pluripotent renal precursors which fail to differentiate normally. Fifteen to 20% of Wilms tumours are associated with *WT1* mutations, often in conjunction with activating beta-catenin mutations. They classically exhibit 'triphasic' histology, consisting of epithelial, blastemal, and stromal elements and occasionally differentiate into ectopic tissues such as muscle or fat. In keeping with this failure of differentiation, Wilms tumours are associated with nephrogenic rests, patches of embryonic kidney tissue that persist in mature kidney.

WT1 in heart development

In the heart, the opposite process occurs, in that epithelial cells of the epicardium undergo an epithelial-to-mesenchymal transition (EMT) to migrate into the heart to form the coronary vasculature,

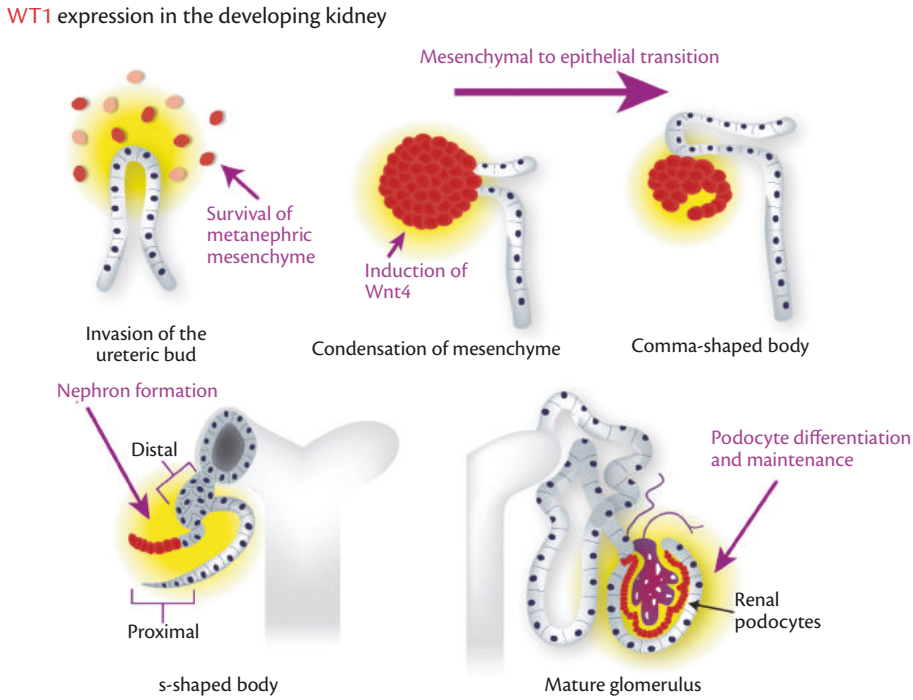

Fig. 329.1 WT1 expression in the developing kidney. Kidney development is detailed in Chapter 343.

endothelial cells, interstitial fibroblasts, and cardiomyocytes. Again, Wt1 controls this process via direct activation of snail, a driver of EMT, and repression of E-cadherin (Martinez-Estrada et al., 2010).

The mechanism behind these dichotomous roles has recently been explained in that Wt1 activates *Wnt4* in the kidney, via recruitment of co-activators and alteration of the chromatin to an active state, to promote MET. Conversely in the heart, Wt1 recruits co-repressors and changes the chromatin of *Wnt4* to an inactive state (Essafi et al., 2011).

WT1 and developmental syndromes

A wide range of human diseases and developmental syndromes have been associated with *WT1* mutations, in keeping with its essential role in embryonic development (Table 329.1).

Genotype–phenotype correlations

WT1 mutations causing Denys–Drash syndrome (DDS) (see Chapter 343) are usually missense mutations found in exons 8 and 9 which code for the zinc finger DNA binding regions. The mutant protein is thought to act in a dominant negative manner, as dimerization with wild type protein prevents normal DNA binding and transcriptional regulation.

Frasier syndrome describes the combination of male pseudohermaphroditism, gonadoblastoma, and progressive renal disease, usually of the FSGS type, reaching end stage by early adolescence, with a relatively low risk of Wilms tumour. Frasier syndrome is caused by mutations in intron 9 which prevent expression of the +KTS isoform (thought to have post-transcriptional functions).

However, it is important to note that these genotype–phenotype correlations are not clear cut. Although the mutations described above are generally associated with the listed diseases, this is not always the case. Intron 9 splice site mutations have been described in patients with clinical DDS but without Wilms tumour (Little and Wells, 1997), and intron 9 mutations which should not have affected splicing have been found in patients with clinical Frasier syndrome (Kohsaka et al., 1999). Again, this supports animal data described earlier which implies a partial degree of redundancy in the differing isoform roles.

Stronger correlation between genotype and phenotype is found with regard to risk from Wilms tumour (see Chapter 173). A recent paper reviewed > 50 patients with nephrotic syndrome. Those with missense or nonsense mutations, as is usually found in DDS, were found to have a high risk of Wilms tumour, whereas splice mutations, as seen in patients with Frasier syndrome, had a very low risk (Chernin et al., 2010).

WT1 in non-syndromic renal disease

There has been increased recognition of the role of WT1 in non-syndromic renal disease over recent years, and *WT1* mutations have been identified in cases of isolated diffuse mesangial sclerosis, FSGS, and membranoproliferative glomerulonephritis (Hahn et al., 2006; Benetti et al., 2010; Megremis et al. 2011).

Sizeable cohort studies have revealed *WT1* mutations to be a significant cause of steroid-resistant nephritic syndrome (SRNS) in children and young adults, with an incidence of 6–7% in a worldwide cohort of 300 paediatric patients with SRNS. Other studies have identified even higher levels, with *WT1* mutations accounting for 12% of cases of SRNS in a cohort of females with SRNS < 18 years of age. This compares with a prevalence of 10–26% for *NPHS2* (podocin) mutations in SRNS, the most common genetic cause (Ruf et al., 2004).

WT1 in clinical practice

Given this relatively significant prevalence, paediatric and adolescent patients with nephrotic syndrome or substantial proteinuria,

Table 329.1 Human diseases and developmental syndromes associated with *WT1* mutations

Disease	*WT1* abnormality	Phenotype
Wilms tumour (see Chapter 173)	*WT1* mutations found in 15–20% of cases (Haber et al., 1990)	Developmental renal tumour—failure of normal renal development
WAGR syndrome (Fischbach et al., 2005)	Deletions of 11p13, to include both *WT1* and *PAX6* (Call et al., 1990)	◆ Wilms tumour ◆ Aniridia ◆ Genitourinary abnormalities ◆ Mental retardation
Denys–Drash syndrome (Niaudet and Gubler, 2006; Scott et al., 2006)	Usually point (missense) mutations in exons 8 or 9 (zinc-finger regions) affecting DNA binding properties	◆ Wilms tumour ◆ Diffuse mesangial sclerosis ◆ Rapidly progressive renal failure ◆ Ambiguous genitalia
Frasier syndrome (Barbaux et al., 1997)	Point mutations in intron 9 resulting in loss of the +KTS WT1 isoform	◆ Male pseudohermaphroditism ◆ Gonadoblastoma ◆ Progressive renal disease (usually focal segmental glomerulosclerosis)
Isolated diffuse mesangial sclerosis and focal segmental glomerulosclerosis (Benetti et al., 2010)	Both missense mutations in exons 8 and 9 and intron 9 mutations identified, with marked phenotypic variability	Renal disease without other features of Denys–Drash or Frasier syndrome
Meacham syndrome (Suri et al., 2007)	Point mutations in zinc finger regions affecting DNA binding properties	Multiple malformation syndrome characterized by: ◆ Male pseudohermaphroditism ◆ Abnormal internal female genitalia ◆ Complex congenital heart defect ◆ Diaphragmatic hernia

especially phenotypic females or males with genitourinary abnormalities, should be screened for *WT1* mutations (Megremis et al., 2011).

Treatment of WT1-related glomerulopathies

Correct identification of SRNS associated with *WT1* mutations is important to guide treatment. The use of ciclosporin, in combination with methylprednisolone, is established in the treatment of idiopathic SRNS; however, when used on patients with *NPHS2* (podocin) mutations, outcomes have been disappointing. These findings were extrapolated to include all genetic causes of SRNS, so it was assumed patients with *WT1* mutations would also fail to respond. Therefore, treatment options remained very limited, with patients expected to progress rapidly to end-stage kidney disease.

However, a number of cases of SRNS with *WT1* mutations have now been reported which demonstrate a favourable response to treatment with a combination of steroids, ciclosporin, and angiotensin-converting enzyme inhibitors. Ciclosporin is thought to prevent calcineurin-mediated dephosphorylation and degradation of synaptopodin, thus maintaining podocyte cytoskeletal architecture (Faul et al., 2008). These children had a variety of previously described *WT1* mutations, varying presentations, and with both diffuse mesangial sclerosis and FSGS found histologically, again emphasizing the lack of specific genotype–phenotype correlation (Stefanidis and Querfeld, 2011). Despite the small numbers this treatment looks promising, although it must be noted there may be an influence due to early timing of treatment and publication bias. A randomized controlled trial would provide useful confirmation of these observations.

WT1 in adult kidney

Until recently, research into the role of WT1 in adult kidney has been limited, due to the confounding developmental effects in traditional knockout or mutant animal models. However, a number of WT1 target genes have been demonstrated in the kidney (Table 329.2).

Models of DDS have demonstrated an abnormal podocyte phenotype, with altered cytoskeletal architecture and a more mesenchymal state (Morrison et al., 2008).

Thus, WT1 appears to have a role in both podocyte differentiation and maintenance of podocyte structure and, therefore function. Clarification of these roles, and therefore identification

Table 329.2 WT1 target genes in the kidney

Gene	Role	WTI mode of action
PODXL (podocalyxin)	Highly charged sialoglycoprotein Maintains the architecture of the podocyte foot process	Transcriptionally activated by WT1, which binds to the podocalyxin promoter
NPHS1 (nephrin)	Transmembrane receptor molecule Key component of the slit diaphragm	Transcriptionally activated by WT1, which directly binds a nephrin promoter
REN (renin)	Peptide hormone secreted by the cells of the juxtaglomerular apparatus Regulates blood pressure and electrolyte balance through the rennin angiotensin system	WT1 and renin co-localize the juxtaglomerular apparatus WT1–KTS transcriptionally represses renin via an upstream regulatory region (Steege et al., 2008)
Sce1 (sciellin)	Novel podocyte protein identified via gene expression profiling of a mouse model of DDS Precursor to the cornified envelope of epithelial cells, thought to confer stress bearing properties on the podocyte	Direct transcriptional target of WT1, downregulated in a mouse model of DDS (Ratelade et al., 2010)
SULF1	Extracellular heparan sulphate 6-0 endosulphatase Regulates the sulphation status of heparin sulphate chains on the cell surface, thus affecting growth factor signalling	Positive direct transcriptional target of WT1, downregulated in a mouse model of DDS (Ratelade et al., 2010)

of potential therapeutic targets remains the subject of intensive research in the field.

WT1 in non-renal disease

Despite WT1 expression being widespread during development, until recently, the only site of WT1 expression in the adult was thought to be the renal podocyte. However, novel roles for WT1 have now been identified in the cardiovascular system, liver, bone marrow, and central nervous system.

WT1 expression is also associated with various cancers, especially leukaemias. In leukaemia, *WT1* mutations are associated with poor prognosis and increased risk of relapse and death. The majority of these mutations affect the zinc-finger regions, presumably affecting DNA binding capacity. Increased WT1 expression is seen in the majority of patients with acute myeloid leukaemia (73–93% at diagnosis) (Owen et al., 2010) making it an obvious target for therapy, and early studies using WT1-targeted immunotherapy have shown some promise, without obvious renal side effects (Rezvani et al., 2008).

Novel WT1 expression is also seen in some solid tumours, where *WT1* activation (rather than mutation) is seen in association with malignant transformation of tissues not known to express WT1 in the adult. EMT is thought to have a central role in cancer invasion and metastasis, providing a rationale for WT1 expression, which is known to regulate key EMT genes including *SNAIL, SNAI2* (*SLUG*) and *CDH1* (E cadherin). WT1 has also been shown to directly regulate *VEGF* expression, in response to tumour hypoxia, at least *in vitro*, providing another explanation for the role of WT1 in tumourigenesis (McCarty et al., 2011).

WT1 and tissue regeneration

An exciting recent development in the WT1 field has been the discovery of the role of WT1 in tissue regeneration. As mentioned earlier, WT1 promotes EMT of the epicardium to form progenitor cells which migrate into the heart and form the coronary vasculature and cardiomyocytes. In models of myocardial ischaemia in the rat, novel Wt1 expression is seen in the border zone of infarcted tissue, thought to represent an EMT of vascular cells in order to promote neovascularization (Wagner et al., 2002). More recently, it has been revealed that adult heart contains a resident progenitor cell population, which express WT1, thought to arise from the epicardium. These cells can form new cardiomyocytes, which can integrate with existing tissue following injury (Smart et al., 2011).

The regenerative potential of podocytes in the kidney appears to be very limited, but potential renal precursors have been identified at the glomerular tuft (Ronconi et al., 2009), and parietal epithelial cells have been shown to migrate onto the glomerulus and take on the characteristics of podocytes (see Chapter 139) As part of this process, these cells start to re-express Wt1 (Appel et al., 2009). However, the exact role of Wt1 in this process is yet to be discovered.

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CHAPTER 330

Tuberous sclerosis complex renal disease

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Introduction

Tuberous sclerosis complex (TSC, OMIM #191100, #613254), an autosomal dominant genetic disorder affecting virtually every organ system, exhibits a birth incidence of approximately 1/6000 and thus affects approximately 1 million patients worldwide (Crino et al., 2006; Yates, 2006). Less than 40% of affected patients exhibit the classical features of Vogt's triad (facial angiofibromas, mental retardation, and intractable epilepsy) (Curatolo, 2003). Although TSC most often presents with neurologic symptoms, only about half of patients demonstrate cognitive impairment, autism, or other behavioural disorders (Franz et al., 2010). Renal findings are expected in TSC as angiomyolipomata occur in approximately 90% and renal cystic disease in approximately 50% of patients. Sheppard et al. noted renal feature was the most common feature associated with adult TSC patient death (Shepherd et al., 1991). Lymphangioleiomyomatosis (LAM) is the third most common cause of TSC-associated morbidity, occurring in approximately 35% of female TSC patients, although it only causes significant symptoms in a minority. The gender predilection in LAM is not understood but a role for oestrogen has been postulated.

There are two loci associated with TSC, the *TSC1* gene, located on chromosome 9, and the *TSC2* gene, located on chromosome 16. Children with TSC are most commonly born with normal kidneys and develop cystic disease and angiomyolipomata as they age. The resolution and limitations of the imaging modality employed determines the degree of detected renal involvement, and possibly all patients have involvement in the form of microscopic angiomyolipomata and cystic disease (Siroky et al., 2011). TSC-associated renal disease, whether angiomyolipomata or cystic disease, leads to at least stage 1 chronic kidney disease (CKD), and some patients experience the associated relentless progression, morbidity, and mortality. Renal function in these patients is important because many of the drugs commonly used to treat patients are renally excreted (Table 330.1).

Renal cystic disease in tuberous sclerosis complex

TSC renal cystic disease can be detected by conventional imaging in approximately 50% of patients with TSC, and is associated with mutations in either the *TSC1* or *TSC2* gene (Ewalt et al., 1998; Dabora et al., 2001; Rakowski et al., 2006). TSC renal cystic disease can be microscopic in the form of glomerulocystic disease (Bissler et al., 2010), or dramatically macroscopic in the form of the polycystic renal phenotype associated with the contiguous gene syndrome (Dixon et al., 2011b). This latter syndrome involves deletions encompassing a portion of the adjacent *TSC2* and polycystic kidney disease 1 (*PKD1*) genes on chromosome 16p13 and accounts for about 2% of TSC patients (Sampson et al., 1997).

Renal cysts in TSC can arise from all nephron segments (Bissler et al., 2010). TSC-associated renal cystic disease may be accelerated following acute kidney injury, as has been reported to occur in animal models (Patel et al., 2008). Risk factors for acute kidney injury in TSC patients include certain anticonvulsant and non-steroidal anti-inflammatory medications as well as rhabdomyolysis and hypoxia induced by status epilepticus (de Chadarevian et al., 2003).

Renal angiomyolipomata

Angiomyolipomata are the prototype of a family of tumours referred to as perivascular epithelial cell tumours, or PEComas. Typically TSC-associated angiomyolipomata contain fat (Dixon et al., 2011b). The cellular components of these lesions can be spindle, epithelioid, or a mixture of both cell types that express alpha smooth muscle actin (α-SMA) and melanocyte markers such as gp100 (a splice variant of Pmel17) and MART-1/melan-A. The expression of these melanocyte-associated genes is driven by the Microphthalmia-associated transcription factor (MITF) family of transcription factors (Levy et al., 2006). This increased activity of MITF transcription factors likely has caused confusion between TSC-associated PEComas and the much more aggressive renal cell carcinomas (RCCs) and PEComas associated with *TFE3* or *TFEB* translocations.

All components of angiomyolipomata, including the vascular cells, immature smooth muscle-like spindle cells, epithelioid cells, and fat cells contain somatic mutations that, combined with their germline mutation, render the cells deficient in either tuberin or hamartin. Such cellular deficiency disrupts the integrated control of cell growth, leading to the development of the angiomyolipoma (Siroky et al., 2011).

A cross-sectional study of children with TSC revealed an increased incidence of angiomyolipomata with age (O'Callaghan et al., 2004). In a longitudinal study of children with TSC, 55%

Table 330.1 Renal clearance of commonly used medications in TSC

Generic drug (brand)	Metabolism/elimination	Renal dosing
Vigabatrin (Sabril®)	Eliminated via urine	Cl_{cr} > 50–80 mL/min: decrease dose by 25% Cl_{cr} > 30–50 mL/min: decrease dose by 50% Cl_{cr} > 10–30 mL/min: decrease dose by 75%
Topiramate (Topamax®)	70% excreted unchanged in the urine.	Cl_{cr} < 70 mL/min/1.73 m^2: administer 50% of the usual dose; titrate more slowly due to prolonged half-life. Significantly haemodialysed; dialysis clearance: 120 mL/min (4–6 times higher than in adults with normal renal function); supplemental doses may be required.
Divalproex (Depakote®)	Extensive in liver via glucuronide conjugation and oxidation	Cl_{cr} < 10 mL/min: no dosage adjustment is needed for patients on haemodialysis
Lamotrigine (Lamictal®)	> 75% metabolized in the liver via glucuronidation	Use with caution; has not been adequately studied; base initial dose on patient's antiepileptic drug regimen; decreased maintenance dosage may be effective in patients with significant renal impairment.
Phenytoin (Dilantin®)	Metabolized in liver; major metabolite (via oxidation) HPPA undergoes enterohepatic recycling and elimination in urine as glucuronides	Phenytoin serum concentrations may be difficult to interpret in renal failure. Monitoring of free (unbound) concentrations or adjustment to allow interpretation is recommended.
Clobazam (Onfi®)	Hepatic	Cl_{cr} ≥ 30 mL/min: no dosage adjustment required Cl_{cr} < 30 mL/min: use with caution, has not been studied
Oxcarbazepine (Trileptal®)	Hepatic	Cl_{cr} < 30 mL/min: initial dose: administer 50% of the normal starting dose; slowly increase the dose if needed, using a slower dosage titration than normal
Carbamazepine (Tegretol®)	Hepatic	Cl_{cr} < 10 mL/min: administer 75% of recommended dose; monitor serum levels
Clonazepam (Klonopin®)	Hepatic	No dosage adjustment provided in manufacturer's labelling; use with caution. Clonazepam metabolites may accumulate in patients with renal impairment. Haemodialysis: supplemental dose not necessary.
Risperidone (Risperdal®)	Hepatic	Cl_{cr} < 30 mL/min: starting dose of 0.5 mg twice daily; titration should progress slowly in increments of no more than 0.5 mg twice daily; increases to dosages > 1.5 mg twice daily should occur at intervals of ≥ 1 week. Clearance of the active moiety is decreased by 60% in patients with moderate-to-severe renal disease (Cl_{cr} <60 mL/min) compared to healthy subjects
Metyrosine (Demser®)	Primarily urine (53% to 88% as unchanged drug)	No dosage adjustment provided in manufacturer's labelling
Quetiapine (Seroquel®)	Hepatic	No dosage adjustment required
Trazodone	Hepatic	No dosage adjustment required
Sirolimus (Rapamune®)	In intestinal wall via P-glycoprotein and hepatic via cytochrome P450 3A4	No dosage adjustment (in loading or maintenance dose) is necessary in renal impairment. However, adjustment of regimen (including discontinuation of therapy) should be considered when used concurrently with ciclosporin and elevated or increasing serum creatinine is noted
Everolimus (Afinitor®)	Hepatic	No dosage adjustment necessary

Cl_{cr} = creatinine clearance.

of children (mean age 6.9 years) had some type of renal abnormality detected by ultrasound, and at follow-up, 80% (mean age 10.5 years) had abnormalities (Ewalt et al., 1998), with the most common form of involvement being angiomyolipomata, suggesting to the authors that renal involvement began during infancy and continued with age.

The imaging method used to monitor TSC renal disease greatly impacts the types of lesions detected. Ultrasound is excellent at detecting the adipose component of angiomyolipomata and was extensively used in the past to monitor disease activity. Fat-poor lesions are common in TSC but are often isodense to kidney and may not be sonographically identified, leading to the previously held misconception that fat-poor lesions are rare in TSC. Computed tomography (CT) is excellent at delineating tissues, and can employ angiography to help detect aneurysms in selected patients, but does have the risk of contrast and radiation exposure. Magnetic resonance imaging (MRI) has truly outstanding detection and delineation of the angiomyolipoma, and can be added on to concurrent brain imaging. Although this technology may require sedation for some patients, research using multiphase arrays may speed the process up to reduce this need. As a result, the 2012 TSC Consensus Conference has recommended abdominal MRI as the recommended method to monitor the renal lesions of TSC.

The incidence of renal involvement in TSC is imprecise in part because of the methods used to detect renal involvement. Even kidney tissue that is radiologically normal by MRI, on cut section, may contain both microscopic angiomyolipomata and cysts (Siroky et al., 2011). These histologic findings raise the possibility that such microscopic lesions become identifiable as the patient ages because of continued slow growth.

Angiomyolipomata inflict significant morbidity and even mortality mainly because they are at risk for aneurysm formation and rupture leading to haemorrhage. However, angiomyolipomata can also invade adjacent normal renal parenchyma leading to CKD and even end-stage renal disease. The vascular component of larger angiomyolipomata frequently develops aneurysms that can rupture causing the haemorrhage (Fig. 330.1) (Adler et al., 1984; Ou et al., 1991; Bissler et al., 2002; Casper et al., 2002). The haemorrhage risk of renal angiomyolipomata in TSC patients is estimated to be between 25% and 50% (Mouded et al., 1978; Kessler et al., 1998), while between 20% and 30% of such patients with haemorrhages present to the emergency room in shock (Pode et al., 1985). The haemorrhage risk is significantly increased for aneurysms > 5 mm (Yamakado et al., 2002).

A traditional urological approach to a patient *without TSC* with a suspicious renal lesion could be nephrectomy. Because the incidence of TSC is about 1/6000, and the physical findings can be variable, familiarity with the renal manifestations of TSC is generally only found in specialized TSC centres. TSC patients in the community with suspected retroperitoneal haemorrhage or atypical renal lesions may undergo elective or emergency, but most often avoidable, nephrectomy in centres with limited TSC experience. Such procedures reduce the functioning renal mass and hasten the requirement for renal replacement therapy. Given the progressive natural history of CKD and the associated increased risk in morbidity and mortality, renal disease in TSC patients poses a significant burden (Fox et al., 2008).

Fat-poor angiomyolipoma

Fat-poor lesions in the TSC patient often raise particular concern, in part because of their potentially aggressive nature in non-TSC patients and in part due to the lack of reliable information about such lesions in the TSC population. Because ultrasound depends upon tissue density and is exquisitely sensitive at detecting adipose tissue, its use as an imaging modality to detect angiomyolipomata has introduced an ascertainment bias such that many clinicians believe that *all* angiomyolipomata *must* have sonographically detectable adipose tissue. The vast majority of solid lesions in the kidney of a patient with TSC are fat-poor angiomyolipomata, but rarely can also be oncocytomas or RCCs. Applying criteria to distinguish fat-poor angiomyolipomata from malignant lesions such as a RCC may help guide therapy. Recent work, not limited to the TSC population, further supports the role for specific MRI techniques and features to help discern angiomyolipomata from other types of lesions. Such imaging features as the signal intensity index, presence of necrosis, and rate of growth may greatly facilitate identifying more ominous lesions from fat-poor lesions in patients with TSC (Patel et al., 2005; Sasiwimonphan et al., 2012).

Fat-poor TSC-associated angiomyolipomata consist of predominantly spindle cells, epithelioid cells (Mai et al., 1996; Eble et al., 1997), and/or vascular elements (Tweeddale et al., 1955; Lin et al., 1994; Karbowniczek et al., 2003). The contribution of each of the different cellular components can vary from lesion to lesion and some lesions can exhibit a dominant cell type (Wong et al., 1981; Obuz et al., 2000). Epithelioid angiomyolipomata are reported to exhibit an aggressive phenotype (Eble, 1998), recur after resection, and very rarely can even be fatal (Hardman et al., 1993; Bjornsson et al., 1996; Eble et al., 1997). While such case reports have been published, a case series of 15 patients demonstrated the course of this variant may be quite benign (Aydin et al., 2009), supporting

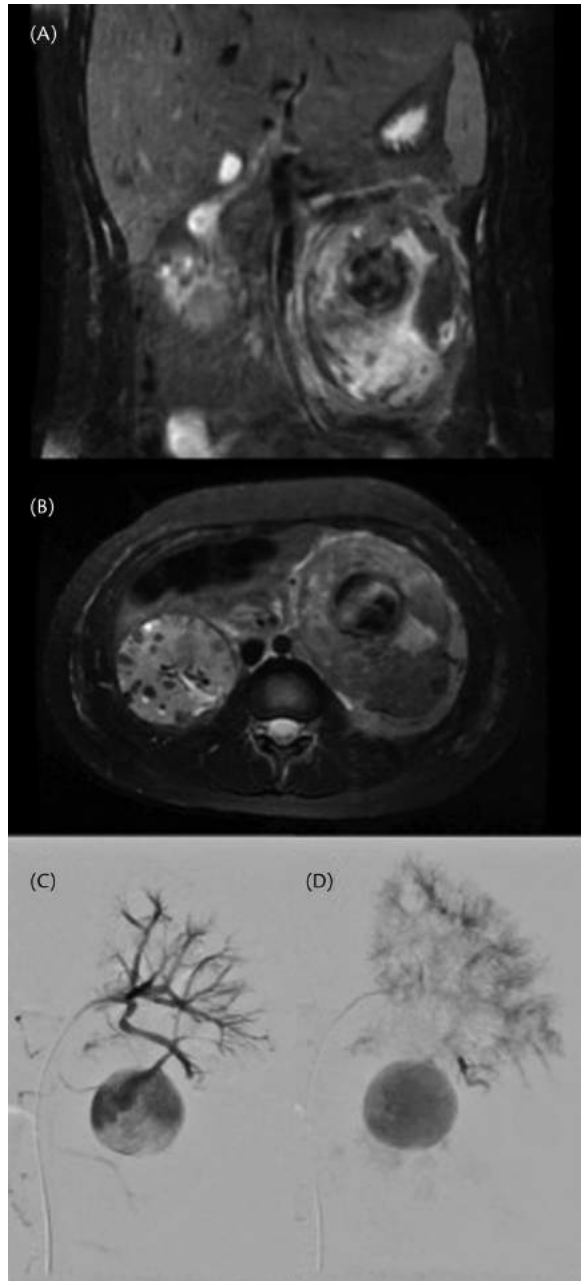

Fig. 330.1 Aneurysm in a fat-poor angiomyolipoma. (A) Coronal fast spin-echo T2-weighted image with fat suppression reveals 4.5 cm aneurysm in a large renal angiomyolipoma (arrow). (B) Axial image of the same lesion as (A). Clot in the aneurysm is seen in both planes of view. (C) Mid-phase angiographic injection reveals the large aneurysm. (D) Late-phase angiographic injection reveals the delayed washout characteristic of aneurysms.

that a more deliberate approach using serial imaging and specific imaging techniques may be used in the TSC population.

The typical haematoxylin and eosin appearance of the epithelioid variant of angiomyolipoma is very similar to that of the RCC (Saito et al., 2002), and the preponderance of RCC reported in TSC patients has not been studied in sufficient detail to exclude misclassification of an atypical angiomyolipoma such as an epithelioid lesion (Pea et al., 1998). The incidence of RCC in TSC patients is estimated to be significantly < 2%.

Immunohistochemical studies aimed at detecting HMB-45 and melan-A, diagnostic markers for angiomyolipoma, are extremely useful for differentiating between RCC and the epithelioid variety of angiomyolipoma in TSC. In fact, although angiomyolipomata are classified as PEComas, these lesions, fat poor or otherwise, are not restricted to mutation in the TSC genes. Mutations in melanoma-associated genes are also associated with fat-poor angiomyolipomata, and this finding may offer novel mechanistic insight into the association of angiomyolipomata with melan-A pathway proteins. Unrestricted mammalian target of rapamycin COMPLEX 1 (mTORC1) activity is associated with increased activity of TFE3 and TFEB, and an increase in these MITF transcription factors has been shown to increase melan-A, HMB-45, and even GPNMB expression (Lim et al., 2007). While translocation carcinomas of the kidney are also associated with overexpression of TFE3 and TFEB, and thus express the melanocyte markers (Bruder et al., 2004; Argani and Ladanyi, 2005; Armah and Parwani, 2010), these are rare tumours and would be extremely uncommon in the TSC population. Sporadic PEComas are likewise associated with such mutations (Folpe et al., 2005; Argani et al., 2010), and it is possible that the genetic event driving the PEComa formation may also have a very large impact on the prognosis. Specifically, that mutations in the TSC genes exhibit a much more benign course that some of the translocation mediated tumours. Thus if the initial mutation is in *TSC1* or *TSC2* the resulting lesion, even with its secondary mutations, is almost always benign, whereas if the initial mutation is in one of the MITF genes, a more malignant tumour such as RCC or the more aggressive form of PEComa is formed.

Hypertension

Renal cystic disease is much more common than once thought in the TSC population, and is a risk factor for hypertension. Hypertension generally responds well to angiotensin-converting enzyme inhibitors and angiotensin receptor blockers. There is evidence that the angiomyolipomata themselves express the angiotensin II type 1 receptor (Siroky et al., 2014). Despite a significant burden of renal parenchymal abnormalities observed by imaging, renal function often is quite preserved. This functional preservation despite the significant imaging abnormalities is due, at least in part, to coalescence of angiomyolipomata rather than growth of a single lesion.

Nephrolithiasis

Patients with TSC are predisposed to nephrolithiasis both because of treatments for their disease manifestations, as well as the renal disease manifestations themselves. Drugs like topiramate have proven to be an effective anticonvulsant therapy for some forms of TSC-associated epilepsy. The drug appears to work by enhancing GABA-activated chloride channels and inhibiting excitatory neurotransmission. A drawback to the medication is that topiramate inhibits carbonic anhydrase, particularly subtypes II and IV, leading to decreased renal citrate excretion and subsequently increased risk of nephrolithiasis. Similarly, although some TSC patients on the ketogenic diet experience a significant decrease in seizure activity, this diet results in both hypercalciuria and hypocitraturia, and thus increases the risk of nephrolithiasis. This stone risk is further enhanced by the resulting decrease in uric acid solubility caused by the low urine pH in patients on the ketogenic diet.

Another risk factor for stone disease in the TSC patient population is significant renal cystic disease, as disruption of distal tubular function by a significant cyst burden can lead to hypocitraturia. Although renal stone disease risk factors appear numerous for the TSC patient population, and some patients may not be able to articulate their symptoms well, successful medical therapy for nephrolithiasis in this patient population is relatively simple, involving adequate hydration and citrate supplementation when required.

Traditional minimally invasive treatment of nephrolithiasis includes extracorporeal shockwave lithotripsy (ESWL), percutaneous nephrolithotomy (PCNL), and ureteroscopic stone removal (URS). ESWL may result in renal subcapsular haematoma formation, and PCNL involves accessing by puncture and dilation of the renal collecting system via the renal parenchyma. Because patients with TSC renal disease have a well-known potential for renal haemorrhage, such approaches may pose an unacceptable risk. USR by stone baskets to remove small stones or a laser fibre to direct pulsed laser energy that will fragment stones are likely better options.

Abdominal imaging

The relative amount of each of the adipose, spindle cell, epithelioid, and vascular tissue components varies in each angiomyolipoma, with a minority of these tumours being classified as minimal-fat or fat-poor angiomyolipomata, as they contain only microscopically detectable fat (Kim et al., 2004). The fat-poor designation depends in part on the imaging modalities employed.

Using a combination of greyscale and power Doppler ultrasonographic modes, Jinzaki et al. evaluated a cohort of patients with small (1.5–3 cm) renal lesions consisting of RCCs, angiomyolipomata, oncocytomas, and pseudotumours. Greyscale ultrasound exhibited an extremely poor diagnostic accuracy (21%) for angiomyolipomata, though the combination of greyscale and power Doppler ultrasound increased the diagnostic accuracy (Jinzaki et al., 1997). Despite the fact that fat-poor angiomyolipomata contain no macroscopic focus of fat by ultrasound or CT scans, these tumours histologically contain an average of 4.1% fat (Hafron et al., 2005).

MRI is a very effective means of detecting both the macroscopic and microscopic adipose components of angiomyolipomata, and is especially successful in the identification of minimal-fat angiomyolipomata. The basic approach has been to assess the difference between fat-suppressed and non-fat-suppressed T2-weighted sequences to locate fat within a mass. This approach is necessary because high T2 signal within a renal mass is not diagnostic for an angiomyolipoma and can be seen with both RCC and haemorrhagic cysts. Comparing the fat-suppressed and non-fat-suppressed sequences can identify the adipose component and reduce the concern about RCC in a patient with TSC.

In addition, utilization of MRI artefacts such as in-phase/out-of-phase techniques can assist in identifying fat in fat-poor lesions (Mitchell et al., 1992). Israel et al. assessed the use of the opposed-phase chemical-shift technique to diagnose renal angiomyolipomata and reported that all of their 23 angiomyolipomata could be correctly identified, though one RCC was mischaracterized as an angiomyolipoma (Israel et al., 2005). Given the differences in the sensitivity of the various imaging modalities, it is also critically important to recognize that switching from ultrasound to either CT or MRI may reveal significantly more involvement especially by a fat-poor component than at first appreciated. As such, this does not necessarily indicate a sudden accelerated growth of a lesion, but merely the resultant detection differences observed by a change in imaging modality.

While MRI offers a superior method to investigate and monitor renal involvement in TSC, there are some aspects of TSC that can prevent the use of abdominal MRI. Vagal nerve stimulators may be implanted in an effort to help control seizure activity. Unfortunately, the presence of a vagal nerve stimulator puts the patient at a low but defined risk for inductive heating, pain and tissue damage and therefore these patients are not eligible for abdominal MRI in most centres. If a solid-appearing lesion is detected and MRI is not possible, CT imaging is a reasonable alternative, and if considerable concern remains, positron emission tomography scans can be helpful because angiomyolipomata are generally not ^{18}F-FDG avid (Jiang et al., 2008; Young et al., 2009).

Treatment of angiomyolipoma

The kidney appears vulnerable to both the renal manifestations of TSC as well as autosomal dominant polycystic kidney disease because both diseases are phenotypically expressed due to a second-hit, or somatic mutation, mechanism (Henske et al., 1996; Brasier and Henske, 1997). Both TSC and autosomal dominant polycystic kidney disease have a particularly impressive renal manifestation, and two separate genetic loci associated with each disease.

The kidney disease associated with the *PKD1* and the *TSC2* loci account for a significant majority of their respective diseases, and both exhibit a more severe phenotype compared to the disease associated with the *PKD2* and *TSC1* loci.

Both the *PKD1* and *TSC2* loci are immediately adjacent, arranged in a tail-to-tail orientation on chromosome 16. The proximity of the genes is important because the *PKD1* gene contains an intronic sequence that has unique structural properties (Blaszak et al., 1999) that can interfere with DNA replication and lead to double-strand breaks and thus an array of somatic mutational effects (Patel et al., 2004; Liu et al., 2012). While the cell has multiple ways to prevent as well as repair the damage (Dixon et al., 2008), this activity depends on the cell's ability to detect the damage. This latter point is critical because DNA damage detection may be the vulnerability point facilitating the renal expression of somatic mutation dependent diseases. The hyperosmolar renal microenvironment uniquely interferes with double-strand break detection that would occur during DNA replication fork blockade (Dixon et al., 2009, 2011a), such as that caused by the intronic sequence in the *PKD1* locus. This renal microenvironmental predisposition to disease may also help explain the multifocal and bilateral nature of the angiomyolipomata in patients with TSC, as well as the cystic disease, and the enrichment of these lesions in the hyperosmolal renal medulla (J. P. Bissler, unpublished observation).

The renal committee at the 2012 TSC consensus conference on diagnosis and therapy recommended pre-emptive treatment of angiomyolipomata that were > 3 cm and enlarging with an mTOR inhibitor. However embolization as the current standard of care to both control active bleeding as well as to prevent lesions with large aneurysms (> 5 mm) from bleeding in the future (Bissler et al., 2002; Williams et al., 2006), using corticosteroid therapy to reduce the subsequent post-embolization syndrome (Bissler et al., 2002). Although embolization is preferable, surgical intervention may be contemplated in selected patients who have cortical or exophytic lesions that have large aneurysms that have not haemorrhaged, or are possible carcinomas. The overarching goal of both surgery and embolization is to do the least invasive procedure directed at a specifically targeted lesion, with the minimum risk to the remainder of the kidney.

Systemic mTOR inhibition

Given the multifocal, bilateral nature of angiomyolipomata, the logical approach is to systemically managing the collective tumour burden with an mTOR inhibitor, rather than trying to individually excise each lesion. The latter approach, given the nature of the disease, runs the risk of nephron loss and iatrogenic acceleration of end-stage renal disease.

Understanding of the TSC proteins and their function has led to the first targeted therapy for TSC renal angiomyolipomata. The TSC gene products, hamartin and tuberin, form a complex that integrates cellular signalling inputs such as growth factors, genomic integrity, cellular energy supply and growth substrate availability to gate growth and proliferation but can also affect cell cycle arrest, senescence, autophagy, or cell death. mTORC1 is an immediately downstream target of the hamartin/tuberin complex, and use of an mTORC1 inhibitor, such as sirolimus (Bissler et al., 2008) or everolimus can reduce the angiomyolipoma burden substantially (Bissler et al., 2013). The first trial used an open-label design and demonstrated an approximately 50% reduction in tumour volume for patients while on the drug (Bissler et al., 2008). This trial also reported an improvement in pulmonary function for female patients with LAM that was confirmed in a placebo-controlled trial (McCormack et al., 2011). The observed results during this trial launched studies aimed at other TSC-related tumours such as the subependymal giant cell astrocytoma, or SEGA. This lesion was confirmed to be responsive to mTORC1 inhibition in a case series (Franz et al., 2006), an open-label trial (Krueger et al., 2010), as well as a placebo-controlled trial (Franz et al., 2013). A follow-up placebo-controlled trial using the 50% reduction in angiomyolipoma volume induced by mTORC1 inhibition confirmed the utility of this therapy (Bissler et al., 2013). Based on these clinical trials, the use of mTORC1 inhibitors for renal angiomyolipomata and SEGAs recently have been approved in several countries.

Further reading

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CHAPTER 331

Hypoxia-inducible factor and renal disorders

Thomas Connor and Patrick H. Maxwell

Introduction

Oxygen is essential for aerobic metabolism. Physicians have long been aware of the complex circulatory, respiratory, and neuroendocrine systems that mediate oxygen homeostasis at a physiological level. We now know that all nucleated cells in the body also sense and respond to changes in oxygen levels. This cellular response shows remarkable evolutionary conservation and is mediated by a family of hypoxia-inducible factor (HIF) proteins.

Hypoxia is a relative decrease of oxygen availability. Physiological oxygenation of tissues varies significantly. Before the circulatory system becomes established, mammalian development proceeds in a relatively low-oxygen environment of about 3%. In the majority of normal adult tissues, oxygen levels vary between 2% and 9% (on average 40 mmHg), as compared to ambient air, which contains 21% oxygen (150 mmHg). However there are a number of tissues with oxygen concentrations < 1%, including regions of the bone marrow, cartilage, thymus, and renal medulla.

Blood flow to the kidneys is high compared to other tissues. However counter-current perfusion of the renal medulla results in a medullary oxygen concentration that is normally in the range 10–20 mmHg (Brezis and Rosen, 1995). Oxygen tensions are also low in the in the renal cortex for two main reasons. First, oxygen diffuses from the blood in the afferent arteriole to the closely apposed venule before it even reaches the glomerulus. Second, oxygen consumption associated with solute transport by tubular epithelium is high. As a result, the mean partial pressure of oxygen is about 50 mmHg and there are steep oxygenation gradients. These patterns of intrarenal oxygenation are important determinants of the distribution of tubular injury after kidney ischaemia.

The cellular response to hypoxia mediated by HIF is highly conserved in evolution. It is present in very simple animals *Caenorhabditis elegans* and *Trichoplax adhaerens*, indicating an important role even in the absence of specialized physiological systems for oxygen delivery (Maxwell, 2003; Loenarz et al., 2011; Semenza, 2011). HIF acts as a master transcriptional regulator of several hundred genes that generally promote adaptation to hypoxia. These adaptations include both oxygen delivery and the regulation of genes involved in glucose uptake and energy metabolism, angiogenesis, erythropoiesis, cell proliferation and apoptosis, cell–cell and cell–matrix interactions, and epithelial barrier function.

Low levels of oxygen can be found in a range of pathological circumstances, including cancer and ischaemic diseases. It is well known that many solid tumours contain regions of hypoxia, and it has been shown that the degree of hypoxia correlates with poor prognosis (Bertout et al., 2008). The great majority of clear cell kidney cancers show constitutive activation of the HIF pathway, resulting in unrestricted stimulation of HIF target genes (Linehan et al., 2010). These include growth factors mediating angiogenesis, which play a critical role in the growth and metastasis of primary tumours.

The cellular response to hypoxia also plays a role in such diverse processes as the homeostatic response to anaemia, acute kidney injury, and the progression of chronic kidney disease. Acute kidney injury due to local ischaemia activates the HIF pathway, and pre-clinical work has suggested that increasing HIF activation results in reduced renal injury. By contrast, chronic kidney disease may involve a maladaptive response to chronic ischaemia that leads to progressive fibrosis and decline in renal function. The erythropoietin (EPO) gene is one of the best understood HIF target genes, and drugs that increase EPO production by activating HIF are now being trialled in humans.

How hypoxia-inducible factor responds to oxygen

HIF is a highly conserved DNA binding transcription factor. The active form is a heterodimer composed of a constitutively expressed beta subunit and one of two oxygen sensitive alpha subunits (HIF-1α or HIF-2α; collectively referred to as HIF-α). Under aerobic conditions, HIF-α is hydroxylated by prolyl hydroxylase domain (PHD) proteins, which use molecular oxygen and 2-oxoglutarate as substrates (Epstein et al., 2001). Hydroxylated HIF-α interacts with the von Hippel–Lindau (VHL) protein, the substrate-recognition subunit of an ubiquitin-protein ligase that targets HIF-α for proteasomal degradation (see Fig. 331.1) (Maxwell et al., 1999; Ivan et al., 2001; Jaakkola et al., 2001).

The hydroxylation reaction acts as a direct sensor of intracellular oxygen levels (Maxwell, 2003; Kaelin, 2007). The oxygen activity of the PHD enzymes is sensitive to oxygen concentration across the physiologically relevant range. VHL only recognizes HIF-α subunits that have been hydroxylated on specific prolyl residues (Ivan et al., 2001; Jaakkola et al., 2001). Proteasomal degradation occurs within seconds to minutes, allowing a very rapid response to fluctuations in oxygen availability (Maxwell et al., 1999).

HIF-α rapidly accumulates when oxygen levels are low, because hydroxylation proceeds less efficiently. HIF-α then translocates to

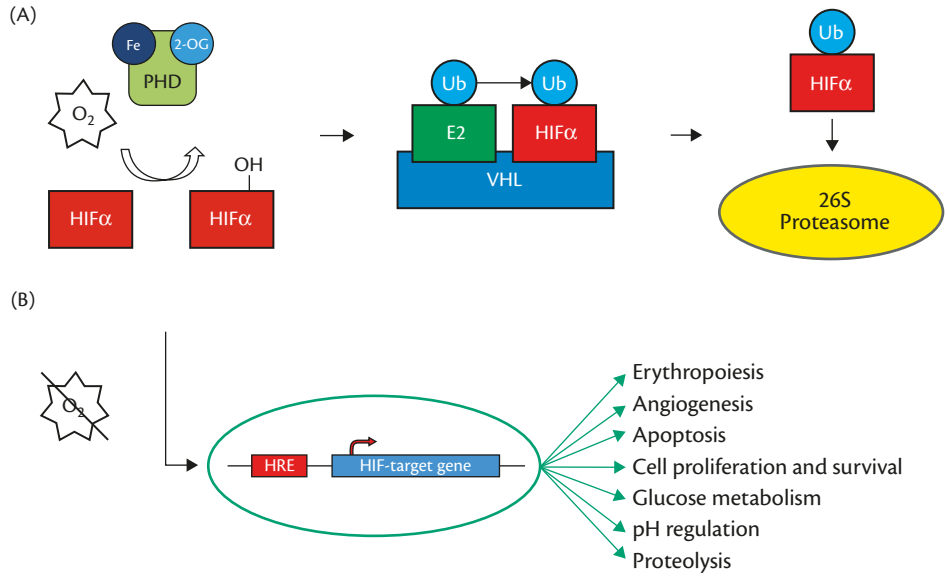

Fig. 331.1 How HIF responds to oxygen. (A) In normoxia, HIFα is hydroxylated by proline hydroxylases (PHDs) in the presence of oxygen (O_2), iron (Fe^{2+}), 2-oxoglutarate (2-OG), and ascorbate. Hydroxylated HIFα (OH) is then recognized by VHL. HIFα is tagged with ubiquitin (Ub) by the ubiquitin-protein ligase (E2), enabling recognition by the 26S proteasome and subsequent degradation. (B) In hypoxia, hydroxylation is inhibited and HIFα accumulates. HIFα translocates to the nucleus where it dimerizes with the beta subunit. The active transcription factor binds to hypoxia-response elements (HREs) within the promoters of target genes that mediate a range of cell functions, as indicated.
Adapted from Carroll and Ashcroft (2005).

the nucleus and forms an active transcription complex with the beta subunit. The active transcription factor directly activates genes containing hypoxia response elements (HREs), as well as interacting with other transcriptional control complexes, and influencing the expression of other transcription factors and signalling pathways.

The rate of HIF hydroxylation is influenced by other parameters besides oxygen tension. The PHD enzymes also require the cofactors ascorbate and reduced iron (Fe^{2+}) for enzymatic activity. As discussed in Chapter 328 on familial renal cancer, perturbations in the Krebs cycle can impact on HIF regulation by increasing the level of metabolic intermediates (e.g. fumarate and succinate) that act as PHD inhibitors. PHD activity can also be modulated by levels of reactive oxygen species (ROS) generated in the mitochondria and by nitric oxide (NO) (Kaelin, 2007).

HIF stabilization has numerous other reported effects in addition to direct effects on HRE-containing HIF target genes. These include altering the activity of p53, c-Myc, and Notch, as well as the transcriptional repressors Snail, TCF3, ZFHX1A, and ZFHX1B (Kaelin, 2007; Haase, 2010). As a consequence, HIF influences cell cycle progression, mitochondrial biogenesis, stem cell behaviour, and can drive epithelial-to-mesenchymal transition (EMT).

Hypoxia-inducible factor and the control of erythropoiesis

The starting point for our understanding of the HIF system was the drive to understand how changes in renal oxygenation were linked to increased production of EPO in the early 1990s. The *EPO* gene is now one of the best-understood HIF target genes, with a well-defined HRE in the 3′ enhancer region. Animal studies suggest that HIF-2α is predominantly responsible for control of erythropoiesis (Haase, 2010). In humans, activating mutations in HIF-2α have been linked with hereditary erythroctyosis (Lee and Percy, 2011).

Circulating EPO protein in the normal adult is produced mainly by the interstitial fibroblasts in the cortex and outer medulla of the kidney. Under normal physiological conditions only a small proportion of these cells located near the corticomedullary boundary produce EPO (Maxwell, 2003; Nangaku and Eckardt, 2006; Haase, 2010). In response to anaemia, individual fibroblasts are recruited to produce EPO in an all-or-none fashion, with recruitment steadily spreading outwards towards the capsule and the inner medulla. In contrast, reducing renal blood flow does not activate EPO production, because oxygen consumption falls in parallel with reduced delivery, due to reduced glomerular filtration and reduced tubular oxygen consumption.

In chronic renal failure there is a marked decrease in the erythropoietin response to anaemia (Nangaku and Eckardt, 2006). It is unclear whether this is because the fibroblasts are less responsive or because the relationship between blood oxygen content and interstitial oxygen tension is altered (Maxwell, 2003). Interestingly in autosomal dominant polycystic kidney disease the EPO response is generally preserved, in contrast to other forms of chronic kidney disease.

There is now considerable interest in using drugs to activate HIF and thus increase EPO expression (Kaelin, 2007; Muchnik and Kaplan, 2011). Most research into small molecule activators is directed towards inhibiting the PHD enzymes via their requirements for cofactors (Bruegge et al., 2007; Yan et al., 2010). The first phase II clinical trial of a PHD inhibitor, FG-2216, completed in 2004, and it has now been used to increase haemoglobin in both dialysis and pre-dialysis patients with anaemia of chronic kidney disease (Bernhardt et al., 2010).

In addition to its role in EPO production, HIF is involved in iron homeostasis (Haase, 2010). The activity of HIF hydroxylases is reduced if concentrations of intracellular free iron are reduced, and this leads to HIF activation. Hypoxia has been shown to alter expression of a number of genes involved in iron metabolism, in particular it suppresses production of the hormone hepcidin by the liver (Mole, 2010). Hepcidin levels are elevated in inflammation and also in chronic kidney disease which leads to impaired iron absorption and mobilization (Ashby et al., 2009). An attractive idea is that HIF activators might be superior to administering EPO when hepcidin levels are elevated.

There are a number of potential problems with using small molecule PHD inhibitors to treat anaemia in humans. In terms of 'on-target' effects, the PHDs may well act on other targets besides HIF, and HIF certainly alters the expression of numerous genes besides *EPO*. In fact, HIF activation is directly implicated in development of kidney cancer, and pulmonary hypertension. 'Off-target' effects are also highly likely since small molecules that inhibit PHD enzymes commonly affect other 2-oxoglutarate dependent dioxygenases that mediate such diverse cellular functions as DNA repair and histone demethylation.

Determining the best use for PHD inhibitors compared to EPO or its analogues (erythropoiesis stimulating agents (ESAs)) will be challenging and will require large-scale clinical trials. A key consideration is the good safety record of ESAs, although normalization of renal anaemia with ESAs is associated with a consistent, although small, increase in mortality compared to partial correction. At present the reason for this is unclear, and it is therefore conceivable that substitution of PHD inhibitors for ESAs would be beneficial from this perspective (See also Chapter 124).

Hypoxia-inducible factor and renal cancer development

Rare individuals have a germline mutation in the *VHL* gene, which causes von Hippel–Lindau disease (see Chapter 332) and is associated with renal cysts and a 70% lifetime risk of clear cell renal cell cancer. The cysts and tumours are caused by somatic mutations which inactivate the remaining wild type *VHL* allele, resulting in a cell without any functional VHL protein and constitutive activation of the HIF pathway. Importantly the great majority of clear cell carcinomas (~80%) occurring in the general population also have mutations which inactivate both copies of the *VHL* gene resulting in HIF activation.

Several lines of experimental evidence suggest that HIF activation, and in particular HIF-2α activation, is the driving pathway in VHL-related kidney cancer. But VHL has other reported actions, besides the destruction of hydroxylated HIFα, and these may contribute to its function as a tumour suppressor. Reported HIF-independent functions of VHL include diverse effects on transcriptional regulation as well as on the extracellular matrix and microtubule cytoskeleton (Frew and Krek, 2008). Understanding the extent to which these other functions of VHL may contribute to tumour suppression in the renal epithelium is a considerable challenge.

It is also uncertain which of the many downstream consequences of HIF activation may contribute to cyst and tumour formation. Interestingly, HIF activation impairs primary cilium formation and ciliary dysfunction is considered important in many other renal cystic disorders.

The development of renal tumours clearly requires additional genetic events besides loss of VHL function. In the mouse, loss of *vhlh* alone does not recapitulate the human VHL disease phenotype (Chen et al., 2010). By contrast, combined loss of *vhlh* and the tumour suppressor *pten* does lead to the formation of renal cysts and hepatic hemangioblastomas. In humans, ultra-deep sequencing has implicated loss of function of *PBRM1* in a significant proportion of cases (Varela et al., 2011).

There has been considerable interest in the possibility that HIF activation might be enhanced by the loss of other tumour suppressor genes besides VHL. For example, loss of function of *TSC2*, which underlies tuberous sclerosis, results in a degree of HIF activation, at least in part via mammalian target of rapamycin complex (mTORC) signalling (see Chapter 330). Likewise, the loss of function of fumarate hydratase or succinate dehydrogenase has been shown to result in HIF activation through decreased PHD activity. In these settings, activation of the HIF system leads to a high constitutive level of expression of angiogenic growth factor and glycolytic enzymes. This increased vascular growth factor production likely explains why these tumours (like VHL-deficient tumours) are highly vascular. However, HIF activation may not be driving tumour formation since the cysts that form in *fh* deficient mice do not require *hif-1a* or *hif-2a*.

Hypoxia-inducible factor and acute kidney injury

The high metabolic demands of renal tubules and basal hypoxia of the renal medulla render the kidney highly vulnerable to ischaemic damage. Acute renal ischaemia leads to modest activation of HIF-1α, in the renal tubular epithelial cells (Rosenberger et al., 2002). HIF-1α is known to upregulate factors that have been shown to be cytoprotective during acute renal injury, including vascular endothelial growth factor, hemeoxygenase-1, and EPO (Horikawa et al., 2002; Vesey et al., 2004; Haase, 2006). Moreover, in mice with genetic knockdown of *hif1a* and *hif2a*, histological injury was more severe after ischaemic reperfusion (Hill et al., 2008; Schley et al., 2011). This raises the possibility that increasing HIF activity might be beneficial in acute kidney injury. Pre-treatment with PHD inhibitors increases HIF activation in the kidney, and ameliorates toxic or ischaemic renal injury (Bernhardt et al., 2006; Haase, 2006; Hill et al., 2008; Weidemann et al., 2008). Treatment with the PHD inhibitor FG-4497 was recently shown to improve graft survival at 24 weeks in a rat model of allogenic kidney transplantation (Bernhardt et al., 2009).

These studies provide evidence that HIF activation can protect the kidney, as has been proposed for other organ systems (Haase, 2006; Semenza, 2011). Increasing HIF signalling with small molecules might ameliorate acute hypoxic injury in other settings, and improve clinical outcome in cardiothoracic surgery, myocardial infarction, and stroke. However, as with exploiting this pathway to treat anaemia, it will be important to consider the possibility of undesirable on- and off-target consequences

Hypoxia-inducible factor and chronic kidney disease

Glomerulosclerosis and tubulointerstitial scarring are the main processes underlying the progression of chronic renal diseases,

regardless of their aetiology (Nangaku et al., 2008). In 1998, Norman and Fine proposed the 'chronic hypoxia hypothesis' to explain this (Fine et al., 1998). According to this hypothesis, glomerular injury is transmitted to the tubulointerstitium and initiates a cycle of progressive tubular injury and decline in renal function.

In this model, glomerular damage produces local ischaemia and activates HIF signalling in hypoxic tubular cells. HIF activation may promote interstitial fibrosis by a number of mechanisms (Haase, 2006; Fine and Norman, 2008; Tanaka and Nangaku, 2010). First, regional ischaemia triggers phenotypic changes in tubular cells such as proliferation rate, EMT, and cell death. Second, HIF has direct effects on profibrotic gene expression. Lastly, HIF influences the inflammatory response, and thus could have distant effects via circulating cell populations which pass through the kidney.

A number of studies have provided *in vivo* evidence for the association between chronic kidney disease and hypoxia. These studies use a variety of imaging modalities, from microelectrodes to direct staining with 2-nitroimidazole compounds, to measure tissue hypoxia. Importantly, they have shown that a variety of renal injuries are associated with an early decline in tissue oxygenation (Fine and Norman, 2008). There is histological evidence from renal biopsies showing increased HIF expression in patients with diabetic nephropathy, immunoglobulin A nephropathy, polycystic kidney disease, and chronic allograft nephropathy (Tanaka and Nangaku, 2010). Moreover the changes in HIF expression correlate with the extent of tubulointerstitial injury. The concept is further supported by the fact that HIF activation in renal tubular cells results in features of EMT.

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CHAPTER 332

Von Hippel–Lindau disease

Thomas Connor and Patrick H. Maxwell

Introduction and epidemiology

Von Hippel–Lindau (VHL) disease (OMIM #193300) is a dominantly inherited familial cancer syndrome. It was named after Eugene von Hippel, who described angiomas in the eye in 1904, and Arvid Lindau, who described angiomas of the cerebellum and spine in 1927 (Melmon and Rosen, 1964). The incidence of VHL disease is approximately 1/36,000 live births, with similar prevalence in both genders and across all ethnic backgrounds (Maher et al., 2011). Eighty per cent of patients with VHL disease have a positive family history, but *de novo* VHL mutations are not uncommon.

Clinical features

The most frequent manifestations of VHL disease are retinal and central nervous system (CNS) haemangioblastomas, clear cell renal cell carcinoma (CCRCC), and phaeochromocytomas. These are commonly multiple and develop at a younger age than similar sporadic tumours in the general population. Patients may also develop non-secreting neuroendocrine tumours of the pancreas, endolymphatic sac tumours (which can result in deafness), epididymal papillary cystadenoma (men), and cysts of the uterine broad ligament (women) (Kaelin, 2007; Maher et al., 2011). In addition to tumours, patients develop multiple cysts of the kidney and other organs including the pancreas (Maher et al., 2011). Mortality is usually due to either metastasis of renal cell cancer or complications of CNS haemangioblastomas, however following the introduction of systematic screening for tumour development, life expectancy of VHL patients has greatly improved.

VHL disease shows a remarkable correlation between genotype and phenotype. Early reports noted that in different families, there were differing patterns of inheritance of haemangioblastoma, phaeochromocytoma, and CCRCC. This implied that different mutant alleles at the VHL locus were associated with distinct tumour suppressor capabilities. There are now > 350 distinct mutations in the *VHL* gene that have been linked to familial VHL disease (Nordstrom-O'Brien et al., 2010).

The clinical phenotype is categorized on the basis of incidence of haemangioblastoma, CCRCC, and phaeochromocytoma, as shown in Table 332.1. Striking aspects of the phenotype–genotype relationship are that loss-of-function alleles carry a low risk of phaeochromocytoma (type 1 disease), while some specific missense mutations predispose to phaeochromocytoma without other manifestations (type 2C disease). Significantly, mutations associated with type 2C disease do not alter the ability of VHL to regulate hypoxia-inducible factor (HIF) (Clifford et al., 2001).

Investigations

Diagnosis of VHL disease is based on clinical criteria or genetic testing (Melmon and Rosen, 1964; Lonser et al., 2003). Patients with a family history, and a CNS (excluding retinal) haemangioblastoma, phaeochromocytoma, or CCRCC are diagnosed with the disease. Those with no relevant family history must have either two or more CNS haemangioblastoma, or one haemangioblastoma of the CNS or retina and a visceral tumour (with the exception of epididymal and renal cysts which are common in the general population).

Mutation analysis is recommended to make a definitive diagnosis (Hes et al., 2001). Screening for germline *VHL* mutations in individuals with apparently sporadic cerebral and retinal haemangioblastoma should be considered since these are rare in the general population (Neumann et al., 2002). Direct sequencing is the gold standard for detecting small germline *VHL* mutations. 'Mutation negative' patients should be screened with techniques capable of identifying deletions, such as multiplex ligation-dependent probe amplification (MLPA), quantitative southern blotting, and fluorescent *in situ* hybridization (FISH) (Hes et al., 2007).

Aetiology and pathogenesis

VHL gene

The *VHL* gene is located at 3p25 (Latif et al., 1993; Iliopoulos et al., 1995; Renbaum et al., 1996). The *VHL* gene encodes two proteins as a result of alternative, in-frame translation initiation codons (Iliopoulos et al., 1998). These two proteins share most biological functions (Kaelin, 2007). The VHL protein contains two functional domains, termed alpha and beta (Stebbins et al., 1999). The alpha domain (residues 155–192) is required for binding elongin C, which results in the formation of a multiprotein E3 ubiquitin ligase complex. The beta domain (residues 63–154) is largely hydrophobic and acts as a substrate-docking site for HIF.

HIF is a highly conserved transcription factor that mediates cellular adaptation to low levels of oxygen (Kaelin and Ratcliffe, 2008; Linehan et al., 2010). Disruption of the VHL–HIF interaction results in constitutive activation of HIF target genes, such as vascular endothelial growth factor (*VEGF*), that play a role in the growth and metastasis of primary tumours. (For more details, see Chapter 331.)

Mutation of the *VHL* gene is detected in nearly all VHL families and, importantly, the great majority (~ 90%) of non-familial CCRCC (Nickerson et al., 2008). *VHL* functions as a classical 'two-hit' tumour suppressor gene. Thus tumour tissue shows inactivation of the remaining normal *VHL* allele in patients with

Table 332.1 Classification of VHL kindreds on the basis of tumour risk

Category	Risk of phaeochromocytoma	Risk of haemangioblastoma	Risk of renal cell carcinoma
1	Low	High	High
2A	High	High	Low
2B	High	High	High
2C	Yes	No	No

High risk = tumour type observed in > 50% affected individuals. Low risk = tumour type observed in< 5% of affected individuals. Yes = tumour type observed in all affected individuals. No = tumour type not observed in affected individuals.

VHL disease, either through mutation, deletion, or methylation. Importantly, reintroduction of a *VHL* gene suppresses CCRCC tumour xenografts (Iliopoulos et al., 1995). The fact that it suppresses growth of fully transformed cells implies a 'gatekeeper' rather than a 'caretaker' role in the renal epithelium. It is notable that mutations in *VHL* are rare in all other tumours apart from CCRCC and haemangioblastoma. This suggests that in other cancers, VHL loss of function does not confer a selective advantage.

VHL and haemangioblastoma

Haemangioblastomas are the commonest manifestations of VHL disease, occurring in up to 80% of patients. They develop earlier than in the general population, with diagnosis almost 20 years earlier than in sporadic cerebellar haemangioblastomas (Wanebo et al., 2003). They are located most commonly in the cerebellum and retina (Melmon and Rosen, 1964; Kaelin, 2007; Maher et al., 2011). These lesions are typically cystic tumours of endothelial cells and lipid-filled stromal cells embedded in capillary networks. It is now clear that the stromal cells drive the neoplastic process by paracrine release of angiogenic factors, as a direct consequence of loss of VHL function (Wizigmann-Voos et al., 1995). They are rarely malignant, but enlargement or bleeding within the CNS can result in neurological damage and death.

The mutations in *VHL* that are associated with haemangioma development (type 1, 2A, and 2B disease) all show defective HIF regulation (Kaelin, 2007; Kaelin and Ratcliffe, 2008). This explains why haemangioblastomas frequently overproduce HIF-responsive growth factors, such as VEGF, platelet-derived growth factor (PDGF), and transforming growth factor alpha (TGFα).

VHL and clear cell renal cell cancer

VHL patients have a 70% risk of developing CCRCC by the age of 60 years (Maher et al., 1991; Whaley et al., 1994). The mean age of onset is 44 years, compared to 62 years for sporadic CCRCC in the general population. Renal cysts are also common in VHL patients, and show a higher rate of malignant transformation than the simple cysts seen in the general population (Kaelin, 2004). VHL-defective CCRCC is highly vascular, due to the overproduction of angiogenic growth factors (Kaelin, 2007).

CCRCCs were believed to originate from the proximal renal tubule. However, premalignant multicellular lesions in VHL patients are almost always in the distal renal tubule, suggesting that the tumours may actually arise in this part of the nephron (Mandriota et al., 2002). Loss of VHL function results in HIF activation and loss of several important characteristics associated with the distal nephron, including expression of E cadherin and Tamm–Horsfall protein (Mandriota et al., 2002; Esteban et al., 2006).

VHL mutations linked to CCRCC (type 1 and 2B) show complete loss of the ability to regulate HIF consistent with HIF activation being critical in tumourigenesis. Conversely, the low or absent risk of CCRCC seen with type 2A, type 2C, and Chuvash polycythaemia mutations is plausibly related to the fact that all of these are associated with at least a partial ability to regulate HIF (Knauth et al., 2006).

The activation of HIF in VHL-associated CCRCC explains why these tumours usually overproduce a wide range of angiogenic growth factors, such as VEGF, PDGF, and TGF (Wiesener et al., 2001; Kaelin, 2007; Kaelin and Ratcliffe, 2008). It may also explain why targeted therapies that block the VEGF pathway have clinical activity as single agents in kidney cancer.

VHL and phaeochromocytoma

Phaeochromocytomas are neoplastic intra- or extra-adrenal gland lesions that appear histologically as an expansion of large chromaffin-positive cells, derived from neural crest cells (Lee et al., 2005). Seven to 18% of VHL patients are afflicted with phaeochromocytomas, with a mean age of onset of 29.9 years (Garcia et al., 1997; Bryant et al., 2003). Untreated phaeochromocytomas can result in severe, episodic hypertension with subsequent heart disease, stroke, or malignant hypertension. *VHL* is one of a number of genes associated with familial phaeochromocytoma. In contrast to sporadic haemangioblastoma and CCRCC, which are associated with bi-allelic *VHL* inactivation, this is unusual in sporadic phaeochromocytoma (Kaelin, 2007).

Phaeochromocytomas are derived from sympathetic neuronal progenitor cells. *VHL* mutations associated with a high risk of phaeochromocytoma (type 2 disease) are relatively conservative missense mutations. Deletions or disruptive mutations carry a low risk of phaeochromocytoma development, implying that this is incompatible with complete loss of function. Mutations associated with type 2C disease, such as L188V, appear not to impair the ability to regulate HIF (Clifford et al., 2001; Hoffman et al., 2001). In addition, bi-allelic *VHL* inactivation is very rare in sporadic phaeochromocytomas, while common in sporadic haemangioblastomas or CCRCC. This suggests that phaeochromocytoma development in VHL disease is due to the loss of a critical VHL function unrelated to HIF, and that this has to occur during development. Notably, the other genes underlying familial phaeochromocytoma are also rarely implicated in these tumours when not mutated in the germline and appear to be mutually exclusive with one another and with *VHL* mutations (Maher and Eng, 2002; Bryant et al., 2003).

A model that potentially unifies all of the familial paraganglioma genes was recently proposed, centring on the role of PHD3 in apoptosis during sympathetic neural development (Lee et al., 2005). It remains to be determined, however, why deletions and highly disruptive mutations in *VHL* are associated with a low risk of phaeochromocytoma.

Familial erythrocytosis

In addition to the association with VHL disease, *VHL* mutations are also associated with congenital polycythemia (also known as familial erythrocytosis-2; OMIM #263400). In this condition,

Table 332.2 Recommended adult screening

Associated tumours	Age range of onset (years)	Screening recommendations
Retinal angioma	1–67 (mean 25)	Early childhood—lifelong Annual ophthalmic exam
CNS haemangioblastoma	9–78 (mean 33)	Adolescence—lifelong MRI brain and spine every 12–36 months
Renal cell cancer	16–67 (mean 39)	16 years—lifelong Annual abdominal MRI (or USS)
Phaeochromocytoma	5–58 (mean 30)	Early childhood—lifelong Annual plasma normetanephrines
Endolymphatic sac tumour	12–50 (mean 22)	See CNS haemangioblastoma
Pancreatic tumour	5–70 (mean 36)	See renal cell cancer
Cystadenoma	Unknown	None

CNS = central nervous system; MRI = magnetic resonance imaging; USS = ultrasound screening.

polycythemia (erythrocytosis) is inherited in an autosomal recessive fashion with either homozygous or compound heterozygous alleles (Ang et al., 2002; Pastore et al., 2003; Semenza, 2009). This is in contrast to classical VHL disease which is inherited in an autosomal dominant fashion. Most mutations have been documented only as isolated case reports, except for several hundred patients with the R200W mutation, first described in the Chuvash Republic.

Patients with congenital erythrocytosis manifest increased red blood cell counts, increased frequency of vertebral haemangiomas, varicose veins, and elevated serum VEGF concentrations, as well as premature mortality related to cerebrovascular events and peripheral thrombosis, but do not develop tumours. Occasional patients with classical VHL disease develop polycythaemia when a CCRCC, haemangioblastoma, or phaeochromocytoma produces significant amounts of erythropoietin.

VHL mutations associated with congenital erythrocytosis are distinct from those associated with classical VHL disease (Pastore et al., 2003). They are typically missense mutations, located at the C-terminus of the protein, which are thought to have only a minor effect on VHL's ability to regulate HIF. It has now been shown that mutations in other components of the oxygen-sensing pathway, affecting *PHD2* and *HIF2A*, can also give rise to congenital erythrocytosis (Semenza, 2009).

Patients with Chuvash polycythaemia exhibit disordered vascular physiology, with an exaggerated physiological response to hypoxia, characterized by abnormalities in respiratory and pulmonary vascular regulation (Semenza, 2009). This response is similar to that seen in association with acclimatization to the hypoxia of altitude and supported by studies of the Chuvash knockin mouse. Furthermore, pulmonary hypertension has also been observed in individual erythrocytosis-causing mutations in *HIF2A* (Gale et al., 2008).

Treatment and outcome

Screening for clinical manifestations in individuals who are known to carry a *VHL* mutation should begin in infancy. *The VHL Handbook* provides guidelines for screening (VHL Alliance, 2012). Early treatment reduces both morbidity and mortality (Choyke et al., 1995; Lonser et al., 2003). Similarly, anyone at risk of inheriting a *VHL* mutation who has not had genetic testing performed should undergo regular clinical screening to identify tumours before they result in avoidable harm (Poulsen et al., 2010). (See Table 332.2.)

New drug therapies have been developed that target some of the consequences of VHL loss-of-function (Linehan et al., 2010). Stabilization of HIF increases the expression of a number of secreted growth factors, including VEGF, which are critical to the angiogenic phenotype. Current therapies, including monoclonal antibodies and small molecule inhibitors, have been successfully designed to inhibit growth factor signalling.

Further reading

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CHAPTER 333

Molecular basis of complement-mediated renal disease

Nicholas Medjeral-Thomas, Anna Richards, and Matthew C. Pickering

Complement

The complement system contributes to pathogen destruction, removal of circulating immune complexes, and augmentation of immune responses. Complement activation is tightly regulated enabling appropriate activation (e.g. on a pathogen) but preventing inappropriate activation (e.g. along healthy glomerular endothelium). It is now clear that the balance between activation and regulation is critical: pathology develops if there is either too little or too much complement activation (Fig. 333.1). The kidney is particularly vulnerable to injury in the setting of complement dysregulation. Examples include C3 glomerulopathy and atypical haemolytic uraemic syndrome (aHUS). Complement dysregulation in these conditions is commonly, but not exclusively, influenced by complement mutations. We now appreciate that polymorphic variation within complement constituents influences the balance between regulation and activation and that this also influences susceptibility to disease (Heurich et al., 2011). This is exemplified by the association between complement polymorphisms and age-related macular degeneration (AMD) (Edwards et al., 2005; Haines et al., 2005; Klein et al., 2005).

The complement activation pathways, activation of C3 and complement regulation are depicted in Fig. 333.2. Complement is activated through the classical, lectin, and alternative pathways (Fig. 333.2A). All pathways result in the generation of enzyme complexes (C3 convertases) that activate C3 (Fig. 333.2B). This activation results in the generation of anaphylatoxin (C3a), opsonin (C3b), and activation of the complement terminal pathway through the generation of enzyme complexes (C5 convertases) that activate C5. Terminal pathway activation results in the production of anaphylatoxin (C5a) and membrane attack complex (MAC, C5b-9), a protein complex that damages cell membranes by introducing pores in the membrane. Important points to note are (1) activated C3 (denoted C3b) is rapidly amplified through a positive feed-back loop, termed the C3b amplification loop. The result is the generation of millions of C3b molecules within minutes of triggering activation. (2) C3b can be further proteolytically cleaved to generate biologically active fragments (iC3b and C3d) that mediate their effects through interaction with complement receptors (Fig. 333.3). However, once C3b is cleaved it can no longer contribute to C3b amplification.

Activation of complement is tightly regulated to prevent damage to host tissues. Complement regulators act in plasma ('fluid-phase') and surfaces at multiple steps in the activation cascade (Fig. 333.2C). It is particularly important to regulate the spontaneously ('always on') alternative pathway and the C3b amplification loop (Fig. 333.2C). The regulators of these pathways are identical and include complement factor H (CFH) and factor I (CFI). In the absence of either of these regulators, alternative pathway activation proceeds unhindered and severe secondary depletion of C3 develops (Pickering and Cook, 2008). Important points to note include (1) CD59 is the major membrane-bound regulator of the MAC. It prevents the assembly of the MAC. In paroxysmal nocturnal haemoglobinuria, acquired deficiency of CD59 in erythrocytes renders the affected cells susceptible to complement-mediated lysis. (2) CFI mediates the proteolytic conversion of C3b to iC3b but only in the presence of an additional protein which is termed a cofactor. These include CFH, CD46 (also called membrane cofactor protein) and CR1 (complement receptor 1, CD35). (3) CFH is part of a family of structurally related proteins. These comprise CFH itself, together with five proteins, encoded by individual genes (Fig. 333.3A), and named complement factor H-related (CFHR) proteins 1, 2, 3, 4, and 5. All of these proteins are comprised of subunits, termed short consensus repeat (SCR) domains, approximately 60 amino acids in size, and common to all complement regulators encoded along a region of chromosome 1 (regulators of complement activation gene cluster). The *CFH-CFHR* loci is characterized by blocks of high sequence homology (long homologous repeats, LHRs). The presence of LHRs results in frequent (polymorphic) and rare (mutations) structural variation (copy number variation, CNV) within this region. A very common CNV results in deletion of both the *CFHR1* and *CFHR3* genes (ΔCFHR3-1) (Fig. 333.3B) and exerts a protective effect in immunoglobulin A nephropathy (Gharavi et al., 2011) and AMD (Hughes et al., 2006). Rarer CNV within this region is associated with aHUS (Venables et al., 2006; Maga et al., 2011; Francis et al., 2012) (Fig. 333.3C) and familial C3 glomerulopathy

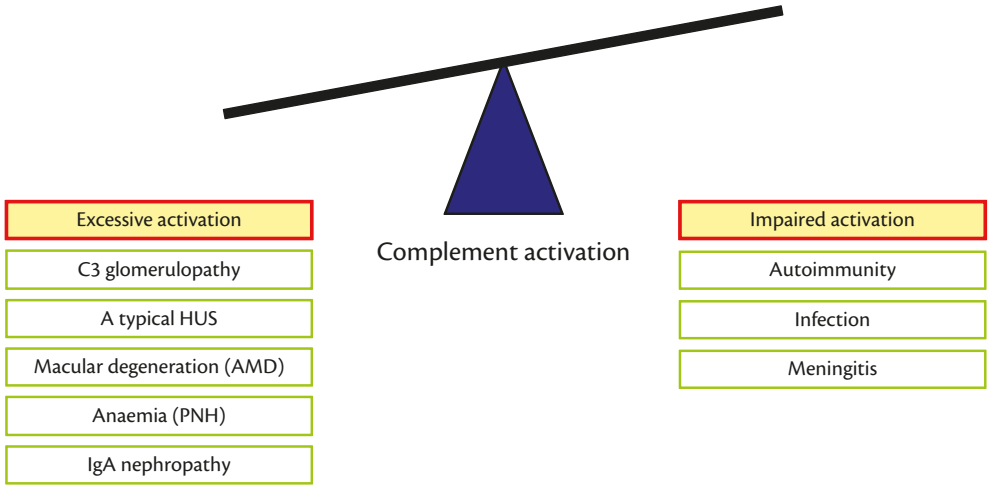

Fig. 333.1 Complement regulation and disease. The balance between complement activation and regulation is influenced by both polymorphisms and mutations. Either too little regulation or too much activation can be associated with disease. AMD = age-related macular degeneration; PNH = paroxysmal nocturnal haemoglobinuria.

(Gale et al., 2010; Malik et al., 2012) (Fig. 333.3D). (4) CFH negatively regulates C3 through its initial four amino-terminal SCR domains (regulatory domains) (Fig. 333.4A) whilst it interacts with polyanions on surfaces through domains 19 and 20 (surface recognition domains) (Fig. 333.4B). This functional localization is important for understanding the pathogenesis of CFH-associated renal disease.

Complement mutations and renal disease

Complement mutations may be conveniently divided into those that result in impaired activation (complement deficiency states) and those that result in impaired regulation (complement dysregulation).

Complement deficiency states

Homozygous deficiency of complement activation proteins is with the exception of C9, mannose-binding lectin (MBL), and C2, extremely rare. The phenotypes are listed in Table 333.1. These deficiencies are associated with increased susceptibility to bacterial infection which is most problematic in childhood before the antibody repertoire has developed. Alternative and terminal pathway deficiency is associated with an increased incidence of neisserial infection. Classical pathway activation protein deficiency is associated with increased susceptibility to a systemic lupus erythematosus (SLE)-like illness (reviewed in Botto et al., 2009). Homozygous C3 deficiency has been associated with membranoproliferative glomerulonephritis (MPGN; see Chapter 80) (Borzy and Houghton, 1985; Borzy et al., 1988; Botto and Walport, 1993) and lupus nephritis can occur as part of the manifestations of the SLE-like illness associated with homozygous classical pathway deficiency (reviewed in Pickering et al., 2000).

Complement dysregulation

The phenotypes associated with abnormalities in complement regulation are summarized in Table 333.1. Increased activation of the alternative pathway is associated with C3 glomerulopathy and aHUS.

C3 glomerulopathy

C3 glomerulopathy (see Chapter 80) is a classification term introduced to describe glomerular pathology characterized by the presence of C3 in the absence of immunoglobulin (Fakhouri et al., 2010). The term is used irrespective of light microscopic (such as the presence or absence of MPGN changes) and ultrastructural changes (such as the location of electron-dense change). The best defined pathological entities within C3 glomerulopathy are dense deposit disease (DDD) and C3 glomerulonephritis (Table 333.2). The presence of C3 in the absence of immunoglobulin implies antibody-independent activation of complement through either the alternative or lectin pathways. Accordingly, C3 glomerulopathy is associated with acquired and genetic factors that result in enhanced activation of the alternative pathway.

C3 glomerulopathy and acquired dysregulation of C3 activation

The commonest acquired abnormality is C3 nephritic factor (C3NeF) which is present in the majority of DDD patients and approximately 50% of patients with C3 glomerulonephritis (for the largest characterized C3 glomerulopathy series see Servais et al. (2012)). C3NeF activity was first described in patients with glomerulonephritis whose serum contained a factor that potentiated C3 activation (Spitzer et al., 1969). The factor was subsequently demonstrated to be immunoglobulin (Davis et al., 1977). C3NeF (more appropriately termed C3 nephritic antibody) may result in predominant activation of C3 alone (properdin-independent C3NeF) or activation of both C3 and C5 (properdin-dependent C3NeF) (Mollnes et al., 1986; Clardy et al., 1989; Tanuma et al., 1990; Varade et al., 1990). C3NeF is consequently typically but not invariably associated with reduced plasma C3 levels (Servais et al., 2012) and, for properdin-dependent C3NeF, a reduction in C5 and other terminal pathway components. C3NeF interact with the C3 convertase and prevent its spontaneous decay and its inactivation by regulators (Daha et al., 1976). They may be detected among individuals with partial lipodystrophy with or without renal disease (Sissons et al., 1976) and even among healthy individuals (Gewurz et al., 1983). As a consequence, whilst their association with enhanced plasma C3 activation was never in doubt, it was

considered that their relationship to renal disease might be an epiphenomenon (Mathieson and Peters, 1994). However, a direct relationship between uncontrolled C3 activation and C3 glomerulopathy has been established in animal models of uncontrolled C3 activation due to complete CFH deficiency (Hogasen et al., 1995; Pickering et al., 2002; Rose et al., 2008). Notably, In CFH-deficient mice, spontaneous C3 glomerulopathy was absolutely dependent on the ability to activate C3 (Pickering et al., 2002). C3 glomerulopathy is also associated with autoantibodies, distinct from C3NeF, that trigger uncontrolled C3 activation. These include inhibitory autoantibodies to the regulatory domains of CFH (Meri et al., 1992; Jokiranta et al., 1999) and stabilizing autoantibodies

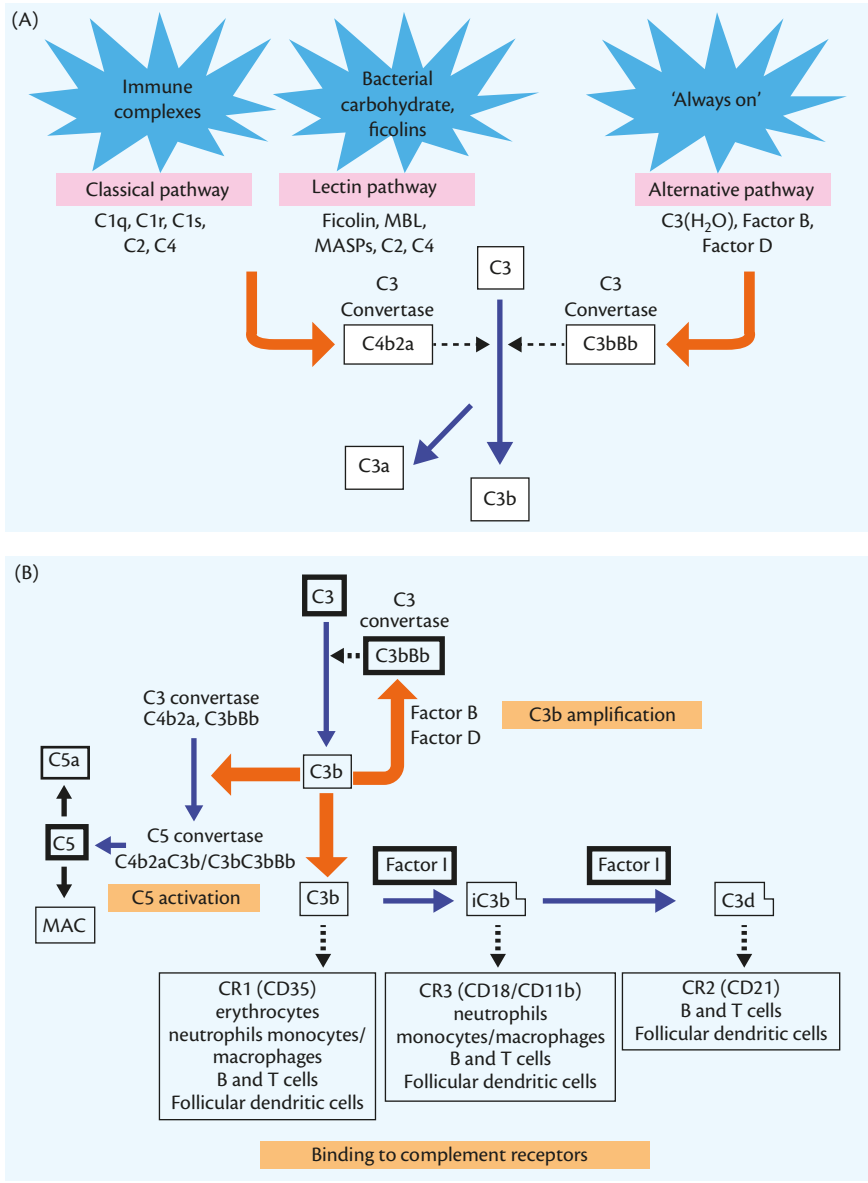

Fig. 333.2 (A) Complement activation pathways. Complement can be activated through three pathways, all of which converge on the central complement component C3. The classical pathway is triggered by the binding of the Fc portion of IgG or IgM with C1q. IgG4 does not bind C1q and hence cannot activate the classical pathway. The Fc portion of IgG4 is frequently used in monoclonal antibody therapy to prevent the therapeutic antibody from activating complement. The lectin pathway is triggered by ficolins and sugar residues on bacterial surfaces binding to mannose-binding lectin. The alternative pathway is continuously activated. Plasma C3 is susceptible to hydrolysis and hydrolysed C3 ($C3H_20$) initiates alternative pathway activation. This pathway can therefore be thought of conceptually as 'always on'. Under physiological conditions it is tightly regulated and therefore only low-level spontaneous activation occurs. MASP = MBL-associated serine protease; MBL = mannose binding lectin. (B) Biological roles of activated C3. C3b can be rapidly amplified through a positive feed-back loop (C3b amplification loop) resulting in rapid generation of millions of C3b molecules. C3b can also generate an enzyme complex (C5 convertase) that is capable of cleaving C5 thereby triggering activation of the terminal complement pathway and the generation of anaphylatoxin (C5a) and membrane attack complex. C3b and its proteolytic fragments, iC3b and C3d, mediate important biological functions by interacting with membrane-bound complement receptors. MAC = membrane attack complex. (C) Complement regulation. Complement activation is tightly regulated by proteins termed complement regulators. These act at different stages of the pathways and may be soluble or membrane-bound proteins. Blue boxes depict soluble regulators; yellow circles depict membrane-bound proteins. C4bp = C4-binding protein; CR1 = complement receptor 1 (CD35); DAF = decay-accelerating factor (CD55); MBL = mannose-binding lectin; MCP = membrane cofactor protein (CD46).

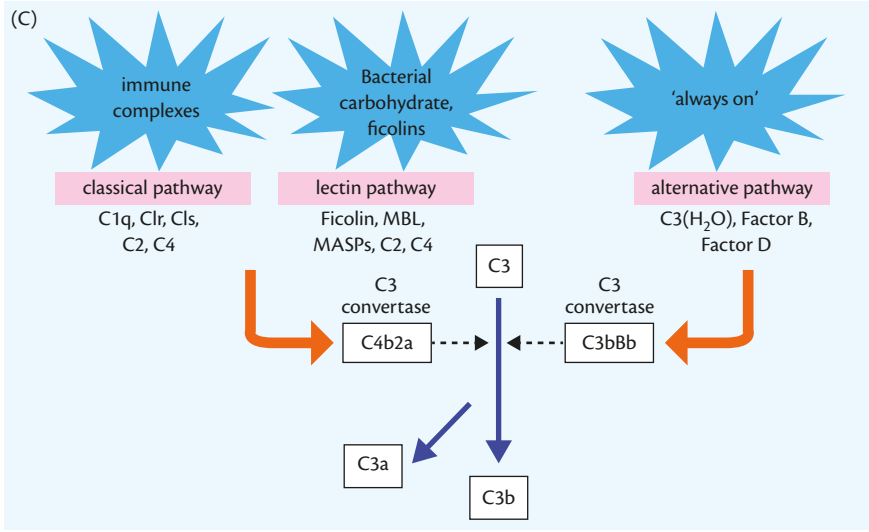

Fig. 333.2 Continued

against factor B (Strobel et al., 2010; Chen et al., 2011) and C3 (Chen et al., 2011).

C3 glomerulopathy and genetic dysregulation of C3 activation

DDD is associated with homozygous CFH deficiency (Levy et al., 1986; Lopez-Larrea et al., 1987; Servais et al., 2012), loss-of-function mutations in the regulatory domains of CFH and a gain-of-function C3 mutation (Martinez-Barricarte et al., 2010) (Fig. 333.4C). The characterization of the gain-of-function mutation in C3 among a family with DDD was especially informative since this mutant protein resulted in specific enhancement of C3 activation in plasma providing, arguably, the most definitive evidence to date that DDD is a disorder of fluid-phase C3 activation (Martinez-Barricarte et al., 2010). Notably, abnormal C3 molecules (characterized at the protein but not genetic level) that generated C3 convertases resistant to inactivation by CFH, have also been described in familial non-DDD C3 glomerulopathy (Marder et al., 1983; Linshaw et al., 1987).

Whilst mechanistically revealing, these mutations are rare among patients with C3 glomerulopathy. In the largest series reported to date, genetic factors were analysed in 56 patients with C3 glomerulonephritis and 29 patients with DDD (Servais et al., 2012). Among the 56 individuals with C3 glomerulonephritis, mutations in CFH, CFI, and CD46 were demonstrable in seven, three, and one case respectively (Servais et al., 2012). With the exception of one individual with homozygous CFH deficiency, all mutations were heterozygous. In the 29 patients with DDD, five had CFH mutations (one homozygous) and none had mutations in either CFI or CD46 (Servais et al., 2012). It is notable that DDD has not been associated with CFI deficiency. Studies in CFI-deficient mice have demonstrated that the CFI-mediated conversion of C3b to iC3b is essential for the accumulation of C3 along the glomerular basement membrane (Rose et al., 2008) suggesting the ability to generate iC3b is important in DDD pathogenesis. This series also included 48 individuals with MPGN type 1, a lesion distinct from C3 glomerulopathy as it is an immune complex-mediated disorder. In the MPGN type 1 cohort, eight mutations were identified, five in CFH and three in CFI. Two of the CFH mutations associated with MPGN type 1 were homozygous. Therefore in this series homozygous CFH deficiency was associated with DDD, C3 glomerulonephritis, and MPGN type 1. This demonstrates that the relationship between mutation and renal phenotype is influenced by other factors. These include environmental factors such as infection and polymorphic genetic variation. Infection is common in CFH and CFI deficiency due to the secondary C3 deficiency. Interestingly, MPGN type 1 can develop in the complete absence of C3: this lesion has been described in homozygous C3 deficiency, where there is an increased infection rate, in both dogs (Blum et al., 1985) and man (Borzy and Houghton, 1985; Botto and Walport, 1993). Consequently, mutations in MPGN type 1 may derive from the association between infection and this lesion. Polymorphic genetic factors among both CFH and CD46 appear to influence susceptibility to C3 glomerulopathy (Smith et al., 2011; Servais et al., 2012).

A major development in C3 glomerulopathy was the recognition that familial C3 glomerulonephritis was associated with CNV mutations within the *CFHR* loci (Fig. 333.3D). The seminal observation was the characterization of an internal duplication within the *CFHR5* gene as the cause of familial C3 glomerulonephritis in Cypriots (Gale et al., 2010). CFHR5 nephropathy is characterized by persistent microscopic haematuria, episodes of synpharyngitic macroscopic haematuria, recurrence in renal transplantation and, curiously, a more severe clinical course in males (Gale et al., 2010; Athanasiou et al., 2011; Vernon et al., 2011). Understanding how this mutation causes C3 glomerulopathy is challenging since, unlike CFH, the biological roles of the CFHR proteins are poorly understood. One clear distinction with DDD is the absence of fluid-phase dysregulation. Low plasma C3 levels, common among DDD patients, were not seen in patients with CFHR5 nephropathy, suggesting that CFHR5 exerts its effects within the kidney. Additional abnormal variation within this loci include a hybrid *CFHR3-1* gene in an Irish family with familial C3 glomerulopathy, originally defined pathologically as membranoproliferative glomerulonephritis type III (Malik et al., 2012) and others will no doubt follow. These novel descriptions illustrate the complex relationship between complement regulation and C3 glomerulopathy. The major challenge is

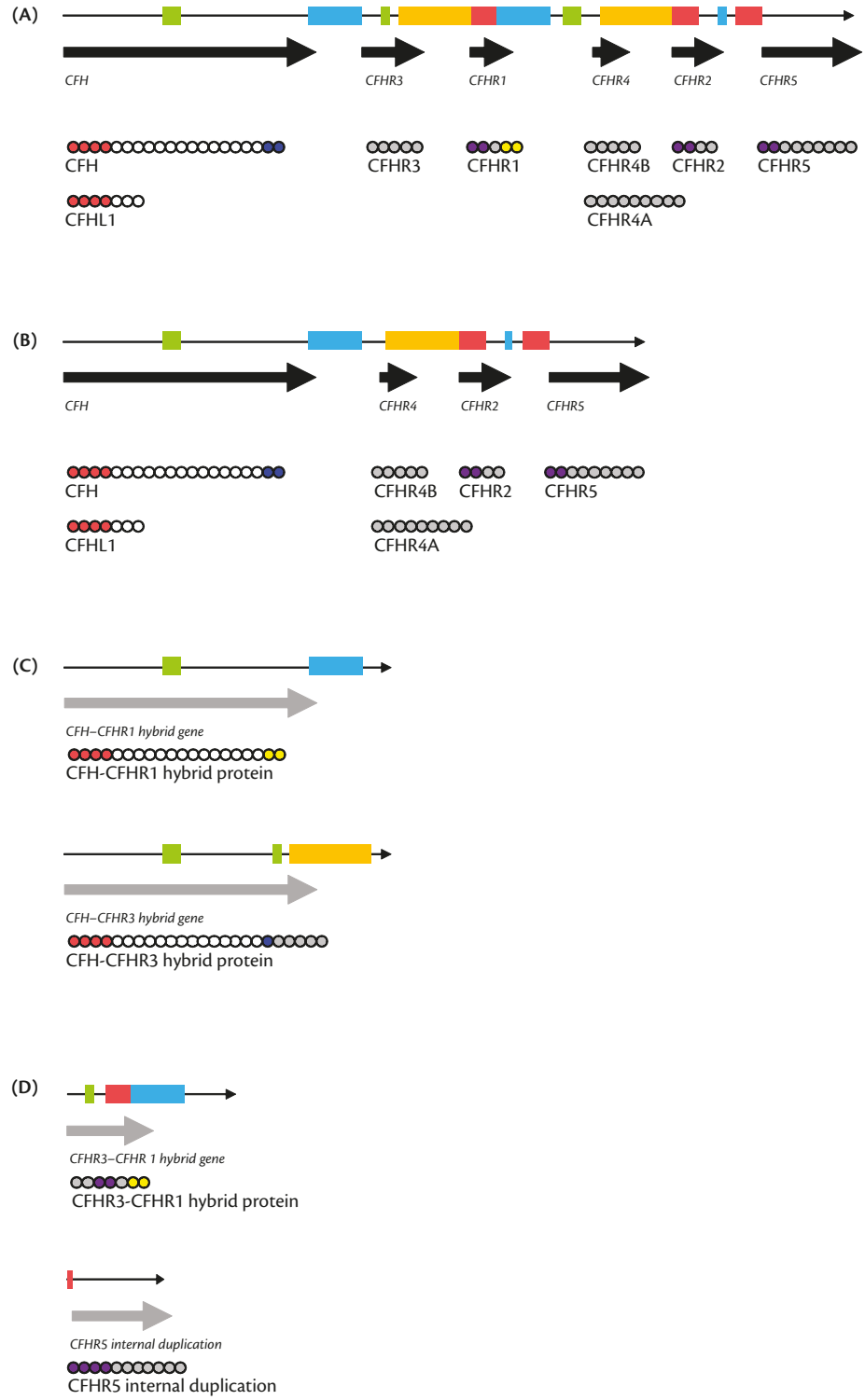

Fig. 333.3 The CFH-CFHR family. In panels A-D, genetic map is shown above corresponding protein products. (A) Complement factor H (CFH) and CFH-related (CFHR) proteins comprise a family of proteins that are composed of subunits termed short consensus repeat (SCR) domains. CFH and CFHR1–5 are each encoded by distinct genes. The *CFH* gene encodes CFH and CFH-like protein 1 (CFHL1) an alternative splice transcript. Similarly the *CFHR4* gene encodes two splice variants: CFHR4A and CFHR4B. C3 regulation domains of CFH comprise the first four amino-terminal SCR domains (●) whilst surface recognition is mediated by domains 19 and 20 (●). CFHR proteins share sequence similarity with each other and CFH. The first two domains of CFHR1, CFHR2, and CFHR5 are almost identical (●). The *CFH-CFHR* locus contains areas of high sequence homology (depicted by colouring) that predispose to structural rearrangements. (B) Deletion of both the *CFHR3* and *CFHR1* genes is a common polymorphism (ΔCFHR3-1) which has been associated with protection against immunoglobulin A nephropathy and age-related macular degeneration.
(C) Rearrangements associated with aHUS include hybrid genes that result in loss of the surface recognition domains of CFH. These include a hybrid *CFH-CFHR1* gene in which SCR domains 19 and 20 (shaded red) were replaced by domains 4 and 5 of CFHR1 (shaded yellow (Venables et al., 2006)) and a hybrid *CFH-CFHR3* gene (Francis et al., 2012).
(D) Rearrangements associated with C3 glomerulopathy include an internal duplication of the *CFHR5* gene resulting in a CFHR5 protein containing two additional SCR domains at its amino terminal end (Gale et al., 2010) and a hybrid CFHR3-1 gene encoding a unique protein containing SCR domains from CFHR1 and CFHR3 (Malik et al., 2012).

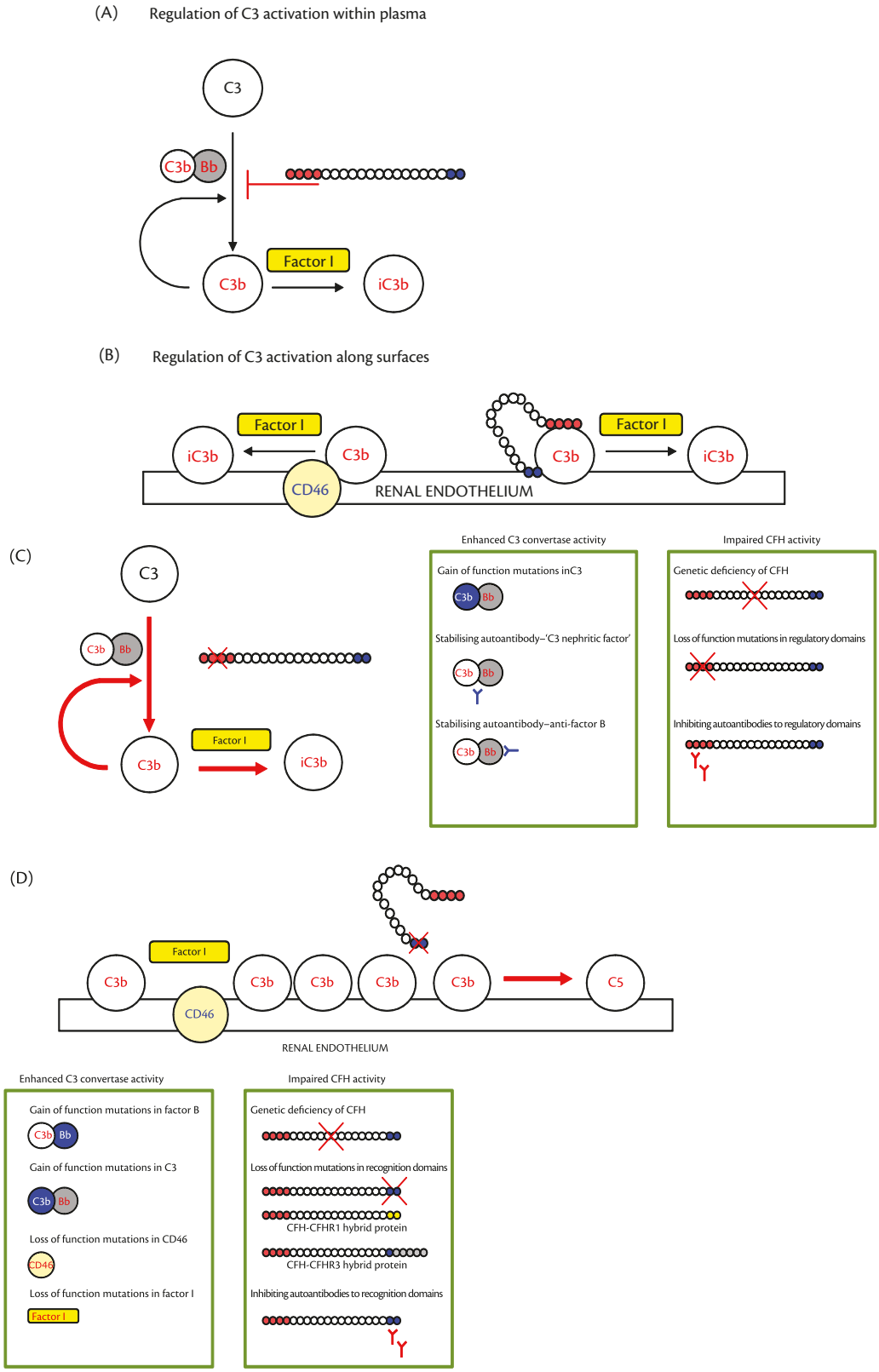

Fig. 333.4 C3 regulation in health and disease. (A) Regulation of C3 activation within plasma is mediated by the combined actions of complement factor H (CFH) and complement factor I (CFI). CFH negatively regulates C3 activation through its first four amino terminal SCR domains (shaded red). CFI converts C3b to iC3b. Unlike C3b, iC3b cannot participate in the further generation of C3b through the C3b amplification loop. (B) Regulation of C3 activation along surfaces. C3b is converted to iC3b by factor I. In both plasma and surfaces this conversion requires a cofactor that can be CD46, CFH and complement receptor 1 (CR1, CD35, not depicted). CFH interacts with surfaces through its recognition domains (SCR domains 19 and 20, shaded blue) which interact with surface polyanions and C3b. (C) Enhanced plasma C3 activation is typically seen in dense deposit disease. Abnormal activation may result from either defective activity of complement factor H (CFH) or enhanced enzymatic conversion of C3 by factors that result in an increased stability of the C3 convertase. (D) Enhanced surface C3 activation underlies the pathogenesis of atypical haemolytic uraemic syndrome (aHUS) where uncontrolled C3 activation along the renal endothelium occurs as a consequence of factors that either impair CFH function or enhance C3 convertase activity. Note that impairment of CFH regulation domains (shaded red) is associated with abnormal plasma C3 activation whilst impairment of surface recognition domains (shaded blue) is associated with abnormal surface C3 activation. For details of the hybrid genes see Fig. 333.3C.

Table 333.1 Complement deficiency and disease

Complement deficiency	Phenotype	Comments
Activation proteins		
Classical pathway deficiency: C1q, C1r, C1s, C2, C4	SLE Recurrent encapsulated bacterial infections	All extremely rare except C2 deficiency where estimated prevalence is 1/20,000 Association with SLE weakest for C2 deficiency
Alternative pathway deficiency: Factor B, Factor D	Recurrent meningococcal infections Recurrent encapsulated bacterial infections	All extremely rare (factor B deficiency only reported in a single incompletely characterized case)
Lectin pathway deficiency: MBL, ficolins, MASP-1, MASP-2 and MASP-3, CL-K1	Increased infection amongst immunocompromised individuals (MBL deficiency) H-ficolin deficiency associated with necrotizing enterocolitis Mutations in the genes encoding CL-K1 (*COLEC11*) and MASP-3 and MASP-1 (*MASP1*) associated with an autosomal recessive developmental syndrome termed '3MC syndrome'[a]	All extremely rare except MBL deficiency where estimated prevalence is 5% in Caucasian populations
Terminal pathway C5, C6, C7, C8, and C9	Recurrent meningococcal infections	All rare except C9 deficiency in Japanese where estimated prevalence is 1/1000
C3	Recurrent encapsulated bacterial infections Membranoproliferative glomerulonephritis (rare) SLE-like illness (rare)	Extremely rare
Regulatory proteins		
C1INH Negative regulator of: classical and lectin pathways, contact system, coagulation system, fibrinolytic system	Hereditary angio-oedema	Estimated prevalence 1/50,000 Angio-oedema results from uncontrolled production of bradykinin due to dysregulation of the contact system, i.e. does not arise from uncontrolled complement activation Associated with low C4 due to uncontrolled classical pathway activation
CFH, CFI, and CD46 Negative regulators of the alternative pathway and C3b amplification loop	Atypical haemolytic uraemic syndrome (aHUS) C3 glomerulopathy (dense deposit disease, C3 glomerulonephritis) Membranoproliferative glomerulonephritis type I (MPGN1)	All rare aHUS typically manifests in heterozygous deficiency states but has been reported in homozygous CFH deficiency (Thompson and Winterborn, 1981) DDD is associated with CFH but not CFI deficiency
Factor H-related protein 5 Putative regulator of C3 processing within the kidney—biological function incompletely understood	Familial C3 glomerulonephritis (also termed CFHR5 nephropathy)	Endemic among individuals with Cypriot ancestry
CD59 Negative regulator of terminal pathway activation	Paroxysmal nocturnal haemoglobinuria	Rare Acquired somatic mutation Renders CD59-deficient erythrocytes susceptible to complement-mediated intravascular haemolysis
Properdin Positive regulator of C3 activation	Recurrent meningococcal infections	Rare X-linked deficiency

C1INH = C1 inhibitor; CD46 = also known as membrane cofactor protein; CFH = complement factor H; CFI = complement factor I; CL-K1 = also known as collectin-11; MBL = mannose-associated lectin; MASP-2 = MBL-associated serine protease.

[a]3MC syndrome is a term used to describe clinically identical syndromes that were independently described: the Mingarelli, Malpuech, Michels, and Carnevale syndromes.

Adapted from Pickering, M. C. and Bakshi, J. (2013). Complement. In R. A. Watts, P. G. Conaghan, C. Denton *et al.* (eds.) *Oxford Textbook of Rheumatology* (4th ed.), pp. 463–8.

to understand the biological role of the CFHR proteins and, of course, how abnormalities within this protein family mediate C3 glomerulopathy.

Atypical haemolytic uraemic syndrome

aHUS refers to the triad of microangiopathic hemolytic anemia, thrombocytopenia, and renal thrombotic microangiopathy in the absence of Shigatoxin-producing *Escherichia coli* or *Streptococcus pneumoniae* infection. aHUS is associated with impaired regulation of the complement that may be inherited or acquired.

Complement mutations in aHUS can be divided into (a) loss-of-function mutations in complement regulators: CFH (Thompson and Winterborn, 1981; Caprioli et al., 2001; Perez-Caballero et al., 2001; Richards et al., 2001; Venables et al., 2006; Francis et al.,

Table 333.2 C3 glomerulopathy

Inclusion criteria	Glomerular C3 deposition
	Absence (or only scanty deposition) of immunoglobulin within glomeruli
Examples	Dense deposit disease
	Idiopathic C3 glomerulonephritis (Servais et al., 2007)
	MPGN1 with isolated sub-endothelial deposition of complement C3
	Familial MPGN type III (Neary et al., 2002a, 2002b; Malik et al., 2012)
	Familial C3GN associated with mutation in CFHR5 ('CFHR5 nephropathy') (Gale et al., 2010)

CFHR5 = complement factor H-related protein 5; MPGN = membranoproliferative glomerulonephritis.

Table 333.3 Complement investigations in C3 glomerulopathy and atypical haemolytic uraemic syndrome

Complement investigations	Measurement of serum complement proteins: ◆ C3 ◆ factor H ◆ factor I ◆ factor B
	C3 nephritic factor
	Anti-factor H autoantibodies CD46 (membrane cofactor protein) expression on peripheral blood mononuclear cells
Mutation screening	Complement regulator genes: ◆ factor H ◆ factor I ◆ CD46 (membrane cofactor protein) ◆ factor H-related protein family (CFHR1, 2, 3, 4, and 5)
	Complement activation genes: ◆ factor B ◆ C3
	Assessment of copy number variation across the *CFH-CFHR* locus

2012), CFI (Fremeaux-Bacchi et al., 2004), and CD46 (Noris et al., 2003; Richards et al., 2003) and (b) gain-of-function mutations: factor B (Goicoechea de Jorge et al., 2007) and C3 (Fremeaux-Bacchi et al., 2008). These mutations enhance C3 activation on the surface of renal endothelium which in turn triggers activation of C5 (Fig. 333.4D). The development of thrombosis is critically dependent on activation of C5 (de Jorge et al., 2011) and eculizumab, an antibody that prevents C5 activation, is effective in aHUS (Gruppo and Rother, 2009; Nurnberger et al., 2009).

CFH mutations preferentially affect surface recognition domains of CFH and comprise both sequence and structural changes (Fig. 333.4D). This results in impaired targeting of CFH to the renal endothelium but does not affect the ability of CFH to regulate C3 levels in plasma. Consequently, plasma C3 levels in patients with CFH mutations associated with aHUS are frequently normal.

Mutations affecting CFHR5, clusterin, and thrombomodulin have been described but are rare and their contribution to the syndrome poorly understood (Delvaeye et al., 2009; Stahl et al., 2009; Maga et al., 2010).

Like C3 glomerulopathy, the relationship between mutations and phenotype is complex. Multiple genetic factors, which include additional mutations or polymorphic variants, may be required for the syndrome to develop in some families (Esparza-Gordillo et al., 2006). Environmental factors such as intercurrent infection (Caprioli et al., 2006), drugs (Caprioli et al., 2006), pregnancy (Caprioli et al., 2006; Fakhouri et al., 2010), and kidney donation (Donne et al., 2002) are important precipitants.

Acquired complement dysregulation and aHUS may be due to autoantibodies against CFH (Dragon-Durey et al., 2005) and, rarely, anti-CFI autoantibodies (Kavanagh et al., 2012). Anti-CFH autoantibodies most commonly develop among individuals with Δ*CFHR3-1* deletion (Jozsi et al., 2008) and target the surface recognition domains of CFH (Jozsi et al., 2007). Functionally, these autoantibodies achieve the same outcome as loss-of-function mutations affecting the surface recognition domains of CFH. The association with Δ*CFHR3-1* is explained by the observation that that CFHR1 contains domains highly similar to the surface recognition domains of CFH; anti-CFH antibodies most likely represent anti-CFHR1 antibodies that cross-react with CFH (Strobel et al., 2011). It is logical to expect that anti-CFHR1 antibodies would be most likely to develop among individuals lacking CFHR1 (i.e. those with the Δ*CFHR3-1* polymorphism) although how these arise *in vivo* remains to be determined.

Investigation of complement-mediated renal disease

The investigation of complement-mediated renal disease requires a combination of serological and genetic investigations (Table 333.3). Routine complement assays include antigenic measurement of C3 and C4. Other investigations are less widely available and we recommend that these are performed in laboratories with the appropriate experience and expertise in complement diagnostic assays.

Complement genetic testing should only be performed in specialist centres since both the detection and interpretation of sequence changes may not be straightforward. Genetic analysis of the *CFH-CFHR* locus is technically challenging since methodologies need to detect not only sequence changes but also structural changes such as gene rearrangements.

When a novel variant is detected then it may require further studies to determine if it is actually disease-related.

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CHAPTER 334

Inherited metabolic diseases and the kidney

Robin Lachmann and Elaine Murphy

Introduction

Table 334.1 summarizes inherited metabolic diseases that affect the kidney. Fabry disease (see Chapters 335–338), cystinosis (see Chapter 339), and mitochondrial nephropathies (see Chapter 340) are discussed elsewhere, as are some disorders such as Lowe syndrome which cause Fanconi syndrome through their effects on the proximal convoluted tubule (see Chapter 41)

The primary renal tubular disorders (Dent disease, Bartter syndrome, Gitelman syndrome, Liddle syndrome, and nephrogenic diabetes insipidus) and the renal stone disorders are also considered elsewhere (see Sections 2 and 9, respectively).

In many inherited disorders of metabolism, renal disease is not usually the primary clinical manifestation, meaning that there are often other multisystem features and biochemical markers which aid diagnosis. Although rare, these conditions are important as early diagnosis and appropriate treatment can often significantly improve outcome.

Consensus guidelines for the management of these conditions are not always available and current management is therefore based on individual experience, case reports, and case series (usually < 150 patients).

Methylmalonic acidaemia

Methylmalonic acidaemia (MMA) is one of the organic acidaemias, a group of disorders resulting from abnormalities in the catabolism of the branched-chain amino acids (Seashore, 1993).

MMA can be caused by:

- a complete or partial deficiency of the enzyme methylmalonyl-CoA mutase (mut^0 or mut^- enzyme subtype, respectively) (OMIM #251100)
- a defect in the transport or synthesis of its cofactor, adenosyl-cobalamin (cblA (OMIM #251100), cblB (OMIM #251100), or cblD (OMIM #277410) type)
- a deficiency of the enzyme methylmalonyl-CoA epimerase (OMIM #251120).

These defects result in a failure to convert methylmalonyl-CoA into succinyl-CoA during propionyl-CoA metabolism in the mitochondrial matrix (Fig. 334.1). There may also be secondary inhibition of propionyl-CoA carboxylase, leading to accumulation of propionic acid and its metabolites.

Estimated prevalence

1/50,000 to 1/100.000,

Clinical presentation

Mut^0 subtype

This is the most common of the isolated MMA phenotypes and typically presents in early infancy with lethargy, vomiting, dehydration, and encephalopathy.

Mut^-, cblA, cblB, and cblD subtypes

These usually present later—in the first few months or years of life. Affected children can have feeding problems (anorexia, vomiting), failure to thrive, and developmental delay. Some children have an aversion to protein-containing foods or symptoms of vomiting/lethargy after eating protein. Other children can appear entirely well until their first presentation with an episode of dehydration, vomiting, respiratory distress, seizures, or coma.

Methylmalonyl-CoA epimerase deficiency

This is a very rare disorder, characterized by persistent moderate MMA excretion. Presentation can range from severe metabolic acidosis to a more chronic neurological picture with dysarthria, hypotonia, ataxia, seizure and a spastic paraparesis.

Biochemical findings

Organic acid analysis will show significantly increased urine and plasma MMA concentrations (typically between 10- to 1000-fold normal).

Other biochemical abnormalities may be seen (particularly during episodes of metabolic decompensation):

- High anion gap metabolic acidosis
- High urinary ketones and lactate
- Hyperammonaemia
- Hyperglycinaemia
- Hypoglycaemia
- Detectable urinary 3-hydroxypropionate, 2-methylcitrate, and tiglylglycine
- Elevated plasma propionylcarnitine (C3), C4-dicarboxylic, or methylmalonic/succinylcarnitine (C4DC).

Diagnostic investigations

Traditionally this required a skin biopsy and establishment of a cultured fibroblast line, although it is now possible to go straight to molecular genetic diagnosis.

Table 334.1 Summary of inherited disorders of metabolism with renal involvement

Group	Specific disease examples	Renal pathology	Further info
Glycogen storage disorders	GSD I	Proteinuria; glomerulosclerosis; tubular atrophy; interstitial fibrosis	
	GSD XI	Fanconi-like	
Lysosomal disorders	Fabry disease	Proteinuria; glomerulosclerosis; tubular atrophy; interstitial fibrosis	See Chapter 336
	Cystinosis	Fanconi-like plus glomerular changes	See Chapter 339
Disorders of carbohydrate metabolism	Hereditary fructose intolerance	Fanconi-like	
	Galactosaemia	Fanconi-like	
Organic acidaemias	Methylmalonic acidaemia (cblA, B, D)	Tubulointerstitial nephritis; tubular acidosis	
Disorders of tyrosine metabolism	Tyrosinaemia type 1	Fanconi-like	
Disorders of copper metabolism	Wilson disease	Calculi; Fanconi-like	
Disorders of amino acid transport	Lysinuric protein intolerance	Glomerulonephritis; chronic tubulointerstitial nephritis; glomerulosclerosis	
	Cystinuria	Nephrolithiasis	See Chapter 203
	Hartnup disease		
Mitochondrial nephropathies	MELAS syndrome	Glomerulosclerosis	See Chapter 340
Disorders of protein glycosylation	Phosphomannomutase deficiency (PMM2-CDG)	Proteinuria, nephrotic syndrome	
Disorders of purine metabolism	Phosphoribosyl pyrophosphate synthetase superactivity	Nephrolithiasis	
	Xanthinuria (xanthine oxidase deficiency)	Nephrolithiasis (xanthine stones)	
	Lesch–Nyhan syndrome	Nephrolithiasis (uric acid stones)	

^{14}C propionate incorporation assay in skin fibroblasts

Following a skin biopsy, patient fibroblasts can be cultured and incubated with ^{14}C-labelled propionic acid. Reduced incorporation of this ^{14}C into protein indicates a block in this pathway.

Complementation analysis

The precise diagnosis is then confirmed by complementation analysis. In this assay a cell line from an affected individual is mixed with a panel of established cell lines of known status (e.g. mut^0, cblA). The cells fuse, allowing cross-correction to occur *in vitro*. A known cell line fusion that fails to rescue ^{14}C proprionate incorporation into protein is of the same 'complementation group' as the patient cell line. These complementation groups (cblA, cblB, cblD, etc.) were originally defined by this biochemical assay, but the molecular bases of all types is now known.

Genetic analysis

Sequence analysis or deletion/duplication analysis (genes *MUT, MMAA, MMAB, MMADHC*, and *MCEE*). All disorders are autosomal recessive in inheritance.

Specific renal complications

The renal complications of MMA include renal tubular dysfunction. The renal pathology is of a tubulointerstitial nephritis with type 4 tubular acidosis, hyporeninaemic hypoaldosteronism, and reduced urine concentrating ability (Walter et al., 1989; D'Angio et al., 1991).

Acute renal dysfunction, often in the context of intercurrent infection or dehydration with metabolic decompensation may also occur (Srinivas et al., 2001; Pela et al., 2006).

Methylmalonic acid is thought to be directly nephrotoxic; end-stage renal disease is more common in the mut^0 (61%) and cblB (66%) subtypes, but less common in the cblA (21%) subtype (Horster et al., 2007).

Other chronic complications

Gastrointestinal

Anorexia, chronic vomiting, and failure to thrive are common and lead to chronic undernutrition with secondary problems such as short stature and osteoporosis. Acute, chronic, or recurrent pancreatitis may occur.

Neurological

Developmental delay and learning difficulties may be significant, even after a single metabolic decompensation. Poor muscle mass and muscle weakness, seizures, and movement disorders have been reported.

Skin

A rash similar to staphylococcal scalded-skin syndrome or acrodermatitis enteropathica can be seen.

Treatment

In some patients, the metabolic block can be reversed by high-dose vitamin B_{12} replacement. All patients should be tested for such vitamin B_{12} responsiveness, for example, by daily injection of 1 mg of

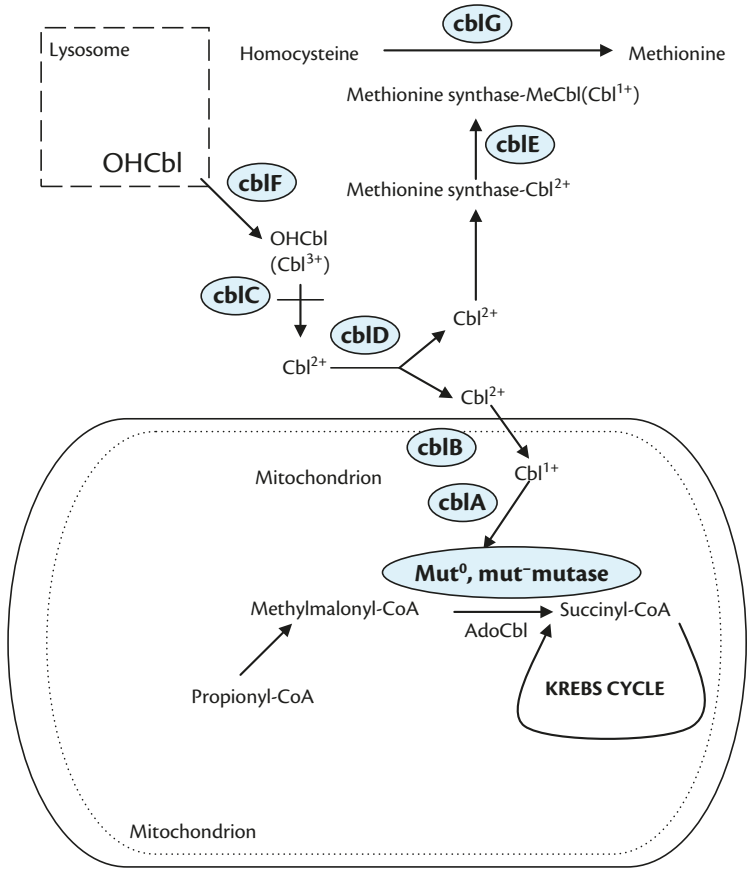

Fig. 334.1 Intracellular cobalamin metabolism. Propionyl-CoA is converted to methylmalonyl CoA, which is isomerized into succinyl Co-A, a Krebs cycle intermediate. The mutase reaction requires adenosylcobalamin (AdoCbl), an activated form of vitamin B_{12}, as a co-factor. The pathway of intracellular processing of vitamin B_{12} (cobalamin) is shown.

hydroxocobalamin (OH-Cbl) for 5 days and serial measurement of MMA and related metabolites.

In vitamin B_{12}-responsive patients, the dose of OH-Cbl required is determined by titrating against plasma MMA levels. Many will require injections daily, or every other day.

Dietary management

A low-protein, high-calorie diet with the aim of avoiding propiogenic amino acid loading of the affected pathway can be prescribed. If natural protein intake is very low, care must be taken to avoid a nutritional deficiency state, with subsequent growth failure. Synthetic amino acid supplements, low in isoleucine and valine, can be given to provide sufficient protein for growth.

Metabolic 'stress' (e.g. prolonged fasting, intercurrent infection) should be avoided or treated promptly to reduce the risk of metabolic decompensation. Fluid status should be carefully monitored. Many patients will have a specific 'emergency regimen', aimed at maintaining high calorie intake in the form of carbohydrate, to avoid catabolism of endogenous protein, and further reducing dietary protein intake, to follow at home, or in hospital, if they become unwell. Further details regarding these emergency regimens can be found on the British Inherited Metabolic Disease Group website (<http://www.BIMDG.org.uk>).

Carnitine supplementation

Carnitine (at a dose of 50–100 mg/kg/day) may increase intracellular CoA pools and hence aid in the excretion of propionylcarnitine.

Rotating antibiotics

Can be given to reduce the production of propionate by gut flora.

Organ transplantation

Limited data exists on liver, combined liver–kidney, or kidney–only transplants in individuals with MMA (Van Calcar et al., 1998; van't et al., 1999; Nagarajan et al., 2005; Mc Guire et al., 2008). Much of the metabolic conversion of propionate occurs in the liver, and the frequency of metabolic decompensation has been shown to be reduced in some individuals post-transplant. However, progression of renal impairment and neurological complications can still occur (Nyhan et al., 2002).

Nonsense mutation suppressor

Nonsense mutations create a premature stop signal in mRNA. This premature stop signal causes the ribosome to halt translation before a functioning protein is generated, creating a shortened, non-functioning protein. Nonsense mutation suppressors are designed to allow the ribosome to ignore the premature stop signal and continue translation of the mRNA, resulting in formation of a functioning protein. A phase II clinical trial for nonsense mutation MMA was commenced in 2010 (<http://clinicaltrials.gov/ct2/show/NCT01141075>).

Hereditary fructose intolerance

Hereditary fructose intolerance (OMIM #229600) is a disorder of carbohydrate metabolism, caused by deficiency of the enzyme

fructose-1,6-bisphosphate aldolase (aldolase B) (Ali et al., 1998; Bouteldja and Timson, 2010). Aldolase B is active in the liver, kidney, and small intestine and its expression is increased by a carbohydrate diet. It catalyses the reversible cleavage of fructose-1-phosphate and frustose-1,6-bisphosphate into 3-carbon sugars that enter the glycolytic or gluconeogenic pathways.

Estimated prevalence

1/11,000 to 1/100,000.

Clinical presentation

Typically babies become unwell following weaning or on exposure to fructose-containing food/medication with vomiting and failure to thrive progressing to coma. There is liver dysfunction, hypoglycaemia, and renal tubular dysfunction. Many patients never have an acute presentation but, because they develop abdominal pain or nausea when exposed to fructose-containing foods, spontaneously develop an aversion to sweet foods. This is why older patients have low levels of dental caries.

Biochemical findings

Biochemical abnormalities will only be present following exposure to fructose—orally or intravenously:

Blood:

- Hyperuricaemia
- Hypoglycaemia
- Hypophosphataemia
- Hypermagnesaemia
- Fructosaemia
- Accumulation of alanine, lactate and pyruvate
- Metabolic acidosis
- Abnormal liver function and coagulation
- Abnormal glycosylation (altered transferrin isoelectric focusing).

Urine:

- Glycosuria
- Albuminuria
- Aminoaciduria
- Phosphaturia
- Bicarbonaturia
- Uricosuria
- Fructosuria (positive test for reducing substances).

Diagnostic investigations

Genetic analysis

This is now the diagnostic method of choice. Sequence analysis, targeted mutation analysis in particular populations (e.g. p.A150P, p.A175D, and p.N335K are prevalent in Europe) or deletion/duplication analysis (gene *ALDOB*).

Enzyme studies

Fructoaldolase activity can be measured in liver tissue.

Fructose challenge

Typical biochemical changes can be measured after intravenous infusion of fructose. This is no longer recommended as a diagnostic investigation due to risks of clinical decompensation.

Specific renal complications

Aldolase B deficiency leads to accumulation of fructose-1-phosphate (F1P). Increased F1P prevents the formation of gluconeogenic intermediates such as fructose-1,6-bisphosphate and glucose 6-phosphate. Krebs cycle precursors, alanine, lactate, and pyruvate, therefore accumulate and contribute to aminoacidaemia and metabolic acidosis.

Impaired function of the proximal renal tubule leads to an acquired Fanconi syndrome with aminoaciduria, phosphate, and renal bicarbonate wasting. Urinary acidification is also impaired.

Other chronic complications

Chronic fructose exposure is associated with irreversible damage to the liver and kidney—cirrhosis and end-stage liver failure can develop. Metabolic bone disease and growth failure can occur secondary to chronic metabolic acidosis and renal impairment.

Treatment

Dietary management

Once the diagnosis is made, and the diet is altered to limit fructose intake then, providing organ damage has not been extensive, the outcome is excellent. Foods high in fructose (fruit, many vegetables, processed/sweetened foods, honey, cakes, biscuits, pastries, some alcohols) need to be avoided. Affected individuals will often have a self-protective learnt aversion to foods which cause symptoms. Intravenous fructose and sorbitol should be avoided; deaths have been caused by use of fructose infusions as a source of parenteral nutrition. Care should be taken with medications that might contain sucrose or sorbitol (both of which can be metabolized to fructose) as coatings or excipients of tablets or as components of syrups.

Vitamin supplementation

In view of the restricted diet, patients are recommended to take supplements of water-soluble vitamins.

Organ transplantation

Successful liver transplantation has been carried out to manage decompensated cirrhosis.

Classical galactosaemia

Classical galactosaemia (OMIM #230400) is a disorder of carbohydrate metabolism caused by deficiency of the enzyme galactose-1-phosphate uridyltransferase (GALT). There is accumulation of galactose-1-phosphate (Gal-1-P), galactose and metabolites which are produced via activation of alternative metabolic pathways (Fig. 334.2) (Bosch, 2006).

Estimated prevalence

1/10,000 to 1/30,000.

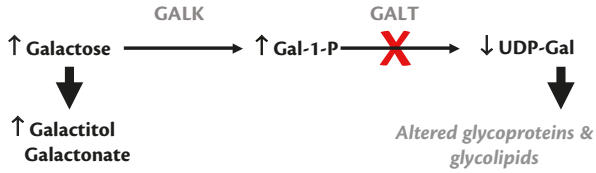

Fig. 334.2 Galactose metabolism. Mutations of the *GALT* gene, result in reduced function of the enzyme galactose-1-phosphate uridyltransferase (GALT) (crossed) and subsequent accumulation of galactose-1-phosphate (Gal-1-P), galactose and derived metabolites, through alternative pathways. GALK = galactokinase; Gal-1-P = galactose-1-phosphate; UDP-Gal = uridine diphosphate-galactose.

Clinical presentation

Typically infants present between day 3 and 5 of life following ingestion of breastmilk or lactose-containing formula milk. Problems include poor feeding, vomiting, failure to thrive, hypoglycaemia, and liver dysfunction with jaundice and coagulopathy. Unless treated, sepsis (particularly with *Escherichia coli*), shock, and death can occur. Renal tubular dysfunction and cataracts may also be evident.

Biochemical findings

Following exposure to galactose (lactose):

Blood:

- Hypoglycaemia
- Hypophosphataemia
- Metabolic acidosis
- Increased erythrocyte galactose-1-phosphate
- Abnormal liver function and coagulation.

Urine:

- Glycosuria
- Albuminuria
- Aminoaciduria
- Phosphaturia
- Increased urinary galactitol
- Galactosuria (positive test for reducing substances).

Diagnostic investigations

Genetic analysis

Autosomal recessive in inheritance. Sequence analysis, targeted mutation analysis (eight common mutations described, most frequent p.Q188R) or deletion/duplication analysis (gene *GALT*).

Enzyme studies

Galactose-1-phosphate uridyltransferase activity can be measured in erythrocytes.

Newborn screening

Several countries have included newborn screening for galactosaemia in their screening programmes for inborn errors of metabolism.

Specific renal complications

Impaired renal tubular reabsorption leads to aminoaciduria, phosphaturia, and glycosuria.

Other chronic complications

Neurological

Developmental delay, speech delay, and learning difficulties can occur. Movement disorders, typically characterized by ataxia, tremor, and dystonia, may develop.

Endocrine

Premature ovarian insufficiency (hypergonadotropic hypogonadism) is common among female patients.

Cataracts

May resolve/stabilize with dietary treatment.

There is no obvious difference in treatment or biological factors between individuals with or without long-term complications. Liver failure and renal impairment are not features of adult patients with galactosaemia—rather it is the cognitive, neurological, and endocrine features that predominate.

Treatment

Dietary management

Once the diagnosis is suspected then sources of lactose (human breast milk, cows' milk, and most infant milk-formulas) should be removed from the diet and replaced with a formula that is free of bioavailable lactose (e.g. soy based). If the diagnosis is confirmed then strict restriction of lactose-containing foods and medicines should be continued for at least the first year of life. How strict the diet should be following that is debatable and some patients have relaxed their diet in later childhood without any overt consequences (Lee et al., 2003).

Vitamin supplementation

In view of the restricted diet, patients are recommended to take supplements of calcium and vitamin D.

Tyrosinaemia type 1

Tyrosinaemia type 1 (OMIM #276700) is a disorder of tyrosine metabolism caused by deficiency of fumarylacetoacetate hydrolase, the final step in the tyrosine catabolic pathway (Fig. 334.3) (Sniderman et al., 1993). The precursor fumarylacetoacetate accumulates and causes cellular hepatic damage. Succinylacetoacetate and succinylacetone also accumulate and inhibit the hepatic enzymes (a) parahydroxyphenylpyruvic acid dioxygenase, leading to plasma tyrosine accumulation and (b) porphobilinogen synthase, leading to reduced activity of δ-aminolevulinic acid dehydratase, and subsequently increased δ-aminolevulinic acid levels.

Estimated prevalence

1/100,000 to 1/120,000.

Clinical presentation

Typically presents within the first year of life with liver dysfunction and coagulopathy (transaminase level and serum bilirubin concentration are not always as abnormal as expected given the degree of clotting factor abnormalities), renal involvement, growth failure, and rickets. Liver disease may be early and severe—untreated patients die from cirrhosis or hepatocellular carcinoma at a young age.

Children may also present with neurologic crises, similar to those experienced in acute intermittent porphyria, with change in mental status, abdominal pain, peripheral neuropathy, and/or respiratory failure requiring mechanical ventilation.

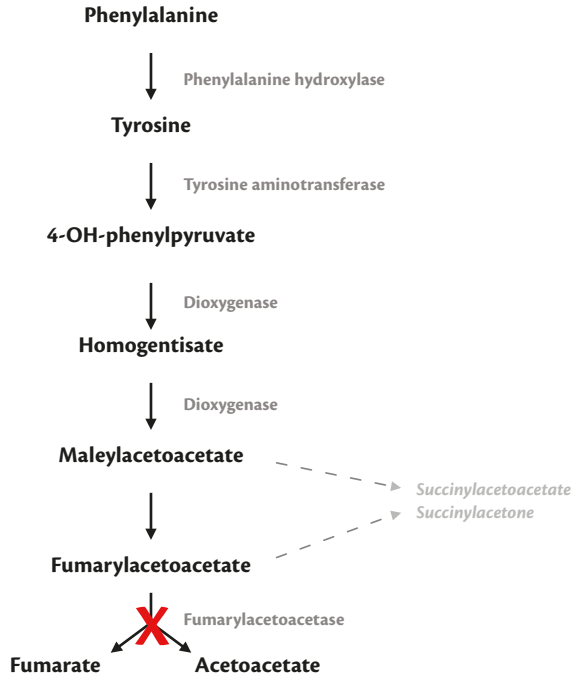

Fig. 334.3 Tyrosine metabolism showing fumarylacetoacetase enzyme deficiency (crossed) and altered metabolites (light grey).

Biochemical findings

Blood:

- Increased succinylacetone
- Increased amino acids—tyrosine, methionine, phenylalanine
- Increased alpha-fetoprotein
- Hypoglycaemia
- Abnormal coagulation—prolonged prothrombin and partial thromboplastin times.

Urine:

- Aminoaciduria
- Phosphaturia
- Abnormal organic acids (succinylacetone, *p*-hydroxyphenylpyruvate, *p*-hydroxyphenyllactate, *p*-hydroxyphenylacetate)
- Increased δ-aminolevulinic acid.

Diagnostic investigations

Genetic analysis

Autosomal recessive in inheritance. Sequence analysis, targeted mutation analysis (four common mutations described in general US population; p.P262L in Ashkenazi Jewish; p.IVS12 + 5 G>A in French Canadian) or deletion/duplication analysis (gene *FAH*).

Enzyme studies

Fumarylacetoacetate hydrolase enzyme activity can be measured in skin fibroblasts.

Newborn screening

Several countries have included newborn screening for tyrosinaemia in their screening programmes for inborn errors of metabolism.

Specific renal complications

In chronic untreated tyrosinaemia type 1, renal tubular involvement can be the major manifestation. This involves a Fanconi-like syndrome with generalized aminoaciduria, phosphate loss, and, often, renal tubular acidosis.

Other chronic complications

Corneal opacities

Blood tyrosine concentration > 600 μmol/L increases the risk of precipitation of tyrosine as subepithelial corneal opacities causing photophobia and itchy, sensitive eyes.

Hepatocellular carcinoma

Is a significant risk in untreated children but early treatment with nitisinone substantially reduces this risk.

Treatment

Medical

Nitisinone (2-(2-nitro-4-trifluoro-methylbenzyol)-1,3 cyclohexanedione, NTBC) is an inhibitor of parahydroxyphenylpyruvic acid dioxygenase and prevents the accumulation of many toxic metabolites (McKiernan, 2006). However, tyrosine levels remain elevated and a phenylalanine-, tyrosine-restricted diet may be required (with the goal of maintaining plasma tyrosine levels < 600 μmol/L to prevent corneal opacities). For individuals on a restricted diet, prescribed low-protein foods and phe-, tyr-free amino acid supplements (with vitamins and minerals) will also be needed. The tubulopathy of tyrosinaemia type 1 is reversible with NTBC treatment (Santra et al., 2008).

Organ transplantation

Liver transplantation was the only definitive therapy prior to NTBC availability. Nowadays, transplant is reserved for patients who failure to respond to NTBC (usually following presentation with late-stage fulminant hepatic failure) or have evidence of malignancy (Arnon et al., 2011). Hepatocyte transplant has also been used as a bridge to liver transplant (Ribes-Koninckx et al., 2012). Follow-up after liver transplant indicates that urinary excretion of succinylacetone remains elevated and tubulopathy can persist in some patients (Pierik et al., 2005).

Wilson disease

Wilson disease (OMIM #277900) is a disorder of copper metabolism caused by deficiency of a copper transporting ATPase, leading to copper overload and a highly variable clinical presentation (Roberts and Cox, 1998).

Estimated prevalence

1/30,000.

Clinical presentation

Patients can present with hepatic disease (~40%), neurologic disease (~40%), psychiatric disease (~ 0%), haemolytic anaemia, or

a combination of these. Individuals with neurologic or psychiatric disease are likely to have Kayser–Fleischer rings. Fulminant hepatic failure can rapidly progress to renal failure.

Biochemical findings

- Reduced serum caeruloplasmin
- Increased urinary copper excretion
- Increased hepatic copper concentration.

Diagnostic investigations

Genetic analysis

Autosomal recessive in inheritance. Sequence analysis, targeted mutation analysis, mutation scanning of selected exons or deletion/duplication analysis (gene *ATP7B*) (Bull et al., 1993).

Specific renal complications

Renal involvement consists of renal calculi, microscopic haematuria, and tubular dysfunction (proteinuria, aminoaciduria, glycosuria, uricaciduria, hypercalciuria, hyperphosphaturia). The gene encoding the ATP7B copper transporter is expressed in kidney glomeruli and medulla (Moore and Cox, 2002). The pathogenesis of the renal lesions has been related to an increase in copper stores in the tubular epithelium and a secondary mitochondrial respiratory chain defect.

Other chronic complications

Chronic liver disease

Hepatosplenomegaly, portal hypertension, ascites, risk of hepatocellular carcinoma.

Neurological problems

Movement disorders: tremors, chorea, choreoathetosis. Spastic dystonia with rigidity and gait disturbance. Dysarthria, difficulty swallowing.

Others

Arthritis, pancreatitis, cardiomyopathy, endocrine problems.

Treatment

Copper chelating agents (penicillamine, trientine) increase the urinary excretion of copper and are the first-line treatment for Wilson disease.

Zinc acetate interferes with the absorption of copper from the gastrointestinal tract. It is usually used after initial decoppering with a chelating agent.

Antioxidants may counteract free radical damage.

Dietary restriction of high copper-containing foods (e.g. liver, chocolate, mushrooms, shellfish) may be useful.

Liver transplantation is reserved for patients who fail to respond to medical therapy and renal tubular function has been shown to improve markedly post-liver transplant (Ozcay et al., 2008; Catana and Medici, 2012). The role of liver transplant in individuals with neurological disease however remains controversial.

Glycogen storage disorders

This group includes disorders of glycogen degradation, glycolysis, glucose release, and glycogen synthesis. Three disorders—glycogen storage disorder (GSD) Ia (OMIM #232200), GSD Ib (OMIM #232200), and GSD XI (Fanconi-Bickel disease) (OMIM #227810)—are associated with renal pathology (Bali et al., 1993; Santer et al., 1998, 2002).

Estimated prevalence

GSD I: 1/20,000 (Ashkenazi Jews) to 1/100,000.

Clinical presentation

GSD I

Typical presentation is in the first 6 months of life with hypoglycaemia (after a short fast), truncal obesity (hepatomegaly), tachypnoea (lactic acidosis), failure to thrive, and short stature. Impaired platelet function can lead to a bleeding tendency (epistaxis).

In addition to the above, patients with GSD Ib develop bacterial infections, diarrhoea, oral and intestinal mucosal ulcers due to associated neutropenia.

GSD XI

Typically presents with fasting hypoglycaemia, abdominal distension (hepatomegaly), growth retardation and rickets (Roy et al., 2011).

Biochemical findings

GSD I

Blood:

- Hypoglycaemia
- Lactic acidosis
- Hyperuricaemia
- Hyperlipidaemia (triglycerides and cholesterol)
- Neutropenia (GSD Ib only).

Urine:

- Albuminuria/proteinuria.

GSD XI

Blood:

- Fasting hypoglycaemia (impaired glucose tolerance)
- Hyperlipidaemia.

Urine:

- Aminoaciduria
- Phosphaturia
- Glucosuria
- Galactosuria
- Proteinuria.

Diagnostic investigations

Genetic analysis

Autosomal recessive in inheritance. Sequence analysis, targeted mutation analysis (ethnic-specific common mutations known) or deletion/duplication analysis (genes *G6PC* (GSD Ia); *SLC37A4* (GSD Ib); *SLC2A2* (GSD XI)).

Enzyme studies

GSD Ia

Glucose-6-phosphatase activity can be measured in liver tissue.

GSD Ib

Glucose-6-phosphate translocase (transporter) activity can be measured in (fresh) liver tissue.

Specific renal complications

GSD I

Glomerular hyperfiltration may initially be the only feature. Subsequently, usually in patients aged 20 years or older, overt renal disease with proteinuria, hypertension, renal stones, nephrocalcinosis, hypocitraturia, and altered creatinine clearance may develop (Weinstein et al., 2001; Mundy and Lee, 2002; Yiu et al., 2008, 2010; Martens et al., 2009). Renal biopsies of patients with GSD Ia reveal tubular atrophy, focal segmental glomerulosclerosis, and, with progressive disease, interstitial fibrosis (Chen et al., 1988). Renal fibrosis is characterized by an increase in the synthesis and deposition of extracellular matrix proteins in the renal cortex, and by tubular basement membrane thickening, tubular atrophy, tubular dilation, and multifocal interstitial fibrosis (Yiu et al., 2008). Some individuals progress to end-stage renal disease.

In a mouse model of GSD Ia, the kidney exhibits enhanced oxidative stress that is mediated by activation of NADPH oxidase and suppression of antioxidant enzymes (Yiu et al., 2010).

The progression from glomerular hyperfiltration to microalbuminuria was significantly delayed by angiotensin-converting enzyme inhibitor therapy in a 10-year retrospective study of a cohort of 95 patients with GSD Ia (Melis et al., 2005). Improved metabolic control has also been reported to ameliorate proximal tubular dysfunction (Chen et al., 1990).

Other chronic complications

GSD I

Reduced bone mineral density.

Delayed puberty. Untreated affected individuals historically showed delayed onset of puberty; however, with good metabolic control, age of onset of puberty can be normal.

Hepatic adenomas. By the second or third decade of life, many affected individuals develop hepatic adenomas. Adenomas may (rarely) haemorrhage or undergo malignant transformation into hepatocellular carcinoma. Occurrence of adenomas may be reduced by good metabolic control.

Treatment

Hypoglycaemia is avoided by (a) frequent meals, (b) overnight continuous tube feeding, or (c) use of uncooked cornstarch (as a 'slow release' form of glucose).

Granulocyte colony-stimulating factor (GCSF) for symptomatic neutropenia with recurrent infections (GSD Ib).

Metabolic 'stress', for example, prolonged fasting and intercurrent infection, should be avoided or treated promptly to reduce the risk of metabolic decompensation with hypoglycaemia and lactic acidosis. Many patients will have a specific emergency regimen to follow at home, or in hospital, if they become unwell. Further details regarding these emergency regimens can be found on the British Inherited Metabolic Disease Group website (<http://www.BIMDG.org.uk>).

Transplantation

Liver transplants alone, liver–renal transplants (kidneys have been transplanted before, after or concurrently with a liver), and hepatocyte transplants have all been successfully performed for patients with GSD Ia (Lee et al., 2004; Panaro et al., 2004; Belingheri et al., 2007; Davis and Weinstein, 2008; Ribes-Koninckx et al., 2012). Unfortunately, liver transplant alone does not seem to eliminate the progression of nephropathy (Davis and Weinstein, 2008).

Liver transplant has also been carried out for GSD Ib—neutropenia persists postoperatively and infectious complications can be problematic (Karaki et al., 2012).

Disorders of amino acid transport

Renal tubular re-absorption is typically ≥ 95–99% for most amino acids. Inherited disorders of these amino acid transport systems may be asymptomatic and detected incidentally by finding elevated amino acids in urine with correspondingly low/normal values in plasma. The more common disorders are briefly summarized here:

Lysinuric protein intolerance (OMIM #222700)

Clinical presentation: typically presents after weaning, with variable findings including recurrent vomiting, episodes of diarrhoea, episodes of stupor and coma after a protein-rich meal, poor feeding, aversion to protein-rich food, failure to thrive, hepatosplenomegaly, and muscular hypotonia (Sebastio and Nunes, 1993). Chronic complications include osteoporosis, and pulmonary, renal, and haematologic abnormalities

Specific renal involvement: glomerular and tubular involvement is described. Elevated creatinine and cystatin C, proteinuria, haematuria, elevated blood pressure, mild to moderate renal insufficiency and end-stage renal disease have all been reported. Proximal tubular disease is associated with generalized aminoaciduria and phosphaturia. Renal histology shows immune-mediated glomerulonephritis and chronic tubulointerstitial nephritis with glomerulosclerosis in the absence of immune deposits.

Estimated prevalence: very rare; 1/60,000 in Finland

Biochemistry: elevated urinary arginine, lysine, ornithine; elevated plasma NH_3 due to disruption of the urea cycle; increased plasma lactate dehydrogenase, thyroxine-binding globulin and ferritin.

Gene: SLC7A7 (Torrents et al., 1999)

Treatment: restricted protein diet, citrulline substitution.

Cystinuria (OMIM #220100)

Clinical presentation: nephrolithiasis (Eggermann et al., 2012)

Estimated prevalence: 1/2500 (Libyan Jews) to 1/100,000 (Swedish)

Biochemistry: elevated urinary cystine, arginine, lysine, ornithine

Gene: SLC3A1 (type A) or *SLC7A9* (type B) (Pras et al., 1994; Feliubadalo et al., 1999)

Treatment: high fluid intake; alkalinization of the urine; penicillamine. (See also Chapter 203).

Hartnup disease (OMIM #234500)

Clinical presentation: skin rash, ataxia, often asymptomatic (Baron et al., 1956).

Estimated prevalence: 1/14,000

Biochemistry: elevated urinary neutral amino acids (alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, histidine)

Gene: SLC6A19 (Kleta et al., 2004; Seow et al., 2004)

Treatment: nicotinamide; sun protection.

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CHAPTER 335

Fabry disease: overview and pathophysiology

Stephen Waldek

Historical perspective

In 1898, Anderson, in London, described a case of angiokeratoma (Anderson, 1898) at the same time as Fabry described a case of purpura nodularis haemorrhagica (Fabry, 1898). Anderson's case had proteinuria as well as deformed fingers and lymphoedema. The presence of proteinuria led him to suggest that that this was a systemic disease and that there were abnormal vessels in the kidney as well as skin. In a follow-up report, Fabry also documented the presence of albuminuria (Fabry, 1930). Several other descriptions followed expanding the clinical associations and in 1947 Pompen and colleagues reported the first post-mortem cases of two brothers who died of renal failure (Pompen et al., 1947). The most important finding was the presence of vacuoles in blood vessels throughout the kidney leading them to postulate that this was a generalized storage disease. Subsequently, this was confirmed by Scriba who established the lipid nature of the storage material (Scriba, 1950). The storage material was characterized as neutral glycosphingolipids, including globotriaosylceramide (Gb3) (Sweeney and Klionsky, 1963) and then Brady and colleagues demonstrated that the accumulation of lipid resulted from a defect in the enzyme ceramide trihexosidase (Brady et al., 1967). The hereditary nature of the condition had been known for some time but it was not until 1965 that Opitz and colleagues documented the X-linked nature of the condition (Opitz et al., 1965) describing it as recessive, something that has now been shown as untrue (see below). In 1986, the gene responsible for the disease was characterized and sequenced (Bishop et al., 1986) paving the way for greater insights into the condition and the possibility of developing effective therapies.

Biochemistry

The α-galactosidase A enzyme is one of many acid hydrolases found in the lysosome. It catalyses the removal of a galactose moiety from neutral sphingolipids, predominantly ceramide trihexoside (Fig. 335.1). The result of deficiency or absence of the enzyme leads to the accumulation of the substrate, predominantly Gb3. The enzyme is a homodimeric glycoprotein with each monomer composed of a (beta/alpha) 8 domain with the active site and an antiparallel beta domain. N-linked carbohydrate appears at six sites in the glycoprotein dimer, revealing the basis for lysosomal transport via the mannose-6-phosphate receptor (Garman and Garboczi, 2004). However, it should be noted that there are other systems that can transport enzyme into a cell (see Chapter 338).

While Gb3 accumulates in Fabry disease some is de-acetylated to form lyso-Gb3 by a mechanism that is, as yet, not understood (Aerts et al., 2008). Lyso-Gb3 has several analogues (Dupont et al., 2013; Lavoie et al., 2013) and these, with lyso-Gb3, may form the basis of a possible biomarker for Fabry disease as well as having a potential role in pathogenesis of disease manifestations (see below).

Glycosphingolipids are widely distributed in the body as constituents of normal plasma membranes (Thompson and Tillack, 1985) and some intracellular membranes such as those of the Golgi and lysosome (Dawson, 1978). In the plasma they are associated with lipoproteins with high concentrations in the low-density lipoprotein fraction (Kundu et al., 1985).

Pathophysiology

The exact mechanisms behind the organ damage in Fabry disease is still not clear. As there are no clinical symptoms in infancy, and rarely in early childhood, the damage must be related to the slow accumulation of Gb3 and allied compounds, starting *in utero* (Tsutsumi et al., 1985), leading to progressive cellular damage (Fig. 335.2). In the kidney, the earliest changes are in the podocytes. Using unbiased stereological quantitative methods to analyse electron microscopic changes in 14 young Fabry disease patients and controls, the podocyte Gb3 inclusion volume density increased progressively with age. Foot process width was increased and also increased with age as did a reduction of endothelial fenestrations (Najafian et al., 2011). While this may be the first stage of renal damage, the ultimate progression to end-stage renal failure is certainly multifactorial.

Valbuena and colleagues hypothesized that the initial trigger may be the rupture of the lysosome after a certain amount of substrate accumulation with subsequent cell death (Valbuena et al., 2008). Some of the damage may be due to local secondary inflammation (Safyan et al., 2006) although crude measures such as C-reactive protein are not elevated (Altarescu et al., 2008). Oxidative stress may also play a role as when Gb3 was added to cultured vascular endothelial cells there was a dose-dependent increase in the production of reactive oxygen species and upregulation of the expression of cell adhesion molecules (Shen et al., 2008). A vasculopathy also seems to occur in many patients where there is little, if any, endothelial deposition but smooth muscle cell hypertrophy occurs and is often one of the first features to appear followed by changes in the neo-intima and subsequent fibrosis. This may be the result of an upregulation of the local renin–angiotensin system. The local

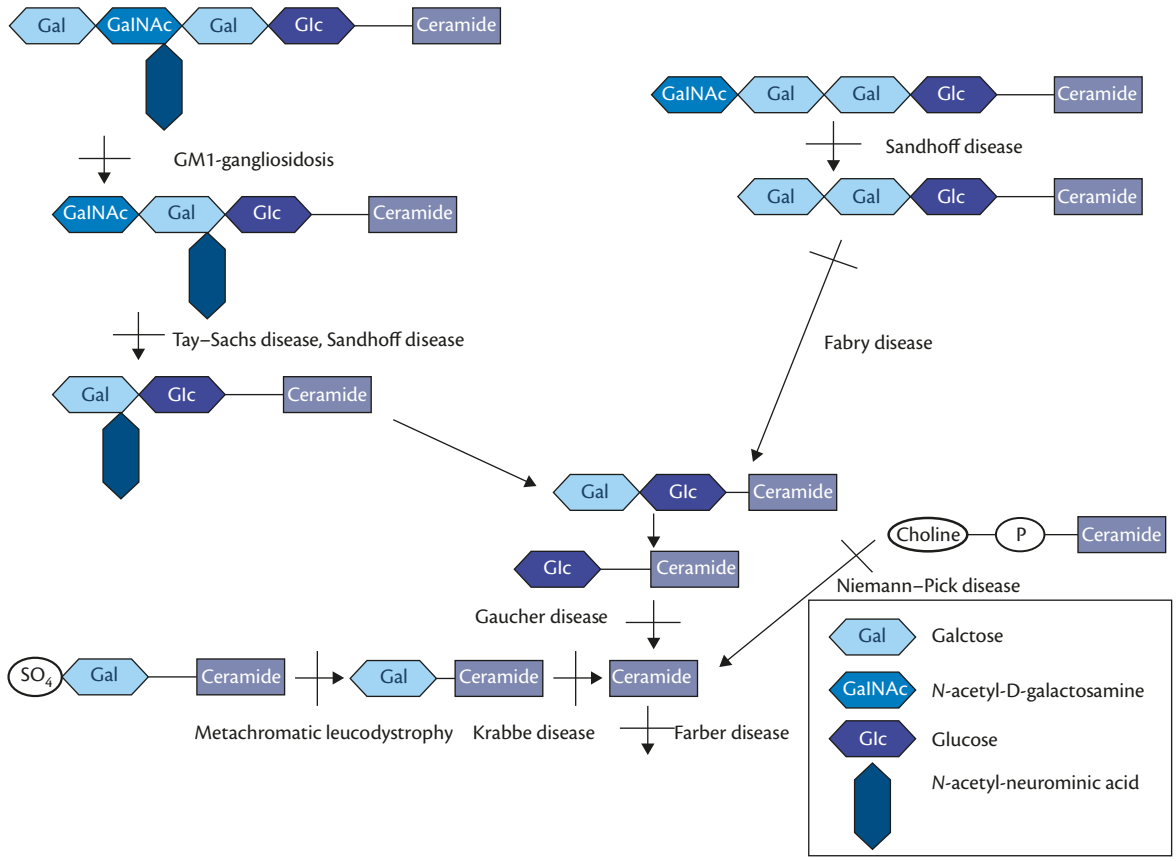

Fig. 335.1 Biochemical pathways for glycosphingolipid metabolism.

increase in angiotensin will then increase adhesion molecules and the production of local cytokines and chemokines resulting in local inflammation and smooth muscle hypertrophy (Rombach et al., 2010). This is further supported by the finding that there is upregulation of transforming growth factor beta 1 (TGF-β1) and vascular endothelial growth factor as a result of Gb3 accumulation (Lee et al., 2012). Gb3 is identical to membranous CD77 and could thus be involved with apoptosis and necrosis of renal cells (Thomaidis et al., 2009). Returning to the podocyte, lyso-Gb3 added to podocytes in culture leads to a dose- and time-dependent increase in TGF-β1, extracellular matrix proteins, and CD74, all of which can cause glomerular injury and cell proliferation (Sanchez-Niño et al., 2011). Interestingly these changes were prevented by paricalcitol, raising the possibility that vitamin D receptor activation may be a potential adjuvant therapy. Although the above processes are almost certainly important in pathogenesis of renal damage in Fabry disease, the question still arises as to how all this follows from the accumulation of Gb3 in the lysosomes of various cell types within the kidney. One clue may lie in the fact that there is dysregulation of autophagy in the podocytes in Fabry disease (Liebau et al., 2013).

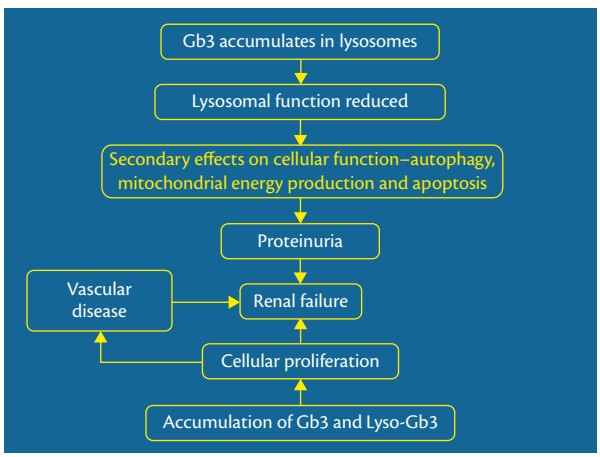

Fig. 335.2 Possible pathophysiological mechanisms underlying Fabry nephropathy.

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CHAPTER 336

Fabry disease: clinical features

Stephen Waldek

Epidemiology

Fabry disease (OMIM #301500) is one of > 50 lysosomal storage diseases and is caused by a deficiency of the enzyme alpha-galactosidase A (EC 3.2.1.22) encoded by the *GLA* gene on the long arm of the X chromosome (Xq22). The gene is seven exons long and consists of 12,436 base pairs (Germain, 2010).

The condition is pan ethnic with an estimated incidence of between 1/40,000 and 1/120,000 live births (Mehta et al., 2004). However, a much higher incidence has been found, consisting mostly of late-onset phenotypes (see below), as a result of newborn screening initiatives. In Italy it was 1/3100 (Spada et al., 2006) and 1/1250 in Taiwan (Hwu et al., 2009). The vast majority of patients have 'private' unique mutations confined to their families with > 600 mutations recorded to date. The majority of classical patients have either nonsense or missense mutation resulting in non-functional, misfolded, or absent enzyme. Other types of serious mutation have also been found (Germain, 2010). However, there are some patients who have more common, 'non-private', mutations that are usually associated with milder disease, and only slightly reduced enzyme levels and a later-onset phenotype that is often confined to a single organ such as the heart (Eng and Desnick, 1994; Spada et al., 2006; Hwu et al., 2009) or the kidney (Nakao et al., 2003).

Although originally classified as an X-linked recessive condition it is now well accepted that female heterozygotes can manifest the same symptoms and signs as their male counterparts so that the disease is now referred to as displaying 'X-linked inheritance'. The symptoms tend to come on at a later age and are generally milder with many women asymptomatic throughout their lives. However, a significant minority of woman end up with the 'classical' picture seen in many male patients (Whybra et al., 2001; Wilcox et al., 2008). This is due to the inactivation of one of the alleles of the alpha-galactosidase gene (Romeo and Migeon, 1970). This inactivation is now known to be random or due to 'Lyonization' (Germain, 2006) that can, however, be 'skewed' towards either the normal or abnormal allele (Dobrovolny et al., 2005).

Heterogeneity

While Fabry disease affects multiple organs there is great heterogeneity both within families sharing the same gene mutation and between families with different mutations but the same level of enzyme deficiency. The reasons behind this phenomenon are not known, but it has major implications for genetic counselling and for predicting final phenotype when seeing patients in the clinic. Although there is some correlation between disease severity and enzyme level, with the most severe cases having very low or absent plasma enzyme, there is no correlation between the enzyme level or mutation and the way that the disease is manifest. This is fully reviewed and discussed by Germain (2010). Generally, patients can be divided into three categories—the classical male patient, the later-onset cases, and the female heterozygotes.

Life expectancy

Irrespective of how the disease manifests, it results in a decreased life expectancy with males dying at a mean of 58.2 years compared to 74.7 years in the general US population for males and 75.4 years compared to 80 years for females with the disease (Waldek et al., 2009). The commonest causes of death from the same publication indicate that cardiovascular and renal events account for the majority of Fabry-related deaths. When considering all the presentations of Fabry disease, 62% of patients present with neuropathic pain, 31% with skin lesions, 19% with gastrointestinal problems, and 17% with renal disease. The mean age of symptom onset is 9 years of age in males and 13 years in females. However, in males the mean age at diagnosis is not until 23 years and in females not until 32 years indicating a significant diagnostic gap (Eng et al., 2007). While this diagnostic gap may have reduced slightly since 2007, it is still important to emphasize, as will be demonstrated below, because early diagnosis gives the best outcomes.

Neuropathic features

The deposition of globotriaosylceramide (Gb3) starts *in utero* (Tsutsumi et al., 1985) but the first manifestations do not start until childhood or adolescence with the involvement of the peripheral and autonomic small nerve fibres (Cable et al., 1982; Dürsch et al., 2002). The median age of symptom onset is 6 years for boys and 9 years for girls (Hopkin et al., 2008).

The most common symptoms are neuropathic pain found in 59% of boys and 41% of girls together with gastrointestinal symptoms present in 18% of children (Hopkin et al., 2008.) Tinnitus, vertigo, fatigue, and angiokeratoma were present in 40% of patients (Ramaswami et al., 2006).

The pain occurs in two forms; there is a chronic burning pain with tingling in the extremities and, superimposed on this, is the 'Fabry crisis' that is characterized as an agonizing burning pain originating in the peripheries and radiating up the limbs to the rest of the body (Burlina et al., 2011). A 'crisis' can be precipitated by several factors including rapid change in temperature, fever, exercise, and other stressful situations—physical and emotional. The pain often decreases in severity later in life and, unless explicitly asked about, can be missed when adult patients are first seen and questioned in clinic (Naleschinski et al., 2009). The abdominal pain is caused by deposition of Gb3 in the abdominal autonomic ganglia and decreased flow in the mesenteric artery, again due to substrate deposition in the endothelium and muscular layer causing narrowing of

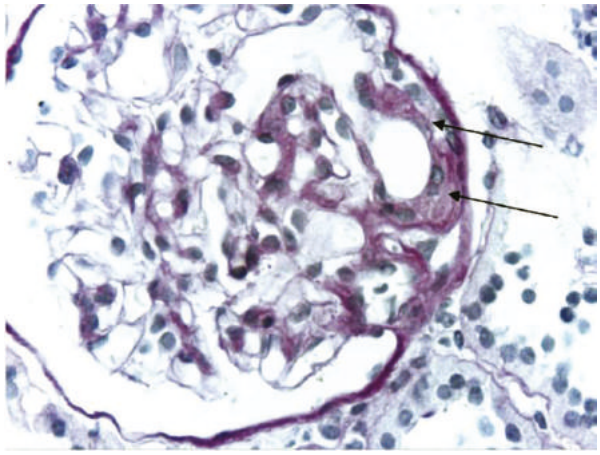

Fig. 336.1 Renal biopsy showing early changes in a young boy. Arrows: early signs of FSGS.
From Tøndel, C., Bostad, L., Hirth, A., *et al.* (2008). Renal biopsy findings in children and adolescents with Fabry disease and minimal proteinuria. *Am J Kidney Dis*, 51(5), 767–76.

the vessel (Seth et al., 1981). The pain is very similar to that found in irritable bowel syndrome which enters into the differential diagnosis (Hoffmann et al., 2007). Also occurring early in the course of disease, due to a mixture of autonomic neuropathy and deposition of Gb3 in the sweat glands, is anhidrosis, or in some cases partial loss of sweating, that can be quite debilitating (Kang et al., 1987). It is estimated that around 53% of males and 28% of females complain of some degree of sweating problems (Orlev et al., 2007).

Skin features

The commonest skin lesions are known as angiokeratoma. Histologically, they are caused by dilatation of vessels—capillaries and venules—associated with deposition of substrate in the endothelium of the dermal vasculature (Brethnach et al., 1980). Clinically, these characteristic lesions are red or purple and can be flat or slightly raised. They are characteristically found between the umbilicus and the mid thigh—the so-called bathing trunk area—and are relatively symmetrical in distribution. However, they can be found anywhere on the body—even on the lips—and may be quite solitary. When concentrated around the umbilicus and on and around the genitalia they can be very unsightly and add to the psychological stress of a patient. In severe cases, where the lesions are very prominent and in large coalescent patches, they can bleed. Some form of skin lesion is found in around 66% of male and 36% of female patients (Orlev et al., 2007). Although less common, lymphoedema can be very debilitating for patients and occurs in 16% of male and 6% of female patients (Orlev et al., 2007). The oedema is caused by blockage of the microlymphatics (Amamnn-Vesli et al., 2003) and affects the limbs—predominantly the legs—as well as causing the mild swelling that many patients have around their eyes (Figs 336.2, 336.3, and 336.4).

Ocular features

Another of the early signs of Fabry disease is the appearance of the characteristic corneal lesion, 'cornea verticillata' seen on slit lamp examination of the eye. While these can be seen as a result of long-term therapy with chloroquine and amiodarone, if patients are not taking these drugs, the presence of theses corneal changes can be considered diagnostic of Fabry disease. Nearly all patients will display these

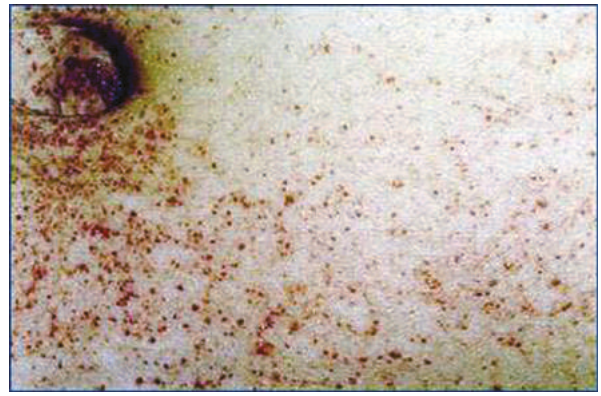

Fig. 336.2 Typical angiokeratoma distribution over the swimming trunk distribution.

lesions. In the cohort of patients managed by the Melbourne Fabry Disease Treatment Centre, 94.1% of males and 71.1% of females had changes (Nguyen et al., 2005) while in the Fabry Outcome Survey (FOS) database the figures are slightly lower with 73.1% of males and 76.9% of females showing this sign (Sodi et al., 2007). Vision is rarely impaired, although a small number of patients do develop Fabry cataracts—23.1% of males and 18% of females (Sodi et al., 2007). Fabry patients also display increased tortuosity of the retinal vessels that has been reported to be associated with an increased severity of the disease (Sodi et al., 2007) and predictive of severer phenotypes in children (Allen et al., 2010) (Figs 336.5 and 336.6).

Cardiac features

Although cardiac and renal involvement do not usually manifest till later in life, there is evidence of early electrocardiogram (ECG) and echocardiogram changes (Havranek et al., 2013) and renal disease, both slight proteinuria and histological changes (Tøndel et al., 2008) in children and adolescents. There have even been reports of early evidence of early microvascular disease affecting the central nervous system (Cabrara-Salazar et al., 2005) (Fig. 336.1).

Cardiac involvement is one of the most common features of Fabry disease, both in hemizygous males and heterozygous females, and is a major source of morbidity and mortality. Deposition of Gb3 occurs in a wide variety of cell types including cardiac myocytes, vascular endothelial cells, cardiac conducting tissue, and fibroblasts of the cardiac valves. The resulting clinical effects are progressive cardiac hypertrophy with reasonably well preserved cardiac function till late in the disease process; a wide range of arrhythmias, mitral valve prolapse, and angina, as well as a dilated aortic root (Linhart, 2006). The left ventricular hypertrophy (LVH) is reported in around 50%

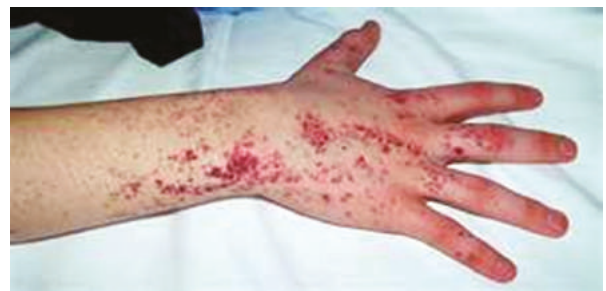

Fig. 336.3 Angiokeratoma mostly on the hand.

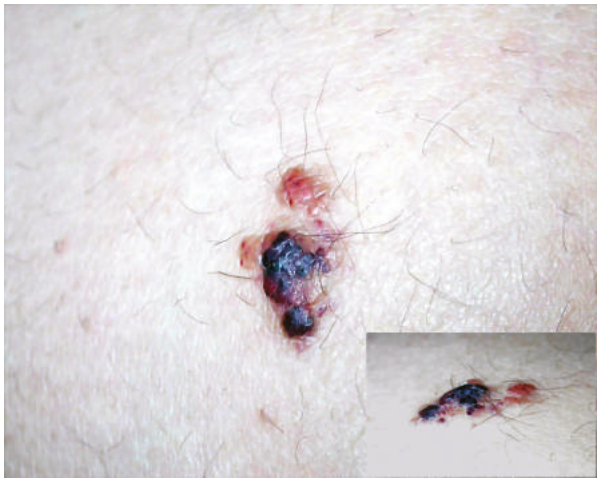

Fig. 336.4 Solitary angiokeratoma.

of classical male patients and about a third of female heterozygotes (Linhart, et al., 2007; Kampmann et al., 2008) and can be detected both by imaging techniques and ECG (Senechal and Germain, 2003). The typical changes start in the posterior lateral section of the left ventricle and during progression there is also evidence of fibrosis in that area. However, in female heterozygotes it is not uncommon to get fibrosis without evidence of hypertrophy (Neimann et al., 2011). Despite the progressive LVH, significant cardiac dysfunction rarely occurs and then only as a very late manifestation (Linhart et al., 2000). However, diastolic dysfunction is common and occurs quite early in the course of the disease (Linhart, 2006). Although LVH is the most common cardiac manifestation of Fabry disease, it is the associated cardiac arrhythmias that cause the most morbidity and mortality. In a retrospective case note study of 447 patients, 42% of male and 27% of female patients had an arrhythmia (Schiffmann et al., 2009). These figures were confirmed in a smaller study (Acharya et al., 2012) where 47% of patients needed some form of implantable intracardiac device. In another study, the patients were divided into the various types of rhythm disturbance and showed paroxysmal atrial fibrillation in 13.3%, sustained atrial fibrillation in 3.9%, non-sustained ventricular tachycardia in 8.3%, and four cases needed a pacemaker because of heart block induced bradycardia. In addition, there was sudden death in one case and a further one

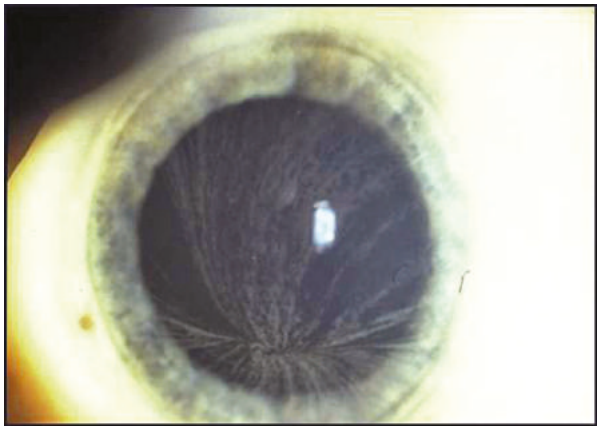

Fig. 336.5 Corneal changes.

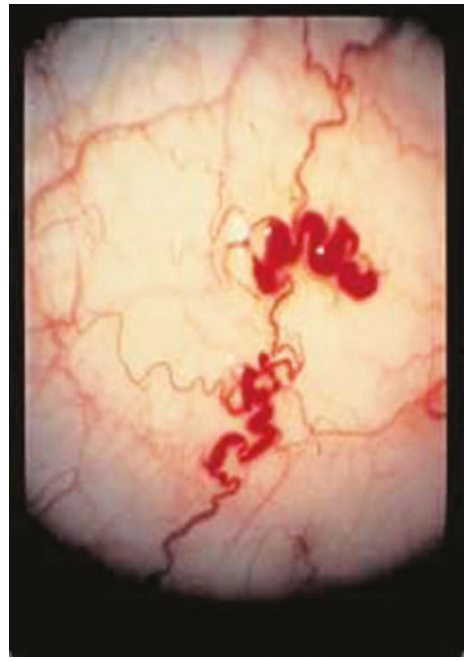

Fig. 336.6 Dilated tortuous vessels seen in the eye and typical of Fabry disease.

patient needed fitting with a biventricular pacemaker with internal cardioverter defibrillator (Shah et al., 2005). The resting ECG will show the changes reflecting the LVH, but early changes can include subtle slowing of the heart and shortening of the PR interval (Pochis et al., 1994). In fact, a short PR interval has been reported in 7% of patients, but 3% had a long PR interval with 6% of patients developing a spontaneous bradycardia requiring intervention (O'Mahony et al., 2011). Syncope may also occur and, although arrhythmia may be partly to blame, cardiac autonomic dysfunction could also be a contributing factor (Lobo et al., 2008) (Figs 336.7 and 336.8).

As with many of the other lysosomal storage diseases, mitral valve involvement is quite common although surgical intervention is rarely required (Sakuraba et al., 1986; Linhart et al., 2000; Kampmann et al., 2002). While there is little aortic valve involvement, dilatation of the aortic root has been reported, but the clinical significance is unclear (Bass et al., 1980).

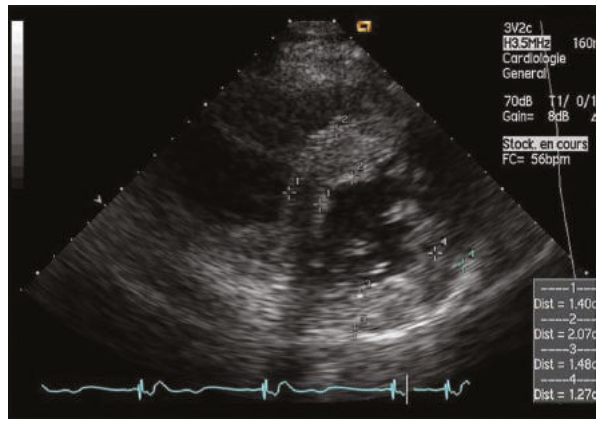

Fig. 336.7 Echocardiogram showing typical left ventricular hypertrophy.
From Germain, D. P. (2010). Fabry disease. *Orphanet J Rare Dis*, 5, 30.

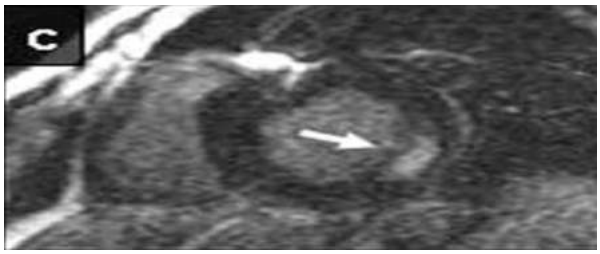

Fig. 336.8 Magnetic resonance scan of heart with gadolinium showing the late enhancement in the posterior wall (arrow) typical of Fabry disease.
From Moon, J. C., Sachdev, B., Elkington, A. G., *et al.* (2003). Gadolinium enhanced cardiovascular magnetic resonance in Anderson-Fabry disease. Evidence for disease specific abnormality of the myocardial interstitium. *Eur Heart J*, 24, 2151–5.

Myocardial infarction is also rare but angina is reasonably common, occurring in around 60% of patients, and is due to reduced flow in the coronary microcirculation as a result of deposition of Gb3 (Elliott et al., 2006; Chimenti et al., 2008). As well as angina, significant numbers of patients also have cough and wheeze due to pulmonary involvement with 36% showing an obstructive ventilation defect (Brown et al., 1997).

Uncontrolled atrial fibrillation certainly contributes to the increased frequency of stoke and TIA in the Fabry population. However, there are other factors involved. A review of 2446 untreated patients in the Fabry Registry showed that 6.9% of males and 4.3% of females had a stroke and of those 86% had an ischaemic stroke while 16.9% of males and 6.9% of females had a haemorrhagic stroke (Sims et al., 2009). The majority of stokes involve the territory of the posterior circulation. While transient ischaemic attacks also occur to excess in Fabry patients, the true incidence is unclear due to problems in definitions and data collection.

Cochlear features

Tinnitus affects many patients in childhood and early adolescence (Reis et al., 2003) and deafness is also common. A review of 109 patients—85 males age range 6–58 years and 24 females aged 22–72 years—showed that 56% had a hearing deficit. The hearing loss in males was more profound in those who had the most evidence of cerebral small vessel disease in the form of white matter lesions. Nevertheless, the predominant defect was found to be cochlear in origin (Reis et al., 2007). Fabry patients often complain of headache and can have symptoms similar to migraine with an aura (Albano et al., 2010).

Renal features

Renal disease is a common complication of Fabry disease, especially in the classical phenotype (Branton et al., 2002). While 17% of patients present with renal problems (Eng et al., 2007), in a cross-sectional study of 1262 adult patient aged 20–82 years 28% of males and 13% of females had chronic renal disease as defined by an estimated glomerular filtration rate (eGFR) < 60 mL/min/m^2. In addition, and very importantly, 62% of males and 33% of females had overt proteinuria (> 300 mg/24 hours). This study also demonstrated that, in males and females, there is a definite association between degree of proteinuria and decrease in eGFR (Ortiz et al., 2008).

Renal involvement has been described early in the course of the disease, especially in children and young adolescents. End-stage renal disease occurred in two young men aged 16 and 24 (Sheth et al., 1983) and early renal changes were found on renal biopsy in a boy of 7 years (with proteinuria) in a cohort of males and females (Gubler et al., 1978). Gubler and colleagues had previously described Fabry disease in children (Desbois et al., 1977). More recently, using registry data, overt proteinuria has been described in boys and girls in their early teenage years (Reis et al., 2003), while microalbuminuria has been described in children as young as 7 years old (Tøndel et al., 2008). Once proteinuria has developed renal function declines at a variable rate between 2.5 and 9 mL/min/year with end-stage renal disease reached by the third to fifth decade in both male and female patients (Branton et al., 2002; Ortiz et al., 2008; Schiffmann et al., 2009; Germain, 2010). It is also of note that patients with significant renal impairment have a far higher incidence of other Fabry complications, and present at a far later stage of the disease, than those without renal involvement (Ortiz et al., 2010).

Even though proteinuria is the most sensitive marker of early renal involvement in Fabry disease, microscopic haematuria has also been described (Sheu et al., 1994; Chen et al., 1990). However, there have also been reports of immunoglobulin A nephropathy and thin basement membrane disease occurring concomitantly in patients with Fabry nephropathy (Whybra et al., 2006; Cai et al., 2011)

An increased incidence of renal cysts in the para-pelvic area has been found on ultrasound scanning of the kidneys (Reis et al., 2004), but these are relatively small and not associated with any symptoms.

Other features

Other clinical features include osteopenia in 50% of patients (Mersebach et al., 2007); depression (Cole et al., 2007); cognitive impairment and dementia (Elsteiin et al., 2012); and 34% with anaemia, partly due to renal impairment (Kleinert et al., 2005), but also contributed to by hypersplensim (Oliveira et al., 2008). Some endocrine abnormalities have also been suggested. Subclinical hypothyroidism is reported (Hauser et al., 2005; Faggiano et al., 2006) as well as cases of azospermia (Faggiano et al., 2006; Paraxanthos-Roche et al., 2007) due to testicular and epididymal involvement (Nistal et al., 1983). Lastly, classical male patient are thought to have slight facial dysmorphism with thick-set features, especially around the orbits and eyebrows (Cox-Brinkman et al., 2007).

Heterozygous females

The traditional view had been that, as an X-linked disease, female heterozygotes would be carriers with a very low incidence of symptoms. However, quite early on it was recognized that females can be affected (Wise et al., 1962). In the first report of a large cohort of female patients MacDermot and colleagues found that, out of 60 obligate carrier females, 70% had neuropathic pain and in 27.5% the pain was such that it significantly adversely affected quality of life while 30% had experienced a serious major organ event. They also reported that survival was 70 years of age some 15 years shorter than the normal population (MacDermot et al., 2001).

The effect of symptoms on quality of life was confirmed by a case–control study in 63 female where there was a highly significant increase in pain in the hands and feet, joint pain, dizziness, fatigue, and loss of libido (Bouwmann et al., 2012). In a larger cohort of female heterozygotes in the Fabry Registry (N = 1077), similar findings were reported—69.4% had some symptom or signs of Fabry disease with a median age of onset of 13 years and

20% had experienced a major clinical event at a median age of 40 years—about one decade later than in men (Wilcox et al., 2008).

From the renal perspective, 19% had an eGFR of < 60 mL/min/m^2, 39% had proteinuria > 300 mg/24 hours, and 22.2% had proteinuria > 1 g/24 hours putting them at high risk of progressing to end-stage renal failure with around 1–2% at end-stage renal disease (Wilcox et al., 2008).

From a cardiac perspective, around 75% of 55 female patients had some degree of LVH with all those > 45 years of age showing LVH. An increase in LVH and slight progressive decline in systolic and diastolic function were also found (Kampmann et al., 2002). However, one aspect of cardiac involvement that differs in females compared to males is that females can have fibrosis and decreased function without LVH (Neimann et al., 2011).

Atypical variants

While most patients have 'private' genetic mutations, some patients have been described with mutations common to several completely unrelated families and these were found to be missense mutations with significant residual, but still low, levels of enzyme and to have disease confined to a single organ as well as presenting at an older age (Eng and Desnick, 1994; Topacoqlu et al., 1999). When screening of 'at-risk' populations started, so-called atypical variants were described with cardiac disease (Nakao et al., 1995) and renal disease (Nakao et al., 2003) without any apparent systemic symptoms or signs. However, a cautionary note is needed at this point. While these late-onset phenotypes are predominantly single organ, the patients will need regular follow-up as they may develop other Fabry complications at a much later stage, such as the cardiac variant patient who developed renal impairment with proteinuria and typical inclusions within his podocytes at the age of 70 years (Meehan et al., 2004). In addition, some patients who have a mutation associated with late-onset single organ disease can present with a classical phenotype (personal observation).

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CHAPTER 337

Fabry disease: diagnosis

Stephen Waldek

Introduction

Like all rare diseases, the key to diagnosis lies in including Fabry disease in the differential diagnosis when, in a renal context, patients present with proteinuria, haematuria, and/or renal impairment. A careful history, paying particular attention to symptoms of neuropathic pain, gastrointestinal upset, and ability to perspire, can be very helpful, remembering that some of these symptoms may have occurred in childhood but spontaneously improved by the time the patient is seen in an adult clinic. A thorough and extensive family history can also be an indicator taking into consideration that some members of the family may have experienced non-renal manifestations of the disease such as cardiac hypertrophy or stroke.

Detailed and meticulous physical examination looking for the tell-tale sign of angiokeratoma can be extremely helpful. If readily available, examination using a slit lamp to detect the typical corneal changes can also be very helpful. Urine microscopy can be useful. The presence of lipid deposits has been reported as a method of diagnosing Fabry disease (Desnick et al., 1971; and see Fig. 337.1). Seventy-five per cent of cells found in the urinary sediment of classical male hemizygous Fabry disease patients are of tubular origin and are loaded with glycosphingolipids (Chatterjee et al., 1984). Examination of urine sediment under polarized light in Fabry patients will demonstrate the typical 'Maltese Cross' configuration. This has been found to have a near 100% diagnostic sensitivity and specificity (Salvarajah et al., 2011) (Fig. 337.2).

Pathology

Renal biopsy is often the first indication that a patient might have Fabry disease (Figs 337.3–337.7). The deposition of Gb3 within all elements of the kidney gives a distinctive and typical picture. If the biopsy is examined under a low-powered light microscope immediately after being taken, the glomeruli appear white due to the lipid deposition rather than the normal red (Fig. 337.6). The vacuoles seen using standard techniques under light microscopy are quite typical of Fabry disease, but can sometimes be misinterpreted as foam cells found in other conditions such as focal segmental glomerular sclerosis. Electron microscopy is, however, diagnostic showing the typical 'zebra body' appearance (Fig. 337.5). This is especially helpful in female heterozygotes (Schatzki et al., 1979). As electron microscopy is not always available, the diagnosis can readily be made by staining semi-thin sections with toluidine blue (Fig 337.7). To aid diagnosis and to allow for the biopsy to be graded, several scoring systems have been developed and their use is to be encouraged (Fogo et al., 2010; Barisoni et al., 2012).

The renal lesions of Fabry disease were described by Colley and colleagues (Colley et al., 1958). The disease is characterized by the progressive widespread deposition of crystalline material containing neutral glycosphingolipid and terminal alpha-galactosyl moieties. These show birefringence under polarized light with the typical 'Maltese Cross' sign. Nearly all cell types are affected although there are no deposits in red blood cells or in liver hepatocytes (deposits are found in the Kupffer cells and the endothelial cells of the liver sinusoids). Within the kidney all cell types are affected, even in patients with normal glomerular filtration rate and minimal proteinuria (Gubler et al., 1978). Vacuolization of podocytes and epithelial cells is characteristic of Fabry disease with mesangial expansion and progressive segmental and global glomerulosclerosis present (Gubler et al., 1978; Alroy et al., 2002; Sessa et al., 2002; Fischer et al., 2006). Deposits are also seen in the endothelial cells of the renal vasculature and vascular smooth muscle cells often giving the appearance of fibrinoid necrosis (Gubler et al., 1978) (Figs 337.3, 337.4, and 337.5).

The tubules are not spared and deposits are seen in the distal tubules and loop of Henle with far fewer in the proximal tubules (Sessa et al., 2001). Although they can be more patchy, similar lesions are seen in all renal cell types in female heterozygotes (Valbuena et al., 2008).

Biochemical and genetic testing

Once Fabry disease is suspected, a definitive diagnosis can be made in male patients by measuring the level of alpha galactosidase in either white cells or plasma using the synthetic substrate 4-methylumbelliferyl-alpha-D-galactosidase (Winchester and

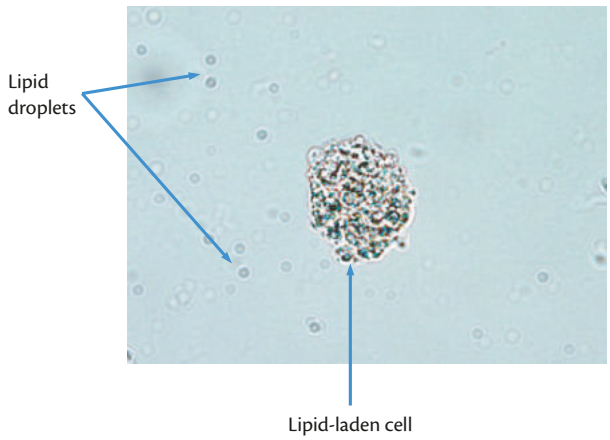

Fig. 337.1 Urine microscopy showing lipid droplets.
From *Journal of Nippon Medical School*, Utsumi K, Mitsuhashi F, Katsura K, Iino Y, and Katayama Y. 'Maltese Crosses' in Fabry Disease, 77(6), p. 284. Copyright 2010 The Medical Association of Nippon Medical School. Reprinted with permission.

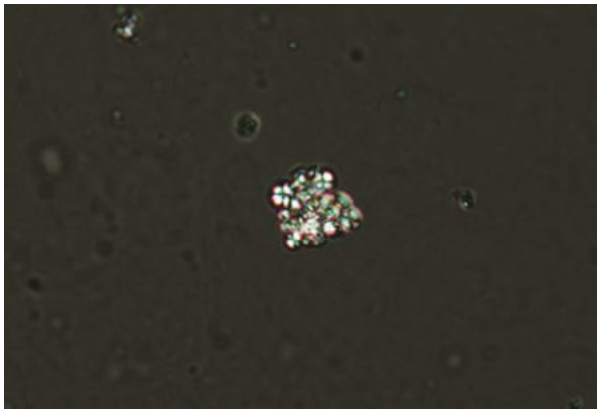

Fig. 337.2 Urine microscopy under polarized light showing 'Maltese Cross' sign. From *Journal of Nippon Medical School*, Utsumi K, Mitsuhashi F, Katsura K, Iino Y, and Katayama Y. 'Maltese Crosses' in Fabry Disease, 77(6), p. 284. Copyright 2010 The Medical Association of Nippon Medical School. Reprinted with permission.

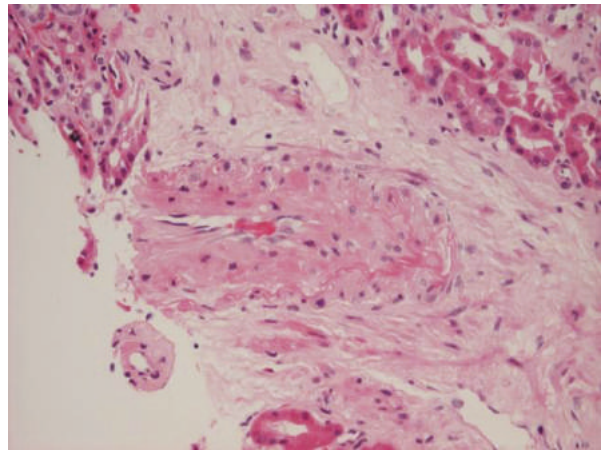

Fig. 337.4 Small renal artery stained with H and E showing the fibrinoid like changes due to deposition of Gb3 in the endothelium and muscle layers. Reproduced from James C.C. Moon, Bhavesh Sachdev, Andrew G. Elkington, William J. McKenna, Atul Mehta, Dudley J. Pennell, Philip J. Leed, Perry M. Elliott, Gadolinium enhanced cardiovascular magnetic resonance in Anderson-Fabry disease: Evidence for a disease specific abnormality of the myocardial interstitium, *European Heart Journal*, 2003, 24/3, by permission of Oxford University Press.

Young, 2006). This process has been facilitated by the development of an assay using dried blood spots (Lukas et al., 2007), which is particularly helpful where samples need to travel over long distances or in screening programmes. Unfortunately, only about 40% of female patients have a low enzyme level and therefore enzyme measurement is not suitable for diagnostic purposes. In this circumstance, gene mutation analysis should be undertaken using standard techniques. In fact, gene mutation analysis should be undertaken in all cases as there are occasional instances when there are polymorphisms present resulting in slightly reduced levels of enzyme but no disease as the enzyme produced is fully functional. For example, the D313Y polymorphism gives low levels of enzyme but no apparent disease and is known as a 'pseudodeficiency' (Neimann et al., 2013). Gene mutation analysis will also be important for family screening (Figs 337.6 and 337.7).

Family screening

Family screening is an essential part of the diagnostic journey and for every index case seen an average of five to six new cases can be found (Laney et al., 2008). By undertaking such testing patients can, hopefully, be identified early in the course of their disease and thus get maximum benefit from therapeutic intervention. While family screening is important, it needs to be done with care and by an experienced genetic counsellor or geneticist—especially where babies and young children are involved—so that the pros and cons can be properly explained. Two reports of neonatal screening have been published (Spada et al., 2006; Hwu et al., 2009). However, both these studies showed the vast majority of patients to have later-onset mutations, raising the issue of whether such screening can be justified for a disease where the manifestations may not appear for many decades. To deal with these issues, the American College of Medical Genetics set up a working group that published guidelines in 2011 (Wang et al., 2011)

Prenatal diagnosis is available (Desnick, 2007) and there is also some successful experience of pre-implantation diagnosis

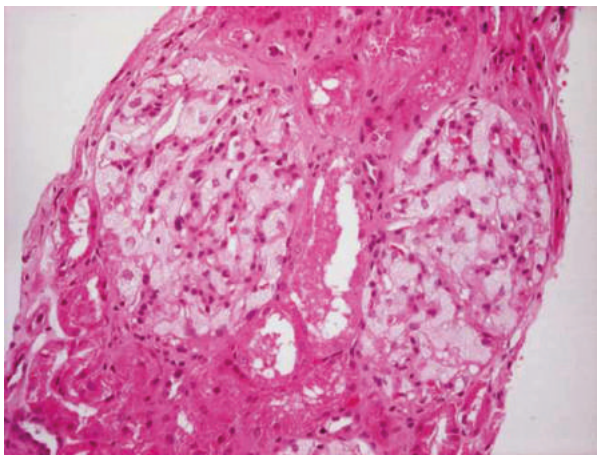

Fig. 337.3 Renal glomerulus stained with H and E showing numerous vacuoles typical of Fabry disease.

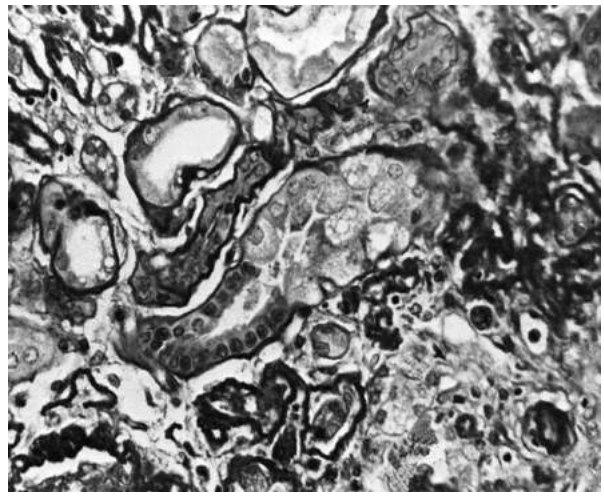

Fig. 337.5 Renal tubules showing Gb3 deposits.

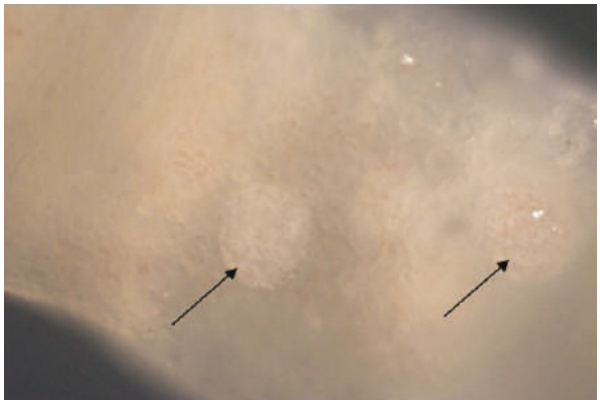

Fig. 337.6 Low power of freshly taken renal biopsy showing the white rather than usual red appearance of the glomeruli (arrows). From Tøndel, C., Bostad, L., Hirth, A., *et al.* (2008). Renal biopsy findings in children and adolescents with Fabry disease and minimal proteinuria. *Am J Kidney Dis*, 51(5), 767–76.

(Altarescu et al., 2012). Prenatal testing is discussed further in Chapter 302.

Population screening

Screening in so-called high-risk populations has been reported. These are difficult to interpret and compare because of differing methodologies—some using enzyme measurements and thus confined to male patients; others looking at males and females via genetic mutation analysis; and some may well have included polymorphisms that may not be disease causing.

A systematic review reports Fabry disease in up to 4% of cases among patients with unexplained left ventricular hypertrophy, an average 0.33% in those on dialysis, 0.1% in transplant patients, and up to 6% in those < 55 years with a cryptogenic stroke (Linthorst et al., 2010). Whether diagnosing Fabry disease through such screening programmes can be justified is debatable. Much will depend on the nature of the population deemed at risk and methods used. For example, a study in the United Kingdom in haemodialysis patients failed to detect any new patients (Wallin et al., 2011) whereas a study in Spain reported 0.55% of their cohort had the disease (Gaspar et al., 2010). However, in the United Kingdom only male patients were studied using enzyme analysis in dried blood spots, but in the Spanish study both males and females were included and the screening was via a combination of enzyme assay and genetic mutation analysis.

In order to try and define the benefits of screening for Fabry disease and to have uniform criteria, guidelines have been produced (Terryn et al., 2013). As has been noted, proteinuria is a cardinal manifestation of renal involvement and should be considered in the differential diagnosis of undiagnosed proteinuria (Fervenza et al., 2008).

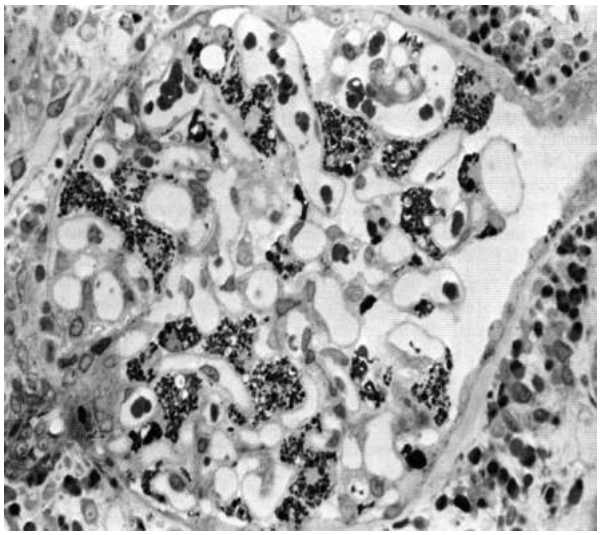

Fig. 337.7 Semi thin section of renal biopsy stained with Toluidine blue. From Tøndel, C., Bostad, L., Hirth, A., *et al.* (2008). Renal biopsy findings in children and adolescents with Fabry disease and minimal proteinuria. *Am J Kidney Dis*, 51(5), 767–76.

Future methods

Unfortunately, there is as yet no definitive biomarker to aid diagnosis, although, as mentioned below, progress is being made and might be used when following certain groups of patients under therapy. Plasma Gb3 has been proposed and used in the biochemical diagnosis of Fabry disease, but the method is time-consuming and not readily available. In addition, plasma Gb3 levels are generally normal in female patients and many of the milder later-onset phenotypes (Vedder et al., 2007). The situation has been reviewed in 2010 (Schiffman et al., 2010). Work on the de-acetylated product of Gb3, Lyso- Gb3, and its analogues may prove helpful (Lavoie et al., 2013) as may the proteomic approach (Kistler et al., 2011) and development of Saposin A and GM_2 activator protein as biomarkers (Manwaring et al., 2013).

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CHAPTER 338

Fabry disease: management and outcome

Stephen Waldek

Introduction

Fabry disease is a multiorgan and multisystem disease. Successful management lies in the establishment of a coordinated, multidisciplinary, and multiprofessional team approach. Guidelines have been published to assist in patient management (Eng et al., 2006) and additionally, several countries have produced their own guidelines or standard operating policies such as these from the United Kingdom: (UK at <http://www.edrep.org/pages/resources/rare-and-metabolic.php#fabry>; Canada at <www.garrod.ca/wp-content/uploads/FD-Treatment-Guidelines-2011.pdf>).

While there are differences between these guidelines and they may not always be updated to include the latest pieces of evidence, those managing Fabry disease are encouraged to review the published guidelines and available evidence to establish their own protocols for the care of these patients who nearly always have multiple problems in addition to their renal disease.

Assessment

Because of the wide variation in phenotype and the heterogeneity of the disease, all patients and potential patients should undergo a full assessment. Table 338.1 lists the assessments recommended (non-renal), or desirable, with the frequency they should be undertaken in those receiving enzyme replacement therapy (ERT) and those not.

General

Quality of life (QOL) questionnaires have been used for Fabry disease, both clinically and in clinical trials, but none of these, including the SF-36, have been specifically validated in Fabry disease. Nevertheless, QOL questionnaires can give interesting results (Wyatt et al., 2012). Pain and gastrointestinal symptoms can either be assessed purely from the history or using scales. Exercise tolerance can be assessed from the history by reference to the ability to perform acts of daily living. However, if an exercise electrocardiogram (ECG) is performed as part of the assessment (see below), this can also act as a measure of exercise tolerance. In addition, this can be helpful in assessing the degree of autonomic nervous system dysfunction by studying the ability of the patient to mount a satisfactory blood pressure and pulse rate response to exercise. As QOL is related to psychological well-being, this should also be assessed and can give an indication that therapy is effective. The Minnesota Multiphase Personality Inventory has been used and found to be helpful (Crosbie et al., 2012).

As depression occurs frequently in Fabry disease patients and is often under-diagnosed (Cole et al., 2007) assessment should include appropriate questioning at regular intervals. In addition, cognitive impairment also occurs (Segal et al., 2010; Schermuly et al., 2011) and needs to be detected at an early stage. Therefore screening for this should be included in the assessments of older patients (Elstein et al., 2012).

Disordered sweating is a significant cause of concern for patients. The Quantitative Sudomotor Axon Reflex Test (QSART) can be fairly easily employed to assess sweating (Schiffmann et al., 2003), but more sophisticated neurophysiological measurements are time-consuming and really not suitable for routine clinical practice.

Audiology

A routine audiogram is helpful at baseline and can be repeated at regular intervals depending on whether there are changes at baseline—especially the typical reduction in high-pitch tone perception.

Ophthalmology

Baseline ophthalmological assessment might show the typical corneal changes, but of more importance is the state of the fundal vessels. Increased tortuosity of the fundal vessels has been associated with a more severe phenotype (Allen et al., 2010).

Cardiology

Cardiac complications account for a significant degree of morbidity and mortality in both males and females and a comprehensive assessment at baseline is essential. An annual ECG may show some of the early signs (O'Mahony et al., 2011) as well as give an indication of left ventricular hypertrophy (LVH). At the same time a Holter 24-hour ECG will indicate the presence of arrhythmias, although in some cases a more prolonged ECG may be needed or even an implantable recorder placed. LVH is common and thus echocardiography, at least annually, is important with special reference to left ventricular mass index (LVMI), posterior wall, and septal thickness. Diastolic and systolic function should be monitored as well as aortic root diameter and the function of the mitral and aortic valves. Cardiac fibrosis, commencing in the interstitial layer of the posterior wall, is important as a marker of Fabry cardiomyopathy. This was initially demonstrated by multislice computed tomography (CT) (Funabashi et al., 2003) but has been superseded by magnetic resonance (MR) scanning with gadolinium enhancement (Moon et al., 2003) and echocardiographic

Table 338.1 Non-renal assessments recommended for Fabry disease with frequency. Italics = discretionary

General well-being	At baseline and annually thereafter
Some measure of QOL (e.g. SF-36)	
Pain, gastrointestinal symptoms (using validated scales)	
Exercise tolerance.	
Sweating (QSART if available)	
Neurological	At baseline and annually thereafter
History—tinnitus, vertigo, transient ischaemic attacks/stroke, at baseline and annually thereafter	
Cognition using standard tests	If indicated
T2-weighted brain scan with or without angiogram.	If indicated
Audiology at baseline and then every 2–3 years	
Ophthalmology at baseline	
The heart	*At baseline and annually if on ERT*
ECG and routine Holter monitoring	
ECHO (and variations)	
Exercise ECG	

tissue Doppler strain imaging (Peroni et al., 2003). While assessment using straightforward two-dimensional echocardiography is essential, many are now advocating MR scanning and/or tissue Doppler strain imaging to give earlier more accurate indications of Fabry cardiomyopathy, especially in the early stages of the disease (Weidemann et al., 2005). More recently, non-contrast MR scanning with myocardial T1 mapping has been shown to have great promise, especially in cases where gadolinium is contraindicated (Sado et al., 2013). Two-dimensional speckle tracking has also been found quite useful and is being adopted by many centres (Saccheri et al., 2013).

Central nervous system

As noted in Chapter 336, central nervous system involvement is common and the early signs are often not clinically detectable. Therefore a T2-weighted brain scan with angiogram is included in the recommended scheme for regular assessment. Particular attention should be paid to the presence of 'white matter lesions' and evidence of micro-bleeds together with the diameter of the basilar artery (Fellgiebel et al., 2009; Reisin et al., 2011) and the volume of the hippocampus (Fellgiebel et al., 2012) (Fig. 338.1).

Renal

From a renal perspective, three investigations need to be considered. Proteinuria, as in other glomerular diseases, is a significant prognostic indicator (Wanner et al., 2010; Warnock et al., 2010b, 2012) and should be measured when the patient is first seen and then every 6–12 months thereafter, either in a timed urine sample or in a spot urine as albumin and/or protein:creatinine ratio. As early renal disease can be present in children (Reis et al., 2005, Tødel et al., 2008), it is also important that they are screened from an early age for the first indications of renal involvement by regular measurement of microalbuminuria/proteinuria. The same is true of older patients, including female heterozygotes that may have varying degrees of disease manifestations, or may be apparently asymptomatic. In fact, microalbuminuria/proteinuria should be measured in all patients.

Measurement of renal function is essential and, again, this should be done when someone first presents and then at regular intervals—6-monthly if on ERT or annually if not. How this is done will depend on clinical circumstances. In adults, Rombach and colleagues recommend the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula for estimated glomerular filtration rate (eGFR) as being the most accurate over the whole range of renal function, but felt that the iohexol method was preferred for direct measurement (Rombach et al., 2010). In children, the new Schwartz formula is recommended (Ramaswami et al., 2010).

Lastly, if not performed in the process of diagnosing the condition, a renal biopsy is strongly recommended in any patient with reduced renal function and/or any degree of proteinuria—even microalbuminuria. At baseline, before starting therapy, this can give valuable information as to the prognosis or help in deciding on the initiation of ERT. Glomerular sclerosis has been reported as indicating a poor outcome and response to ERT (Germain et al., 2007) and early changes in children and young adults have been an indication to start therapy (Tøndel et al., 2008, 2013). In cases of heterozygous females, renal biopsy can also be very helpful in deciding on whether there is renal involvement and whether treatment should be started (Farge et al., 1985). Renal biopsy can also be helpful to rule out concomitant disease such as diabetes of immunoglobulin A nephropathy—especially in those patients with significant microscopic haematuria. As mentioned in Chapter 336, haematuria can be found in Fabry disease and biopsy will distinguish patients with Fabry disease from those where this is another superimposed condition. Biopsy can also be helpful in if there is a sudden deterioration in renal function during treatment (e.g. from a superimposed second renal disease) or if there is a poor response to therapy where a comparison with a baseline biopsy can show if there has been significant clearing of globotriaosylceramide (Gb3) deposits.

Although not essential, a renal ultrasound may be performed and might demonstrate parapelvic cysts. Although they are not of any clinical significance, they are found in up to 50% of patients (Reis et al., 2004).

While no true validated biomarker has been identified in Fabry disease (unlike many of the other lysosomal storage disorders where specific treatment is available), much progress has been made (Kistler et al., 2011; Lavoie et al., 2013; Manwaring et al., 2013). As this is an important area for future development, those caring for Fabry patients are to be encouraged to 'bio-bank' samples for future use to measure biomarkers as they become available.

Adjuvant therapies

Table 338.2 details the therapies and interventions that are available for many of the non-renal manifestations of Fabry disease.

While most patients, especially those in the early stages of the disease, have relatively low blood pressure (Branton et al., 2002), hypertension does occur (Kleinert et al., 2006) and should be aggressively managed as in other cases of chronic kidney disease as it has been shown to accelerate the decline in renal function in Fabry patients (Ortiz et al., 2008). However, care should be taken when choosing antihypertensive agents because of possible cardiac involvement. Angiotensin converting enzyme inhibitors (ACEIs)

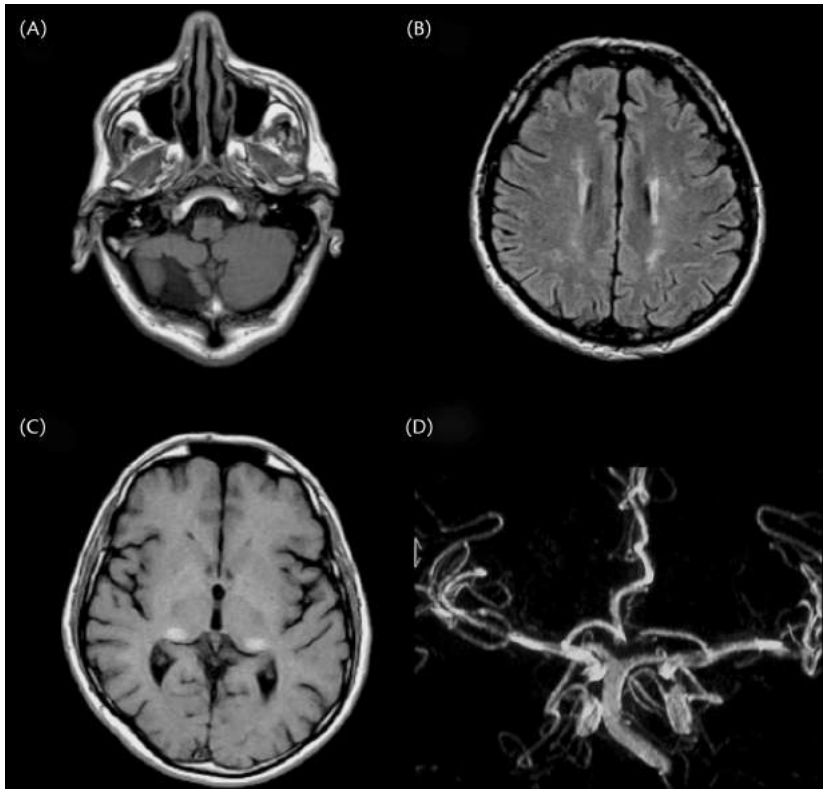

Fig. 338.1 Neuroimaging with magnetic resonance showing four typical changes in Fabry disease. (A) Haemorrhage or infarction. (B) White matter lesions. (C) Pulvinar sign. (D) Irregularity of the basilar artery system.
Reprinted from *The Lancet Neurology*, 5/9, Andreas Fellgiebel, Matthias J Müller, Lionel Ginsberg, CNS manifestations of Fabry's disease, pp. 791–795, Copyright 2006, with permission from Elsevier.

Table 338.2 Adjuvant therapies for non-renal complications of Fabry disease

Symptoms	Management options
Pain crises, acroparaesthesias	Avoid physical exertion or exposure to other triggers
	Phenytoin, carbamazepine, gabapentin, amitriptyline
Gastrointestinal symptoms	Small frequent meals, metoclopramide, H_2 blockers
High degree of AV block or significant tachy/brady arrhythmia	Permanent cardiac pacing
Other arrhythmias	Appropriate drugs as indicated
Angina	Nitrates, beta blockers, calcium channel blockers etc.
Cardiac failure	Beta blockers, ACEIs/ARBS, diuretics
Stroke prophylaxis	Antiplatelet agents, ACEIs, statins
Dyslipidaemia	Statins
Hearing loss	Hearing aids
Angiokeratoma	Advice on 'masking' agents. Rarely laser therapy
Depression/Anxiety	Psychiatric or psychology referral. Social support
Lymphoedema	Symptomatic therapy only

or angiotensin receptor blockers (ARBs) are the treatment of choice. Beta blockers should be used with caution because of possible bradycardia and for the same reason non-rate limiting calcium antagonists are preferable. Atherosclerosis is not a particular feature of Fabry disease, but statins may help because of their pleotropic effects (Politei, 2009).

Proteinuria is a major prognostic indicator for outcomes in Fabry disease (Wanner et al., 2010; Warnock et al., 2010b, 2012) and the use of ACEIs or ARBs to reduce the level of proteinuria is recommended. (Tahir et al., 2007). In fact, the recommendation is to use ERT with antiproteinuric and antihypertensive medication to reduce the urine protein to < 500 mg/24 hours, the blood pressure to 130/80 mmHg or less, so as to reduce the decline in renal function to −1.0 mL/min/1.73 m^2 (Jain and Warnock, 2011).

Enzyme replacement therapy

Two recombinant enzyme replacement products are available. Agalsidase beta (GenzymSanofi)) is manufactured in hamster ovary cells, is administered in a dose of 1 mg/kg body weight intravenously every 2 weeks (AgalB), and was approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in 2001. Agalsidase alpha (Shire Corporation) is produced in human fibroblasts, is administered in a dose of 0.2 mg/kg body weight intravenously every 2 weeks (AgalA), and was also approved in 2001 by the EMA but not by the FDA. Efficacy data for these two products was based on small clinical trials, because of the rarity of the disease, and used surrogate markers based

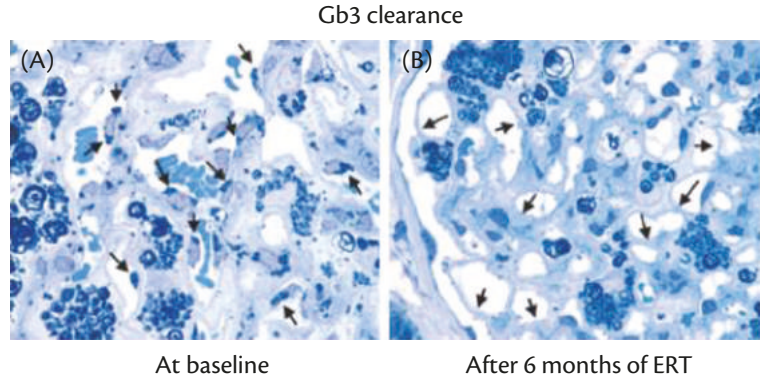

Fig. 338.2 Representative renal biopsies from the agalsidase beta phase III study at baseline and after 6 months of treatment showing clearance of Gb3.
Reprinted by permission from Macmillan Publishers Ltd: *Kidney International*, Beth L Thurberg, Helmut Rennke, Robert B Colvin, Steven Dikman, Ronald E Gordon et al., Globotriaosylceramide accumulation in the Fabry kidney is cleared from multiple cell types after enzyme replacement therapy, 62/6, copyright 2002.

predominantly on substrate clearance on histological examination of kidney (and in some studies heart and skin). The phase III randomized placebo controlled trial of AgalB over 6 months in 58 symptomatic patients with relatively uniform mild renal involvement showed complete clearance of Gb3 from interstitial capillary and mesangial cells (Eng et al., 2001) as well as partial clearance from arterial smooth muscle cells (Thurberg et al., 2002). This clearance was maintained at the end of a 54-month extension (Fig. 338.2) where all patients were receiving ERT with at this stage partial clearance from podocytes that had been unaffected at 6 months (Germain et al., 2007). A smaller placebo controlled study in 26 quite heterogeneous patients treated with AgalA over 6 months showed a 12.5% reduction in mesangial widening compared to a 16.5% increase in the placebo group (Schiffmann et al., 2001).

Renal outcomes

The AgalB 54-month extension study showed that eGFR remained stable, except in a few patients where there was proteinuria of >1 g/24 hours and/or glomerulosclerosis of 50% or more (Germain et al., 2007). Over the 6 months of the AgalA study (Figs 338.3 and 338.4) there was an apparent increase in creatinine clearance in the treated group compared to those receiving placebo but there was no difference in the inulin clearance (Schiffmann et al., 2001). When 25 patients on AgalA were followed for up to 5 years, renal function in CKD stages 1 and 2 was fairly stable declining at between 1.5 and 2 mL/min/1.73 m^2 per year while it was approximately 5.2 mL/min/1.73 m^2 per year in those in CKD stage 3 (Schiffman et al., 2006). Only one clinical trial has looked at outcomes in patients with significant renal impairment at baseline (Fig. 338.5).

A placebo controlled study in 74 patients with significant renal impairment resulted in a 61% reduction in renal, cardiac, cerebrovascular events and death in a per protocol analysis that adjusted for baseline proteinuria (Banikazemi et al., 2007).

Although, from a renal perspective, there have been no other controlled clinical trials, results have been published of data accumulated from two disease registries. The Fabry Outcome Survey (FOS) reported 5-year outcomes and reported declines in renal function of 2.83, 2.17, and 3.8 mL/min/m^2 per year for CKD stages

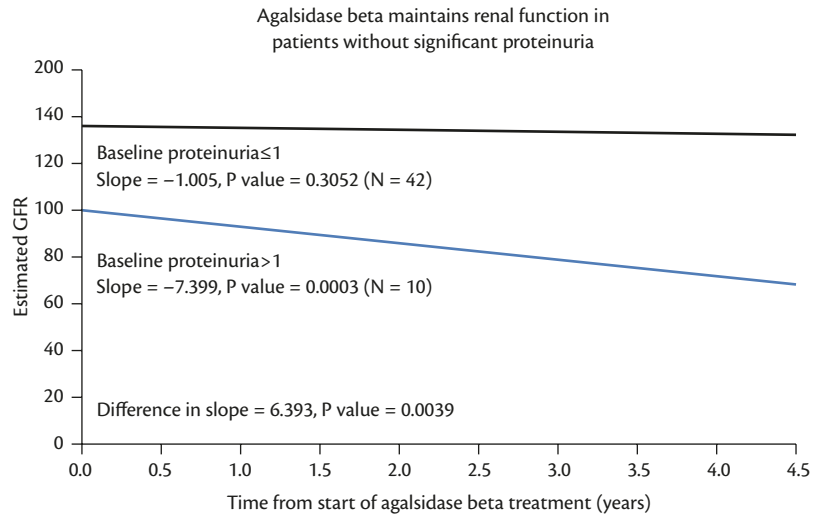

Fig. 338.3 Influence of proteinuria on renal function in patients receiving ERT—from the agalsidase beta phase 3 extension study.
Reproduced from Germain, D. P., Waldek, S., Banikasemi, M., *et al.* (2005). Sustained long term renal stabilization after 54 months of agalsidase beta therapy in patients with Fabry disease. *J Am Soc Nephrol*, 18(5), 1547–57.

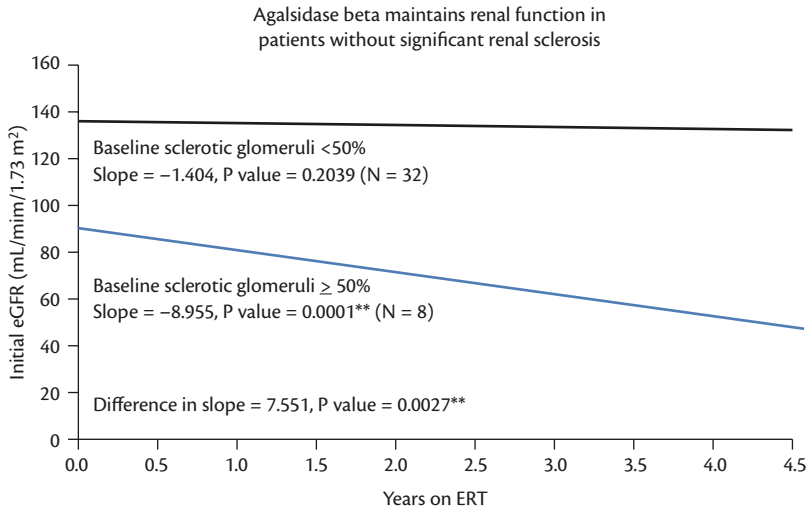

Fig. 338.4 The negative influence of glomerulosclerosis on renal function in patients receiving ERT—from the agalsidase beta phase 3 extension study.
Reproduced from Germain, D. P., Waldek, S., Banikasemi, M., *et al.* (2005). Sustained long term renal stabilization after 54 months of agalsidase beta therapy in patients with Fabry disease. *J Am Soc Nephrol*, 18(5), 1547–57.

1, 2, and 3 respectively (Mehta et al., 2009). More recently, also from FOS, data has been reported on patients treated for 5 years or more (Feriozzi et al., 2012). This showed similar results with a mean yearly decline of −2.2 mL/min/1.73 m^2 per year with proteinuria again an important factor. In the group with proteinuria < 500 mg/24 hours the mean decline was −1.7 mL/min/1.73 m^2 per year. This compares with a decline of −1.01 mL/min/1.73 m^2 per year in patients treated with AgalB who had < 1 g of proteinuria per 24 hours and were followed for 54 months (German et al., 2007). Further evidence comes from the Fabry Registry. Renal function was assessed in 151 men and 62 women receiving AgalB for at least 2 years. Patients were divided into quartiles based on their degree of proteinuria. The mean eGFR slope in men within the first quartile with the lowest amount of proteinuria was only −0.1 mL/min/min/1.73 m^2 while in the fourth quartile with the highest protein excretion the mean slope was −6.7 mL/min/1.73 m^2. The conclusion reached was that treatment with agalsidase beta stabilized renal function if proteinuria was controlled, especially if protein excretion was < 1 g/24 hours. This study also found that the sooner a patient started therapy after the onset of symptoms, the better the renal outcome (Warnock et al., 2012).

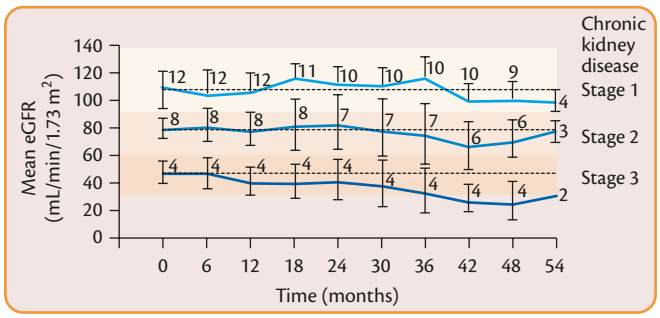

Fig. 338.5 Outcome in patients treated with agalsidase alpha over 54 months. Lines show change in eGFR over time for patients with three different levels of function at time zero.
Reproduced from Schiffmann, R., Ries, M., Timmons, M., *et al.* (2006). Long-term therapy with agalsidase alpha for Fabry disease: safety and effects on renal function in a home infusion setting. *Nephrol Dial Transplant*, 21, 345–54.

Effects on other organs

Heart

After 6 months of AgalA therapy in a double-blind placebo-controlled randomized trial there was a 20% decrease of myocardial Gb3 compared to a 10% increase in the placebo group. This was accompanied by a slight decrease of left ventricular mass as measured by MR scanning (Hughes et al., 2008). Examination of the myocardial histology after 6 months of AgalB showed almost complete clearance of Gb3 deposits from all cell types except the cardiomyocytes (Thurberg et al., 2009) and this was maintained after 54 months (Germain et al., 2007). Further evidence for AgalB comes from the Fabry Registry where 115 men who had received ERT for at least 2 years were compared to 48 untreated men. For those < 30 years of age the mean improvement in left ventricular mass was 3.6 g/year compared to an increase of 9.5 g/year in the untreated group. Overall, there was a significant improvement in left ventricular mass or a stabilization. Multivariate analysis showed that the two factors that were associated with the lowest degree of improvement were the age of the patient at baseline and degree of LVH at start of ERT (Germain et al., 2013). Results from a single cohort of 42 patients, receiving AgalB, also demonstrated an improvement in left ventricular morphology and function as well in ECG changes (Motwani et al., 2012). Similar findings have been reported in patients receiving AgalA. Data on patients with LVH from the FOS using echocardiographic measurements in patients followed for 1–2 years revealed a reduction on mean ventricular wall thickness ($P < 0.5$) and in LVMI (Beck et al., 2004). More recently, the effectiveness of AgalA on LVMI in male and female patients with and without LVH at baseline was assessed and after 12 months there was a significant reduction of 9.2 ± 7.9 g/m^2 (Kampmann et al., 2009).

Stroke

No specific data has been published on stroke, although the phase IV AgalB study did show an overall reduction of risk, when

corrected for proteinuria, and that included stroke (Banikazemi et al., 2007). Nevertheless, strokes can occur while on ERT and are often associated with an increased disease burden such as significant renal impairment. However, data does exist showing improvement in pain and other neuropathic symptoms as well as QOL in patients receiving both products. Three reports are from formal clinical trials (Germain et al., 2005; Wraith et al., 2007; Schiffmann et al., 2010) while others from FOS and the Fabry Registry also confirm improvements in pain and well-being (Wilcox et al., 2004; Hoffmann et al., 2005; Breunig et al., 2006; Pintos-Morell et al., 2009; Watt et al., 2010).

Enzyme replacement in patients with end-stage renal disease

There is little data on ERT in patients with end-stage renal disease, either on dialysis or with a functioning renal transplant. Renal transplantation was initially thought to be a potential treatment for alleviating some of the symptoms of Fabry disease by providing a source of enzyme, However that is not the case with circulating alpha-galactosidase remaining the same after as before transplantation (van den Berg et al., 1976).

The results of renal transplantation are excellent. The 5-year graft survival has been reported as 74% and superior to a non-Fabry group (69%) but comparable with a matched group. Patient survival at 5 years was 81%, a little less than the 90% in a matched group (Shah et al., 2009). An earlier study showed similar 5-year results (Ojo et al., 2000). In fact, the results are so good that renal transplantation should be considered in all Fabry patients with end-stage renal disease (Cybulla et al., 2013).

Despite these good results, especially in terms of patient survival, Fabry disease confers a higher risk of death—odds ratio 2.15 (Mignani et al., 2010)—and, although there are not clinical trials or other evidence to support the use of ERT post transplant, this is now recommended (Mignani et al., 2010).

ERT given during haemodialysis with either of the two available products is safe, is not removed by dialysis, and requires no change of dose (Kosch et al., 2004; Pastores et al., 2007).

For those on dialysis, 3-year survival is 60% in Europe (Tsakiris et al., 1996) and 63% in the United States (Thadhani et al., 2002). This is better than the diabetic population but not as good as the general non-diabetic patients, and is almost certainly due to the added disease burden from the cardiac and central nervous system complications of Fabry disease (Mignani et al., 2010). Thus, ERT should be considered, on an individual patient basis, in an attempt to reduce the cardiovascular risk.

When should treatment be commenced?

Reviewing this, Warnock and colleagues are clear that treatment should be started as soon as a diagnosis is made in patients where no enzyme is detected as these are the severe classical cases. In other patients, treatment should start as soon as the first signs or symptoms appear and, from a renal perspective, this would be when proteinuria (or micro-proteinuria) is first detected (Warnock et al., 2010a). It has been known for some time that typical renal lesions can be identified on histology in children (Tondeur and Resibois, 1969; Gubler et al., 1978). More recently these early changes have been correlated with small degrees of proteinuria in children, adolescents, and young adults (Tøndel et al., 2008) and these early changes can resolve with ERT (Tøndel et al., 2013). Therefore, it would seem prudent to start ERT as soon as the first signs of renal involvement occur in the form or proteinuria aided by renal histology (Warnock et al., 2010a).

If a decision is taken to start ERT in children, both available enzyme replacement therapies have been shown to be safe (Ramaswami et al., 2007; Wraith et al., 2007). A study in children between the ages of 9 and 16 who had received ERT for between 1 and 8 years showed improved headache, acroparaesthesias, and abdominal pain as well as an increase in physical activity. There was no progression of organ damage during follow up (Borgwardt et al., 2013).

Women

Having recognized that many female heterozygotes can display manifestations of the disease and that some of those may have renal involvement, do female patients respond differently than their male counter parts? The answer is that the results for women are not dissimilar to males (Hughes et al., 2011).

Pregnancy

There is no evidence of reduced female fertility in Fabry disease although there has been a report of disorders of the menstrual cycle (Faggiano et al., 2006). Similarly, there is no evidence that Fabry disease affects pregnancy although there has been a report of an increased incidence of proteinuria in pregnant women with Fabry disease (Bouwmann et al., 2012). Although neither of the available enzyme replacement therapies are licensed in pregnancy, there is a report of a successful pregnancy in a lady who continued her ERT throughout her pregnancy (Germain et al., 2010).

Antibodies to recombinant enzyme products

There is little doubt that antibodies to both licensed enzyme replacement products are produced, especially in those classical patients who have little or no residual native enzyme. The antibodies cross react so that there is nothing to be gained from switching products if antibodies to one product are thought to be clinically significant. Although 'reactions' to AgalB did occur in a significant number of patients these had subsided by 6–12 months and had no apparent negative influence on efficacy (Wilcox et al., 2012). However, in another study the antibodies had a negative impact on urinary Gb3 with the implication that this would be detrimental to clinical outcome (Linthorst et al., 2004). Similar results were found when skin biopsies were examined with re-accumulation of Gb3 deposits in the presence of high antibody titres (Hollak and Linthorst, 2009).

In a comparative study between agalsidase alpha and beta, beta-galactosidase A antibodies were measured as well as clinical outcome and Gb3 levels in urine and plasma in 52 patients after 12 months of treatment. Antibodies were found in 18/28 patients and interfered with urine Gb3. At a dose of 0.2 mg/kg body weight every 2 weeks it would appear that, where antibodies persist, there is impairment of enzyme activity as measured by urine Gb3, but at a dose of 1 mg/kg body weight every 2 weeks there was a significant reduction in urine Gb3 with less of an impact of antibodies with better stability of renal function and reduction in left ventricular mass (Vedder et al., 2008). While there was some criticism of the methods used in this study (Mehta et al., 2008), it does seems that

antibodies are important and that higher doses of ERT might overcome some of the potential negative effects on long-term outcomes.

However, there is, as yet, no standardized method for measuring antibodies to these enzymes as well as the difficulty in the interpretation of results. This has been well reviewed by Deegan (2012).

Comparison of AgalA with AgalB

There is virtually no difference between the two products when their structure and composition is considered. AgalB has slightly more sialylated oligosaccharides and a higher level of phosphorylation, but in a mouse model this did not seem to be significant (Lee et al., 2003). However, this does not take into consideration the fact that mannose 6-phosphate receptors are not the only mode of entry into the cell for enzymes and that the two products may enter different cell types in different ways and to different degrees (Prabakaren et al., 2011). When 0.2 mg/kg every 2 weeks of both enzymes were compared there was seemingly no difference. However, there were many treatment failures in the relatively small number of patients studied and this was almost certainly due to the age of the patients included in the study and the severity of the disease at baseline (Vedder et al., 2007). When patients who had been receiving 0.2 mg/kg every 2 weeks of AgalA increased the dose to 0.2 mg/kg every week the decline in renal function was slowed (Schiffmann et al., 2007).

Hughes and colleagues looked at three dosing regimens of AgalA—0.2 mg/kg body weight every 2 weeks; 0.1 mg/kg every week; and 0.2 mg/kg every week. They enrolled 18 patients who were treated for 4 weeks on each dose. At the end of the study there was no statistical difference between the doses but there was a trend towards the highest dose being most effective (Hughes et al., 2013).

Turning to AgalB, it is worth noting that in the phase I/II study three doses were used—0.3, 1, and 3 mg/kg body weight every 2 weeks—and there was a dose-dependent response in both clinical, biochemical, and histological terms (Eng et al., 2001), but 1 mg was chosen for further clinical trials because of the adverse reaction profile to the higher dose. A trial has also been reported where 21 male patients were treated for 6 months with AgalB at a dose of 1 mg/kg body weight every 2 weeks and then the dose reduced to 0.3 mg/kg body weight every 2 weeks for a further 18 months. The usual renal parameters and Gb3 were measured as well as examining renal and skin histology. While 100% of patients cleared deposits from interstitial capillary endothelial on the 1 mg dose, only 90% remained clear on the reduced dose and when seven other renal cell types were examined, 70% remained clear on the lower, 0.3 mg, dose (Lumbanda et al., 2009).

In June 2009 there was an interruption to the supply of AgalB and many patients either had their dose of enzyme reduced or were changed to AgalA in the standard dose of 0.2 mg/kg body weight every 2 weeks. Seven male and three female patients who had received AgalB at the standard 1 mg/kg dose for at least 48 months were changed to AgalA again at the standard dose of 0.2 mg/kg with no ill effects (Pisani et al., 2013). In Australia, 40 patients who had been on AgalB for at least 2 years had their dose initially reduced by 50% and then by a further 30%. When assessed there was no deterioration in disease severity scoring or QOL although the male patients did complain of decreased energy levels (Ghali et al., 2012). A similar study was performed in the Dutch cohort where 35 patients were receiving AgalB at the time of the shortage and either received a lower dose of AgalB or changed to the standard 0.2 mg/kg dose of AgalA. Clinical event rates were compared before and after the changes together with lysoGb3 levels, QOL and pain scores. No increase in clinical events was founds. There was a minimal but significant reduction in two of the SF-36 QOL subscales and, importantly, there was an increase in lyso-Gb3 levels suggesting that there was recurrence of the disease (Smid et al., 2011). These studies are, however, only slightly indicative of the possibility that a dose of 1 mg of AgalB is better than 0.2 mg of AgalA per kg body weight.

A study in children and young adults reported from Norway (Tøndel et al., 2013), although dealing with a small number of patients, does show a significant relationship between the ability to clear Gb3 deposits from the kidney and the total dose of agalsidase received. Despite this, more studies are needed and, in time, it may be that patients will require different doses at different periods of their management or with different degrees of organ involvement. The message should be that whatever dose of agalsidase is used there needs to be careful monitoring of progress with repeat renal biopsies if there is a poor clinical effect despite optimum adjuvant therapies.

Home therapy

Receiving ERT by intravenous infusion every 2 weeks can be an added burden for patients and their families. However, this can be mitigated by the use of home therapy that is now standard practice in many countries and has been reported as safe and efficacious (Schiffmann et al., 2006; Cousins et al., 2008).

Further reading

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CHAPTER 339

Cystinosis

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Epidemiology

Cystinosis is a rare lysosomal storage disease occurring with an incidence of 1/100,000 to 1/200,000 live births per year with clustering observed in some populations, especially in those with high degree of consanguinity. The worldwide prevalence of cystinosis is unknown as the disease still remains underdiagnosed in many parts of the world.

Clinical features

Clinical forms of cystinosis

Depending on the age of presentation and the degree of disease severity, three clinical forms of cystinosis are distinguished:

1. Nephropathic infantile form (OMIM #219800), which is the most frequent and most severe form of the disease
2. Nephropathic juvenile form (OMIM #219900); synonyms: intermediate cystinosis, late-onset form, adolescent form
3. Non-nephropathic adult form (OMIM #219750); synonyms: benign non-nephropathic cystinosis, ocular non-nephropathic cystinosis.

All three forms of the disease are caused by mutations of the *CTNS* gene and have phenotypic overlap.

Clinical presentation of renal disease in cystinosis

Nephropathic infantile cystinosis

Patients with infantile cystinosis are generally born from uneventful pregnancies and have normal birth weight and length. Despite cystine accumulation starting *in utero*, clinical symptoms are absent at birth and gradually develop during the first months of life. The kidneys are the first affected organs, which progressively lose function of their proximal tubular transporters, resulting in urinary loss of water, Na^+, K^+, bicarbonate, Ca^{2+}, Mg^{2+}, phosphate, amino acids, glucose, proteins, and many other solutes reabsorbed in this nephron segment. This generalized proximal tubular dysfunction is called 'deToni–Debré–Fanconi syndrome' or shortened to 'renal Fanconi syndrome', named after the paediatricians who first described the disorder in the last century (Fanconi et al., 1949) (see Chapter 41). Asymptomatic aminoaciduria can appear during the first weeks of life and is followed by glucosuria, phosphaturia, and urinary bicarbonate losses during the second trimester (Brodehl et al., 1965; Levtchenko and Monnens, 2006). At the age of 6 months, full-blown Fanconi syndrome is usually present and causes clinical symptoms of polyuria, thirst, failure to thrive, growth retardation, vomiting, periods of dehydration, constipation, developmental delay, and rickets in some patients. Biochemically, patients present with hypokalaemia, hypophosphataemia, metabolic acidosis, low serum uric acid, low carnitine, and, sometimes, hyponatraemia (Gahl et al., 2002). Occasionally, hypokalaemia in combination with hypochloraemic metabolic alkalosis and elevated plasma renin activity can mimic Bartter syndrome (Caltik et al., 2010). Proteinuria can reach grams per day and consists of low-molecular-weight proteins, albumin, and high molecular weight proteins (Wilmer et al., 2008). Excessive losses of calcium and phosphate can cause the development of nephrocalcinosis and the formation of renal stones (Saleem et al., 1995).

In most patients, the glomerular filtration rate remains normal during the first 2 years and then progressively deteriorates towards end-stage renal disease (ESRD) at the end of the first decade of life (Markello et al., 1993). Renal transplantation is the treatment of choice in patients with ESRD, as the disease does not recur in the grafted organ. Three independent studies demonstrated superior renal graft survival in cystinosis, compared to other renal diseases (Ehrich et al., 1991; Kashtan et al., 1995; Van Straelen et al., 2011).

Nephropathic juvenile form

The nephropathic juvenile form of the disease is diagnosed in a minority of patients (~5%) and manifests with a spectrum of the symptoms, varying from milder (compared to the infantile form) proximal tubulopathy to an apparent nephrotic syndrome. In terms of the age at presentation, there is a continuum between the infantile and late-onset form, however, most of the described patients are > 10 years old. The deterioration of renal function also occurs in the late-onset form, but the rate of renal disease progression is mostly slower compared to the infantile form of cystinosis.

Non-nephropathic adult form

Ocular non-nephropathic cystinosis manifests only with complaints of photophobia due to cystine accumulation in the cornea of the eye. The kidney, retina, and other organs are spared in these patients (Anister et al., 2000). Recently, a coexistence of juvenile and ocular forms of cystinosis was described in one family, suggesting that there might be a continuum between mild forms of cystinosis and thus warranting the follow-up of renal function in patients with ocular cystinosis (Servais et al., 2008).

Renal pathology

Renal biopsy is not required for the diagnosis of cystinosis, and therefore the descriptions of renal histology at early stages of the disease are limited. Serial renal biopsy in two cystinosis patients demonstrated atrophy of the proximal convoluted tubules, called

a 'swan neck deformity', which appeared after 6 months of life (Mahoney et al., 2000). In a larger series of kidney specimens the most striking features were irregularities of the tubular brush border and very large cells with a prominent and hyperchromatic cytoplasm. Cystine crystals are mostly seen within interstitial cells and rarely in podocytes, but not in tubular cells. Giant multinucleated podocytes and parietal epithelial cells are frequently observed and are pathognomonic for the disorder (Gubler et al., 1999). Electron microscopy demonstrates podocyte foot process retraction, especially in patients with pronounced proteinuria (Wilmer et al., 2008). These podocyte abnormalities explain the levels of proteinuria and albuminuria seen in this condition; it is not solely a tubulopathy.

The deterioration of renal function is accompanied by progressive tubulointerstitial and glomerular lesions, consisting of interstitial fibrosis, tubular atrophy, segmental or global collapsing of the glomerular tuft, and accumulation of mesangial matrix material (Gubler et al., 1999).

Renal histology in patients with late-onset cystinosis may demonstrate glomerular lesions, undistinguishable from idiopathic focal and segmental glomerulosclerosis (FSGS) (Servais et al., 2008), and therefore the diagnosis can be missed in older patients presenting with nephrotic syndrome.

Characteristic morphologic features of cystinotic renal tissue are shown in Fig. 339.1.

Extrarenal features of cystinosis

The advent of renal replacement therapy and transplantation has uncovered the continued cystine accumulation in extrarenal organs and has emphasized the multisystemic nature of cystinosis, which may additionally involve the eyes, thyroid, liver, spleen, pancreas, muscle, and central nervous system.

Ocular symptoms

The severity of eye involvement differs from one patient to another (Dureau et al., 2003). Corneal deposits accumulate progressively in the stroma of the cornea and iris in all patients and on the surface of the anterior lens and retina in some (Fig. 339.2). Photophobia, watering, and blepharospasm may become disabling; these symptoms are often related to erosion of corneal epithelium, leading eventually to keratopathy. Photophobia may be prevented and even completely cured by cysteamine eye drops (Gahl et al., 2000). Sight may be progressively reduced, leading to blindness in a few patients who already had major ocular symptoms at an early age and a severe retinopathy. Cataract and glaucomas have been also reported (Wan et al., 1986; Fahey et al., 2001).

Endocrine disturbances

Hypothyroidism

Thyroid dysfunction caused by thyroid gland fibrosis, atrophy and crystal formation in follicular cells, usually appears between 8 and 12 years of age in up to 75% of the patients (Chan et al., 1970; Lucky et al., 1977; Brodin-Sartorius et al., 2012). It is rarely overt with clinical symptoms, but rather discovered by systematic assessment of thyroid function (Broyer et al., 1987). Hypothyroidism in cystinosis may be associated with pituitary resistance to thyroid hormone (Bercu et al., 1980); hypothyroidism may be partly responsible for the growth impairment. Cysteamine was reported to delay or prevent thyroid dysfunction (Kimonis et al., 1995; Brodin-Sartorius et al., 2012).

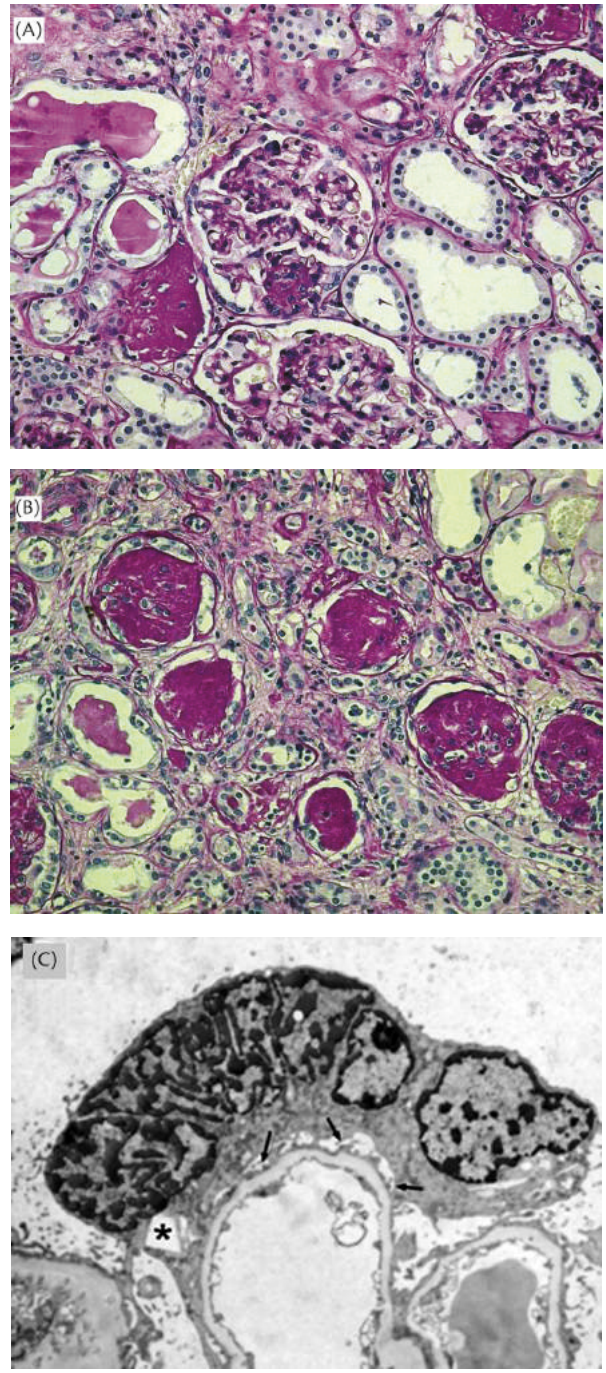

Fig. 339.1 Morphological characteristics of the cystinotic kidney. (A) Light microscopy (PAS) shows marked dilatation, irregularity and atrophy of proximal tubules and a glomerulus with an FSGS lesion. (B) More advanced stage of cystinotic nephropathy with a global collapse of glomeruli and pronounced interstitial fibrosis. (C) Electron microscopic image shows a giant multi-nuclear podocyte pathognomonic for cystinosis and podocytes foot process effacement (arrows). *Cystine crystal within the cytoplasm of the podocyte.
Images (A) and (B) are kindly provided by Prof. Dr. E.Lerut, University Hospital Leuven. (C) is adapted from Wilmer et al. (2008).

Gonadal function

Abnormalities in the pituitary testicular axis with a low plasma testosterone and high follicle stimulating hormone/luteinizing hormone levels, termed hypergonadotropic hypogonadism, are

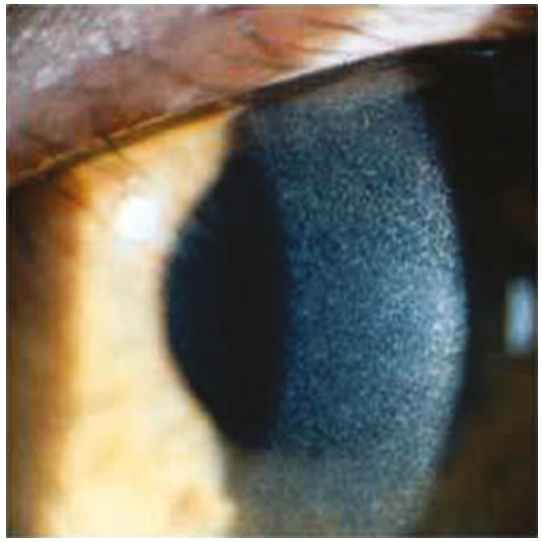

Fig. 339.2 Split-lamp examination showing corneal cystine accumulation characteristic for cystinosis. Ocular crystals can be detected by an experienced ophthalmologist starting from the age of 1 year.

common in male patients with cystinosis (Chik et al., 1993). It is due to testicular fibrosis and atrophy and can be treated with testosterone replacement therapy Older male patients can experience erections, but seven patients who produced ejaculate all proved to be azoospermic (Besouw et al., 2010). The finding of spermatogenesis in the testis biopsy of one patient may provide opportunities for male cystinosis patients to produce their own offspring by *in vitro* fertilization after testicular sperm extraction (Besouw et al., 2010).

Female patients might exhibit pubertal delay but seem to have more normal gonadal functions and there are several reports of successful pregnancies.

Endocrine pancreas

The pancreas may also be affected by long-standing cystine accumulation. Although non-suppressible insulin-like activity is normal in young children with cystinosis, several patients have developed type 1 diabetes mellitus after the age of 10 (Gahl and Kaiser-Kupfer, 1987). Poor glucose tolerance results from impaired insulin production and is exacerbated by antirejection steroid therapy (Robert et al., 1999). The exocrine pancreas is usually not affected except in one reported case with steatorrhoea (Fivusch et al., 1988).

Liver and gastrointestinal involvement

Hepatomegaly and splenomegaly occur in one-third to one-half of the cases after 15 years of age who did not receive cysteamine (Broyer et al., 1987). Hepatomegaly is related to enlarged Kupffer's cells that transform into large foam cells containing cystine crystals. In general, it is not associated with elevated serum liver enzymes or other clinical abnormalities.

In a few patients, portal hypertension with gastro-oesophageal varices and cholestatic liver disease were reported (Broyer et al., 1987; Cornelis et al., 2008). Splenomegaly is also related to the development of foam cells in the red pulp. Haematological symptoms of hypersplenism may be noted. Cystine crystals also occur in the appendix, rectal mucosa, and intestinal mucosa.

Morning nausea and vomiting are frequently observed among some children with cystinosis, many of whom are also poor eaters.

Muscle disease

A distinctive myopathy, potentially leading to a severe handicap, has been reported in many patients with generalized muscle atrophy and weakness, mainly of distal muscles of all limbs but with more severe involvement of the interosseous muscles and those of the thenar eminence (Gahl et al., 1988; Vester et al., 2000). Muscle cystine content increases with age among patients with cystinosis who are not receiving cysteamine therapy; weakness and atrophy progress distal to proximal. Pharyngeal and oral dysfunction, which may also cause voice changes, is often observed and has been attributed to muscle dysfunction (Sonies et al., 1990; Trauner et al., 2001). Pulmonary dysfunction with an extraparenchymal pattern of restrictive lung disease was reported in a series of adult nephropathic cystinotics up to 40 years of age; it was directly correlated with the severity of myopathy (Anikster et al., 2001). It is not clear if one reported case of cardiomyopathy was directly related to cardiac cystine accumulation (Dixit and Greifer, 2002).

Neurologic complications

Patients with cystinosis generally have normal intelligence, however, specific neurocognitive dysfunction consisting of visual processing deficits and disturbed sustained attention, planning, and motor coordination has been reported starting from early age (Trauner et al., 2010; Besouw et al., 2010) and might be attributed to the abnormalities in cerebral white matter microstructure (Bava et al., 2010). Major central nervous system involvement is a late complication that occurs after age of 20. The main symptoms are difficulty in walking and swallowing, progressive loss of speech, memory loss, diminished intellectual function, and dementia. Pyramidal signs, cranial nerve defects, dysphagia, and dysarthria also may be seen. Findings detected by computer tomography or magnetic resonance imaging include cortical atrophy, dilated ventricles, basal ganglia calcifications, ischaemia, and patchy areas of demyelination (Brodin-Sartorius et al., 2012).

Investigations

Cystinosis should be suspected in all patients with failure to thrive and signs of renal Fanconi syndrome, as it is the most common cause of inherited Fanconi syndrome in children. The detection of elevated intracellular cystine content is the corner stone for the diagnosis. Cystine measurement in isolated polymorphonuclear (PMN) leucocytes are superior to mixed white blood cells (WBCs), as PMN cells preferentially accumulate cystine in blood. This method increases the sensitivity of cystine detection during the monitoring of cysteamine treatment (Levtchenko et al., 2004). The cystine-binding assay has been a gold standard for cystine measurements for years; however, at present, most laboratories switched to the other methods of detection, because of lower price and avoidance of radioactivity. In this respect, tandem mass spectrometry is the most sensitive method and is currently widely used for cystine determination in cystinosis (Chabli et al., 2007). Each laboratory performing cystine measurements should provide their own reference values in patients at the time of diagnosis, and also for heterozygote and healthy subjects. Reference values, provided by our laboratory are indicated in Table 339.1 (Wilmer et al., 2010). Molecular analysis of the *CTNS* gene allows early diagnosis and can be used for prenatal diagnosis of the disease. Prenatal diagnosis of cystinosis can be also made by measuring ^{35}S-labeled cystine

Table 339.1 Reference values for intracellular cystine[a]

Healthy subjects	
PMN leucocytes	0.04–0.16 nmol cystine/mg protein[b]
Mixed leucocytes	0.05–0.17 nmol cystine/mg protein[b]
Fibroblasts	0.0–0.23 nmol cystine/mg protein[b]
Patients at diagnosis	
PMN leucocytes	> 2 nmol cystine/mg protein
Fibroblasts	1.6–3.7 nmol cystine/mg protein
Heterozygotes	
PMN leucocytes	0.14–0.57 nmol cystine/mg
Patients under cysteamine therapy	
PMN leucocytes	< 0.6 nmol cystine/mg protein

[a]Cystine is sometimes expressed as nmol ½ cystine per mg protein. Conversion factor ½ cystine/mg protein = 2 × cystine/mg protein

[b]Values are presented as percentile 5 and 95.

Provided by Laboratory of Genetic and Metabolic Diseases, Radboud University Nijmegen Medical Centre, the Netherlands.

accumulation in cultured amniocytes or chorionic villi sample (CVS); and by a direct measurement of cystine in uncultured CVS (Jackson et al., 2005).

Aetiology and pathogenesis

The molecular basis of cystinosis

The Mendelian inheritance of cystinosis was recognized very early after the initial descriptions. In 1998, the *CTNS* gene was mapped to the 17p13.2 region and was cloned (Town et al., 1998). The gene spans 23 kb, is composed of 12 exons, of which the first two represent non-coding regions, and encode a 367-amino acid protein, containing seven putative transmembrane domains and two lysosomal targeting motifs (Cherqui et al., 2001). Kalatzis et al. have deleted the lysosomal targeting motif, re-directing cystinosin to the cell membrane with the intralysosomal domains facing the extracellular medium. Using this model, they were able to measure specific cystine transport when they applied a proton gradient by lowering the pH of the extracellular medium. Furthermore, the lysosomal localization was proven by co-localization studies using GFP-tagged cystinosin and the lysosomal marker LAMP-2 (Kalatzis et al., 2001). Overall, these studies have confirmed the results obtained by the pioneering studies of the late 1970s and early 1980s indicating that cystinosin is a lysosomal cystine-proton co-transporter (Schneider et al., 1967; Gahl et al., 1982; Jonas et al., 1982).

Molecular diagnosis in the following years have allowed the identification of > 100 different mutations including nonsense, missense, and splice-site, small deletions/duplications, and mutations in the promoter region (Shotelersuk et al., 1998; Anisker et al., 1999; Phornphutkul et al., 2001; Taranta et al., 2010). The most common mutation, accounting for approximately 75% of the affected alleles in Northern Europe, is a 57 kb deletion removing the first nine exons and a part of the exon 10 of the *CTNS* gene, the upstream 5′ region encoding for the *CARKL* gene and the first two non-coding exons of the *TRPV1* gene (Fig. 339.3A) (Forestier et al., 1999; Wilmer et al., 2010). The effective contribution of the *CARKL* or *TRPV1* genes in the pathogenesis of the disease in patients with homozygous 57 kb deletion has not yet been studied.

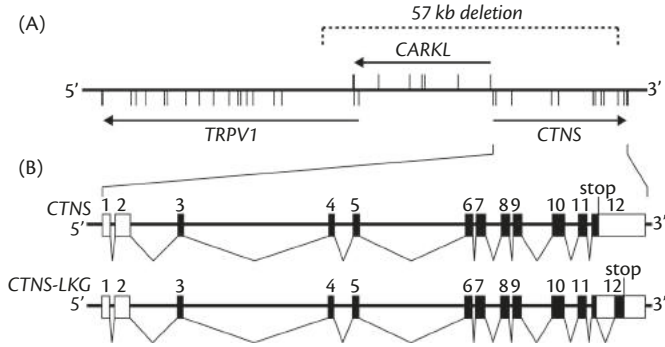

Fig. 339.3 The *CTNS* gene is mapped at chromosome 17p13.2. (A) The *CTNS* gene is adjacent to the *CARKL* and *TRPV1* genes, which are mutually affected in the common 57 kb deletion detected in cystinosis patients. (B) The *CTNS* gene exon 1 and 2 are non-coding and alternative splicing of exon 12 results in two isoforms, previously reported as *CTNS* and *CTNS-LKG*. The *CTNS-LKG* isoform is lacking the lysosomal targeting motif GYDQL (Wilmer et al., 2010).

In vitro studies of residual cystine transport activity have shown that infantile cystinosis generally results from severe mutations that lead to complete loss of cystine transport activity while less severe mutations are causing milder forms of cystinosis (Kalatzis et al., 2004). Of notice, cystine levels in WBCs increase up to 100-fold in affected individuals in comparison to control subjects, whereas heterozygous carriers of *CTNS* mutations demonstrate only a slight increase in cystine levels without clinical consequences.

Recently, a transcript variant of the *CTNS* gene termed *CTNS-LKG* has been reported (Taranta et al., 2008). This isoform originates from an alternative splicing of exon 12, which replaces the lysosomal targeting motif GYDQL at the C-terminus by a longer amino acid sequence (Fig. 339.3B). Overexpression of the LKG transcript in renal HK-2 cells showed expression of cystinosin LKG in the plasma membrane, in lysosomes, in the endoplasmatic reticulum, in the Golgi apparatus, and in small intracellular vesicles. The physiological role of this isoform is not yet known.

Pathogenesis of cystinosis

Despite the fact that the *CTNS* gene was cloned almost 15 years ago, the pathogenesis of the disease is not yet fully understood.

Initial studies performed after exposure of perfused renal tubules or cell lines to cystine dimethyl ester (CDME) for mimicking lysosomal cystine accumulation, have led to hypothesize altered ATP metabolism as the primary cause for diminished tubular reabsorption in Fanconi syndrome (Baum, 1998). Later it has been demonstrated that CDME loading did not adequately reflect lysosomal cystine accumulation and that CDME had a direct toxic effect on cellular respiration and survival (Wilmer et al., 2007). Nonetheless, several *in vitro* studies have reported slightly decreased levels of ATP in cystinotic cells, including fibroblasts and renal epithelial cells (Levtchenko et al., 2006; Wilmer et al., 2011). However, because reduced activity of Na,K-ATPase has not been documented in cystinotic cells and because a significant overlap in cellular ATP levels between cystinotic and control cells was found, altered ATP metabolism is unlikely to represent a valuable explanation for the impaired sodium-dependent reabsorption that characterizes Fanconi syndrome (Wilmer et al., 2010).

High apoptotic rates have been reported in cystinotic fibroblasts and in proximal tubular cells (Park et al., 2002). Hypothetically, cystine accumulation alters lysosomal integrity causing leakage of the lysosomal membrane and the release of cystine in the cytosol, where it binds the proaptotic protein kinase PKC-δ stimulating apoptosis (Park et al., 2006). Apoptotic cell death may explain the 'swan-neck' deformity, which has been described in cystinotic proximal renal tubules. Progressive development of atubular glomeruli could then lead to progressive renal failure (Larsen et al., 2010). This hypothesis is further substantiated by the observation of increased caspase-4 expression in areas of cystinotic renal tissues with reduced number of proximal tubules (Sansanwal et al., 2010a). Caspase-4 is a member of the cysteine proteases that play an important role in programmed cell death. Moreover, increased numbers of autophagosomes and autophagic vacuoles have been observed in cystinotic fibroblasts and renal epithelial cells, suggesting that altered autophagy also plays a role in cystinosis. These observations were associated with structural mitochondrial abnormalities and with increased production of reactive oxygen species (ROS) (Sansanwal et al., 2010b). Further supporting enhanced apoptosis in cystine-loaded cells, an increased apoptotic rate was demonstrated in primary renal proximal epithelial cells pretreated with cystinosin small interfering RNA, mediated in part by adenosine monophosphate (AMP) kinase (Taub and Cutuli, 2012). Another recent study evaluated the expression of a protein clusterin, in conjunction with apoptosis and autophagy proteins *in vitro* in renal proximal tubular epithelial cells from normal and cystinotic patients and *in vivo* in cystinotic renal biopsy tissue. In cystinotic cells, there were low levels of the cytoprotective secretory form of clusterin and elevated levels of the nuclear proapoptotic form; the expression of the nuclear form co-localized with apoptotic proteins (apoptosis-inducing factor and cleaved caspase-3) and autophagy proteins (microtubule-associated protein 1 light chain 3 (LC3) II and p62). Thus, clusterin may link cellular stress with the Fanconi phenotype and progressive renal injury in nephropathic cystinosis (Sansanwal et al., 2015). Interestingly, while macroauthopasomal flux wasn't impaired in cystinotic cells, chaperon-mediated autopaphy was shown to be defective (Napolitano et al., 2015).

Another mechanism related to enhanced cell death in cystinosis is altered metabolism of glutathione (GSH) (Levtchenko et al., 2005), which is the main intracellular antioxidant, protecting cells against oxidative stresses. Decreased levels of GSH have been reported in cystinotic fibroblasts and proximal tubular cells (Chol et al., 2004; Laube et al., 2006). Another study performed on human fibroblasts showed no differences in basal GSH levels, but documented decreased GSH levels in cystinotic cells upon inhibition of ATP synthesis and after exposure to oxidative stimuli, suggesting that the activity of the ATP-dependent gamma-glutamyl cycle might be compromised in cystinosis (Mannucci et al., 2006). Furthermore, several studies demonstrated increased ROS generation in cystinosis cells pointing to oxidative stress being one of the proposed pathogenic mechanisms (Sansanwal et al., 2010; Wilmer et al., 2011).

Several laboratories have shown an increase in the S-S/SH glutathione ratio in cystinotic cells indicating altered redox state and speculated that it results from sequestration of cystine in lysosomes, thereby limiting the availability of cysteine in the cytosol with a subsequent decrease in GSH (Levtchenko et al., 2005). A strong association between cystinosin mRNA levels and the [Cys]/[CySS] redox couple has substantiated this hypothesis (Bellomo et al., 2010).

Contradicting these results, one study has demonstrated normal GSH/GSSG ratio in cystinotic fibroblasts and suggested intact redox status (Vitvitsky et al., 2010).

So far, studying pathogenesis of cystinosis has been focused on the deteriorative effects of cystine accumulation. Consequently, cysteamine treatment restored altered glutathione metabolism, improved cell capacity to deal with oxidative stress, and attenuated apoptotic rate in *in vitro* studies (Park et al., 2006; Wilmer et al., 2011).

However, more recent research start to provide emerging evidence that cystinosin can have alternative functions in the cell not-necessary related to cystine transport. Because cystinosin has structural similarity to the PQ-loop family of heptahelical membrane proteins that are predicted to be involved in vesicle formation and protein trafficking (Saudek, 2012), it has been postulated that cystinosin disruption may cause cellular trafficking defect that may underlie renal Fanconi syndrome in cystinosis.

Indeed, in a mouse model of cystinosis (*Ctns*−/− mouse) a decreased expression of the multi-ligand receptors megalin and cubilin responsible for proximal tubular reabsorption, and increased dedifferentiation and proliferation rates has recently been demonstrated (Raggi et al., 2014). Moreover, studies on mouse and human proximal tubular epithelial cells showed an accumulation of enlarged, dysfunctional lysosomes and reduced expression of endocytic receptors reflected by decreased uptake of specific ligands (Ivanova et al, 2015; Raggi et al., 2014).

An altered distribution of motor protein kinesin-1 found in human cystinotic proximal tubular cells might be involved in abnormal microtubule-based vesicle movements (Ivanova et al., 2015). Another study observed that the expression of a small GTPase Rab27A was downregulated in kidneys from Ctns −/− mice and in human proximal tubular cells from cystinotic patients (Johnson et al., 2013). This finding was associated with significant endoplasmic reticulum expansion and a marked increase in the unfolded protein response-induced chaperones Grp78 and Grp94. Upregulation of the Rab27A-dependent vesicular trafficking mechanisms rescued the defective lysosomal transport phenotype and reduced endoplasmic reticulum stress (Johnson et al., 2013).

Another elegant study analysed the correlation between structural and functional changes in proximal tubular cells in Ctns −/− mice and delineated a typical sequence of events, starting with the formation of amorphous lysosomal inclusions and cystine crystals. At the nephron level, lesions started at the glomerulotubular junction and then extended distally. This was associated with progressive loss of expression of megalin, cubilin, sodium-glucose cotransporter 2, and type IIa sodium-dependent phosphate cotransporter, suggesting apical dedifferentiation accounted for Fanconi syndrome before atrophy (Gaide Chevronnay et al., 2014).

Altogether these studies confirmed the role of cystinosin in cellular trafficking mechanisms indicating that the deficiency of this protein can only partially be rescued by decreasing lysosomal cystine accumulation (Ivanova et al., 2015).

Treatment and outcome

Lifestyle matters

All patients with cystinosis should have free access to water and toilet privilege, because of pronounced polyuria and polydipsia. Prolonged exposure to heat and sun should be avoided because

of photophobia, the risk of dehydration, and/or heat stroke due to impaired sweating.

Symptomatic therapy

The aim of symptomatic therapy in patients presenting with Fanconi syndrome is the maintenance of fluid and electrolyte balance, good nutrition, and prevention of rickets. The dose of potassium, sodium, bicarbonate, and phosphate varies substantially and needs to be regularly adapted according to serum values. 1,25-dihydroxycholecalciferol supplementation should be used starting from early childhood. However, excessive administration of phosphate, 1,25-dihydroxycholecalciferol and bicarbonate may aggravate the development of nephrocalcinosis or stimulate renal stone formation (Wilmer et al., 2011). Calcium supplementation is generally not indicated. Carnitine replacement normalizes plasma and muscular carnitine levels, however, it is not established whether carnitine administration results in improved muscular performance (Gahl et al., 1993).

Poor appetite, vomiting, and oral motor dysfunction often require nasogastric tube or gastrostomy feeding, especially in young children. Treatment with recombinant growth hormone results in catch-up growth and further maintenance of growth velocity (Wühl et al., 2001). Growth hormone is frequently not required in patients treated with cysteamine, especially when started at early age, as cysteamine by itself improves growth (Kleta et al., 2004).

Other complications such as hypothyroidism, diabetes, or hypogonadism are considered for treatment with levothyroxine, insulin, and testosterone, respectively.

Inhibition of the renin–angiotensin system (RAS) results in a decrease of albuminuria (Levtchenko et al., 2003) and might be associated with slowed renal disease progression (Greco et al., 2010); however, RAS inhibitors can have an acute deteriorative effect on kidney function in patients with extracellular volume and/or salt depletion.

Specific treatment with cysteamine

The amino thiol cysteamine depletes lysosomal cystine content by a disulphide exchange reaction with cystine, resulting in the formation of cysteine-cysteamine mixed disulphide and cysteine. Cysteine-cysteamine mixed disulphide exits lysosomes via a recently identified cationic amino acid transporter PQLC2 also belonging to the PQ-loop family of proteins (Jézégou et al., 2012) and the remaining cysteine via a cysteine carrier (Fig. 339.4). The administration of cysteamine at 1.3–1.9 g/m^2 in four daily doses drastically lowers cystine content of the lysosomes, and postpones or even prevents the deterioration of renal function and the development of extrarenal complications. Furthermore, cysteamine treatment improves growth. Cysteamine should be administered as soon as the diagnosis of cystinosis is made, and continued lifelong, as well as after renal transplantation for protecting extrarenal organs.

As the target tissue cystine levels necessary to prevent the progression of renal disease and the occurrence of extrarenal complications are still unknown, the 0.9 percentile of heterozygote values in the PMN cells is mostly recommended as an upper cystine limit before the next dose of cysteamine is given (Table 339.1). Because leucocyte cystine content returns to the initial high levels 6 hours after cysteamine administration, the drug should be taken every 6 hours including the night (Levtchenko et al., 2006). For monitoring cysteamine therapy, blood should be drawn 6 hours after the last intake of the drug.

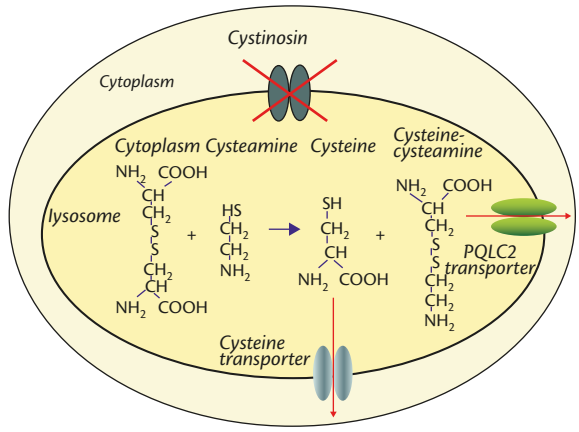

Fig. 339.4 Mechanism of cysteamine action in cystinotic lysosome. Cysteamine reacts with the disulphide bond of cystine resulting in formation of cysteine and cysteamine-cystine mixed disulphides that can exit the lysosomes bypassing defective or absent cystinosin.

Because systemic cysteamine treatment has no effect on corneal cystine crystals, topical 0.5% cysteamine eye drops are indicated. These drops are highly effective and when administered 6–12 times daily are able to dissolve completely corneal cystine crystals within 8–41 months, even at a later age (Gahl et al., 2000).

The current development of an enteric-coated slow-release cysteamine formulation that can be administered twice daily will remarkably ameliorate compliance with cysteamine therapy and improve the quality of life, when its long-term efficiency and safety will be confirmed in ongoing clinical trials. In terms of lowering WBC cystine levels, a non-inferiority of this novel formulation compared to conventional cysteamine has recently been demonstrated (Langman et al., 2012).

Difficulties with cysteamine therapy

The side effects of cysteamine are mostly restricted to gastrointestinal discomfort (due to the release of gastrin and the resulting stimulation of H^+ secretion in the stomach) (Dohil et al., 2003), and bad breath and sweat odour (due to formation of dimethylsulphide and methanethiol, which are metabolites of cysteamine) (Besouw et al., 2007). Gastric acid hypersecretion and ulcerogenity of cysteamine can be improved by the administration of proton pump inhibitors (Dohil et al., 2005). Allergic reactions, fever, seizures, and neutropenia are also reported, especially when the dose of the drug is abruptly increased. Recently, eight European patients treated with cysteamine were reported to exhibit endothelial proliferative lesions on the elbows, skin striae, and bone and muscular pain, which improved or disappeared after lowering the cysteamine dosing (Besouw et al., 2011). Because of these adverse events, using cysteamine doses above the recommended 1.9 g/m^2 should be discouraged. Cysteamine dose calculation per kg body weight (60–90 mg/kg/day) results in dosing above the recommended range in children weighing > 20 kg.

Cysteamine toxicity *in utero* with the development of cleft palate and kyphosis, as well as intrauterine growth retardation and fetal death, without signs of maternal toxicity, was observed in rats treated with a high cysteamine dose (100–150 mg/kg/day) (Beckman et al., 1998). Although the effect in humans treated with lower doses is unknown, it is generally recommended to discontinue cysteamine in female patients planning pregnancy.

Novel therapies

A recent pioneering study using *Ctns*-knockout mice demonstrated a beneficial effect of syngeneic bone marrow and haematopoietic stem cell transplantation on cystine accumulation in various organs and on renal function survival, emphasizing novel potential therapeutic possibilities for cystinosis patients (Syres et al., 2009). However, because of the potential life-threatening side effects of allogenic stem cell transplantation, recent research has focused on possibilities to restore cystinosin expression *ex vivo* for subsequent autologous stem cell transplantation (Harrison et al., 2013).

Because oxidative stress seem to play an important role in the pathogenesis, the potential benefit to use additional antioxidants simultaneously with cysteamine should be further studied.

Another possible therapeutic target in cystinosis is inflammation, as cystine crystals have been show to provoke an activation of macrophages via inflammasome-related and unrelated pathways (Elmonem et al., 2014; Prencipe et al., 2014).

Outcome

More than 20 years ago, the mortality rate of cystinosis in adulthood approximated one-third before 30 years of age. Patients died from uncontrolled water and electrolyte disturbances, as well as from uraemia, infections, or events related to neuromuscular complications. The causes of death have evolved during recent decades. Although the effects of cystine accumulation do not seem to be reversible, they can be prevented by long-term cysteamine administration, which has been associated with lower frequencies of hypothyroidism, swallowing abnormalities, vascular calcifications, and posterior eye segment defects in patients of all ages with cystinosis. The influence of the age at introduction of cysteamine treatment on the onset of hypothyroidism, diabetes, and neuromuscular disorders was assessed according to whether patients were treated before or after 5 years of age, or not treated before the event. Survival curves indicated that treatment started before 5 years of age was associated with significant delay, compared with untreated patients, in the occurrence of hypothyroidism, diabetes, and neuromuscular disorders (Nesterova and Gahl, 2013).

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CHAPTER 340

Mitochondrial diseases and the kidney

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Introduction

The terms mitochondrial disease and mitochondrial cytopathy refer to clinical conditions that are caused by mutations in DNA encoding RNA or proteins required for normal mitochondrial function; this definition distinguishes them from diseases where mitochondrial dysfunction plays a central role in the pathogenesis, but is caused by non-genetic insults, such as ischaemia or drug toxicity. Mitochondrial disease can be deleterious to the function of any cell in the body, but aerobic organs with high energy demands such as the brain, heart, kidney, and skeletal muscle are typically affected. Mitochondrial disease was once considered a rare entity; however, with improvements in diagnostic techniques (notably rapid sequencing of the entire mitochondrial genome) it is becoming increasingly clear that disease-causing mutations are more prevalent than previously realized. As yet, the exact relationship between underlying genotype and renal phenotype remains unclear, as is the case in mitochondrial medicine in general. However, understanding of basic mitochondrial genetics and biology is moving forward rapidly, and it is hoped that this will translate into useful information for the clinical nephrologist in the near future.

Mitochondrial genetics

Mitochondria contain their own DNA (mtDNA), which is approximately 16.5 kb long and is inherited maternally. The mitochondrial genome encodes 13 out of > 80 peptides that form the five oxidative phosphorylation (OXPHOS) complexes, along with 22 tRNA molecules and two rRNA subunits that are necessary for mitochondrial protein synthesis. The majority of mitochondrial proteins required for mitochondrial function, including synthesis of OXPHOS complexes and replication and maintenance of mtDNA, are encoded by nuclear DNA (nDNA), and must be successfully imported into mitochondria. Abnormalities in mtDNA can take the form of point mutations (typically maternally inherited), single deletions/rearrangements (often arising spontaneously), and multiple deletions and/or decrease in copy number (mtDNA depletion syndromes) due to impaired mtDNA replication machinery. Unlike nDNA, there are numerous copies of mtDNA within each cell (100–100,000), and the proportion of copies that contain a pathogenic mutation may vary from tissue to tissue. This phenomenon is known as heteroplasmy, and it helps to explain in part why the severity of disease may vary between different organs. When cells containing mitochondria with mutations in mtDNA divide, the mutant load can be distributed unevenly among daughter cells, and those with a lower mutant burden may have a selective survival advantage. Thus, in a population of rapidly dividing cells the mtDNA mutant load may decrease over time, a process termed mitotic segregation. This explains why mtDNA mutant load is typically lower in cells with a high turnover (e.g. leucocytes) in comparison to post-mitotic tissues (e.g. skeletal muscle).

Mitochondrial function

The five OXPHOS complexes are situated on the inner mitochondrial membrane (Fig. 340.1). Metabolic fuels are oxidized in the citric acid cycle and fatty acid beta oxidation, and electrons are passed to complexes I and II in the respiratory chain by NADH and $FADH_2$, respectively. From there, they pass along the OXPHOS complexes, until they finally combine with oxygen to form water at complex IV. The energy released by electron transport is utilized by complexes I, III, and IV to pump protons out of the mitochondrial matrix, and into the inter-membrane space. This creates a proton gradient that drives ATP synthesis from ADP and inorganic phosphate at the ATP synthase (also referred to as complex V).

It is now appreciated that mitochondria have a range of important roles beyond generating ATP, including maintenance of cellular redox state, generation of reactive oxygen species, biosynthesis of molecules (e.g. haem, steroid hormones, and neurotransmitters), modulation of intracellular calcium signals, and regulation of cell death (Duchen and Szabadkai, 2010). Therefore, mitochondrial dysfunction can be deleterious to cells in a number of different ways, and significant disease can occur even in the absence of a detectable OXPHOS defect. Mitochondrial morphology, proteome and function vary among different organs (Johnson et al., 2007), implying that mitochondria have adapted to meet the specific demands of their host cells.

Clinical phenotypes in mitochondrial disease

The relationship between genotype and clinical phenotype in mitochondrial disease is complex. The same point mtDNA or nDNA mutation can produce entirely different clinical phenotypes. For example, the mtDNA point mutation m.3243A>G is associated with a range of clinical phenotypes, including isolated deafness, maternally inherited diabetes and deafness (MIDD), cardiomyopathy, and the syndrome of mitochondrial encephalomyopathy with lactic

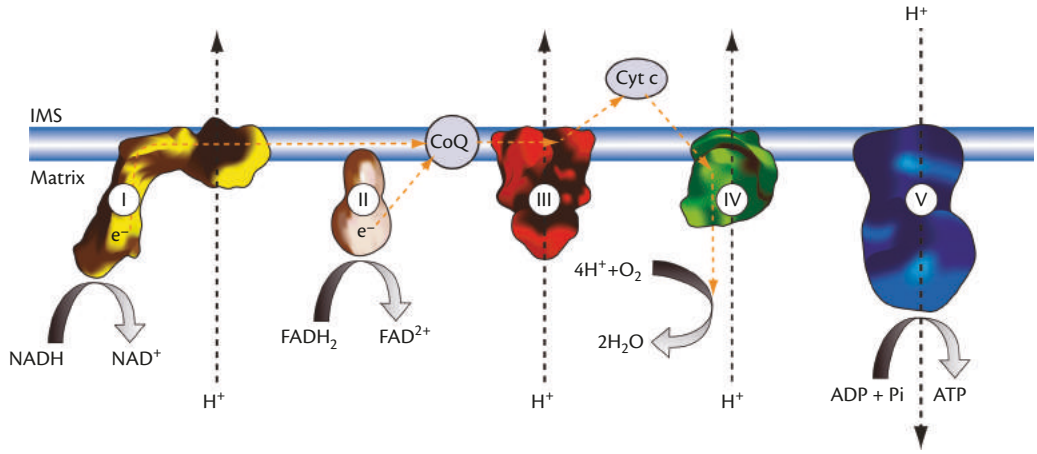

Fig. 340.1 The mitochondrial respiratory chain. NADH and $FADH_2$ are oxidized by complexes I and II, respectively. Electrons (e^-) are shuttled from complexes I and II to complex III by co-enzyme Q (CoQ, also known as ubiquinone). From complex III electrons pass to complex IV via cytochrome c oxidase (Cyt c), before combining with protons and oxygen to form water. Protons are pumped out of the mitochondrial matrix, across the inner mitochondrial membrane and into the inter-membrane space (IMS) by complexes I, III, and IV; this generates an electrochemical gradient that is used to drive the formation of ATP from ADP and inorganic phosphate (Pi) at complex V (the ATP synthase).

acidosis and stroke-like episodes (MELAS) (DiMauro and Hirano, 2010). Conversely, identical diseases can be caused by different underlying genetic abnormalities in mtDNA or nDNA. The classic example of this is Leigh syndrome (subacute necrotizing encephalomyelopathy), which so far has been associated with mutations in 12 mitochondrial and approaching 60 nuclear genes (Thorburn and Rahman, 2014). However, certain mutations seem to target particular tissues (e.g. the optic nerve in Leber's hereditary optic neuropathy) for reasons that remain unclear (Yu-Wai-Man et al., 2011).

Clinical heterogeneity in mitochondrial disease may be explained to an extent by factors such as heteroplasmy in mutation load and mitotic segregation (both explained above), tissue-specific expression patterns of nDNA encoded mitochondrial proteins, nDNA/mtDNA interactions, and environmental factors (e.g. drug toxicity) (DiMauro and Schon, 2003), but there are likely to be other important determinants yet to be discovered. Nevertheless, certain patterns of disease are commonly recognized and are listed in Table 340.1.

Incidence

The mutation rate of mtDNA is thought to be about ten times higher than that of nDNA (Brown et al., 1979), and the prevalence of mtDNA mutation-related disease in the adult population in the United Kingdom has been estimated at about 1/10,000 (Schaefer et al., 2008). The minimum birth prevalence of mitochondrial disease is thought to be 1/5000 (Thorburn, 2004).

Kidney involvement in mitochondrial disease

Mitochondrial disease can lead to abnormalities in function in all sections of the nephron (for reviews, see Niaudet and Rotig, 1997; Niaudet, 1998; Hall et al., 2008; Emma et al., 2012; Rahman and Hall, 2013). Overall, there appears to be a dichotomy in renal presentation between children and adults, with the former typically developing tubular dysfunction (Fanconi syndrome in the most severe cases), whilst glomerular disease (focal segmental glomerulosclerosis (FSGS)) predominates in the latter. However, it is important to appreciate that there are notable exceptions to these generalizations, and the limited literature concerning adults may not accurately reflect the true situation, consisting as it does of isolated case reports and case series. Apparent differences between children and adults, if genuine, may simply reflect the severity of underlying mitochondrial disease; patients presenting early in life typically have large-scale deletions in mtDNA or widely expressed nDNA mutations, whilst patients surviving into adulthood will by definition have milder abnormalities, such as point mutations in mtDNA.

Table 340.1 Common clinical features of mitochondrial disease

Organ	Features
Systemic	Lactic acidosis, hypoglycaemia
Central nervous system	Stroke-like episodes, encephalopathy, seizures, dystonia, ataxia
Neuromuscular	Muscle weakness, exercise intolerance, peripheral neuropathy
Cardiac	Cardiomyopathy (hypertrophic or dilated), conduction defects
Kidney	Proximal tubulopathy (renal Fanconi syndrome), tubulointerstitial nephritis, focal segmental glomerulosclerosis
Gastrointestinal	Vomiting, diarrhoea, exocrine pancreatic dysfunction, hepatomegaly, liver dysfunction
Endocrine	Diabetes mellitus, adrenal insufficiency, hypothyroidism, hypoparathyroidism
Ear	Sensorineural deafness, auditory neuropathy
Eye	Ptosis and progressive external ophthalmoplegia (PEO), cataract/corneal opacities, optic atrophy, pigmentary retinopathy
Haematopoietic	Sideroblastic anaemia, neutropenia, thrombocytopaenia

Mitochondrial disease is an under-diagnosed cause of renal dysfunction, and should be considered in cases of unexplained kidney disease; much remains to be learnt about the relationship between underlying genetic mutations and resulting renal phenotype.

Tubular disease

The most frequent renal phenotype in children with mitochondrial disease is proximal tubulopathy (Niaudet et al., 1997; Niaudet, 1998; Rotig, 2003), which may range from asymptomatic tubular proteinuria and solute loss to overt Fanconi syndrome. The proximal tubule is intrinsically vulnerable to aerobic insults since it has high solute transport demands but very limited anaerobic glycolytic ATP-generating capacity (Bagnasco et al., 1985).

The frequency of renal involvement has been quoted as around 5% of paediatric mitochondrial disease in one large centre (Rotig and Munnich, 2003). However, a systematic study of 42 children with mitochondrial disease found that eight had overt kidney disease and a further 13 had a mild tubular disorder (Martin-Hernandez et al., 2005), suggesting that the prevalence of renal dysfunction may actually be higher.

Fanconi syndrome typically occurs in the first years of life in the setting of severe multisystem disorders, and is only rarely the presenting feature (Mochizuki et al., 1996); it is often associated with diseases caused by large-scale mtDNA deletions, such as Pearson syndrome (refractory sideroblastic anaemia, pancreatic insufficiency, and lactic acidosis) or Kearns–Sayre syndrome (progressive external ophthalmoplegia, retinopathy, myopathy, and ataxia) (Martin-Hernandez et al., 2005).

Proximal tubulopathy is also the most common renal phenotype in patients with nuclear genes linked to mitochondrial disease; of the > 170 nuclear genes so far reported to have mutations causing mitochondrial disease (Rahman 2015), 28 (>15%) have been associated with kidney disease in at least a proportion of patients (Table 340.2).

More distal segments of the nephron can also be affected and a range of tubular disorders have been reported, including tubulointerstitial nephritis (Tzen et al., 2001), Bartter-like syndrome (Goto et al., 1990; Emma et al., 2006) and renal tubular acidosis (Eviatar et al., 1990; Shimizu et al., 2008). In the recently described HUPRA syndrome (hyperuricaemia, pulmonary hypertension, renal failure in infancy, and alkalosis), due to a mutation in the nuclear gene *SARS2* encoding the mitochondrial seryl-tRNA synthetase (important in mitochondrial protein synthesis), affected patients displayed renal impairment, hyperuricaemia, salt wasting, hypochloraemic metabolic alkalosis, and hypomagnesaemia, consistent with impairment of distal tubular function (Belostotsky et al., 2011). Isolated electrolyte disturbances, such as hypomagnesaemia, can also occur, probably due to impaired tubular reabsorption.

Topographical patterns of tubular dysfunction observed in mitochondrial diseases might be explained by factors such as intrinsic energy demands for solute transport, embryological origin (mtDNA deletions may occur early in development), heteroplasmy of mutant load, and differential expression patterns of nDNA.

Glomerular disease

In contrast to children, Fanconi syndrome is rarer in adults with mitochondrial disease (although in our experience it can still occur), and glomerular abnormalities are more common.

m.3243A>G and other mutations

Most reported cases have been in patients harbouring the m.3243A>G point mutation in the leucine^{UUR} tRNA gene, which is the most prevalent pathogenic mtDNA mutation in humans, with an estimated frequency of approximately 1/400 (Manwaring et al., 2007). This mutation is associated with a variety of clinical phenotypes, including MIDD, MELAS, cardiomyopathy, and ophthalmoplegia, as discussed above. A range of renal abnormalities have been described in patients with the m.3243A>G mutation, including tubulointerstitial nephritis and enlarged cystic kidneys, but the most frequently observed pathology is FSGS (Doleris et al., 2000; Guery et al., 2003; Dinour et al., 2004; Lowik et al., 2005; Li et al., 2007; Fujii et al., 2008). It has been suggested that isolated FSGS may represent a distinct phenotype associated with this mutation (Jansen et al., 1997). Typical features of the cases described include female preponderance, proteinuria (but rarely nephrotic syndrome), and progressive renal failure. In the patients who have undergone a kidney biopsy, glomerular abnormalities are indistinguishable from primary FSGS (see Chapter 57) and are pauci-immune. Hyaline changes in vascular smooth muscle in afferent arterioles have been described in some cases and proliferation of dysmorphic mitochondria in podocytes is a typical finding on electron microscopy. Renal dysfunction can be the presenting feature of mitochondrial disease in adults (Hotta et al., 2001), and this should be considered in cases of unexplained kidney disease. Hearing loss is common in patients with m.3243A>G and kidney disease and can lead to diagnostic confusion with Alport syndrome (Jansen et al., 1997), but patients with the latter may be distinguished by the presence of haematuria and other features (see Chapter 322).

Patients with FSGS associated with the m.3243A>G mutation do not respond to steroids, and since these drugs may precipitate diabetes they should be avoided (Guery et al., 2003).

It is currently unclear why patients with the m.3243A>G mutation tend to develop glomerular dysfunction. One explanation might be that podocytes are differentiated cells and probably have limited ability to regenerate and reduce mutant mtDNA load over time by mitotic segregation. Of interest, abnormal mitochondrial function in podocytes has been described in hereditary forms of nephrotic syndrome due to mutations in nephrin (Solin et al., 2000). Diabetes is highly prevalent in patients harbouring the m.3243A>G mutation, but it often develops some years after the appearance of overt kidney disease, and biopsy findings suggest that diabetic nephropathy is not primarily responsible for the glomerular abnormalities that occur (Guery et al., 2003; Guillausseau et al., 2001). Nephropathy is more prevalent in patients with MIDD compared to matched diabetics (Massin et al., 2008), again implying that kidney disease in these individuals is more likely to be due to underlying mitochondrial disease rather than diabetes per se.

In a screening study of Japanese diabetic patients on dialysis, 8/135 (5.9%) were found to have the m.3243A>G mutation (Iwasaki et al., 2001), suggesting that mitochondrial disease makes a significant contribution to the burden of end-stage renal failure in diabetics.

Although tubular dysfunction is the typical renal phenotype of children with mitochondrial disease, glomerular disease can also occur (Hameed et al., 2001; Gucer et al., 2005), and FSGS has been reported in children with the m.3243A>G mutation (Inui et al., 1992; Mochizuki et al., 1996; Yorifuji et al., 1996).

Table 340.2 Nuclear genes associated with mitochondrial disease and renal dysfunction

Gene	Function of protein	Clinical phenotypes	Renal phenotype	Respiratory chain defect	References
PDSS2	Biosynthesis of CoQ_{10}	Leigh syndrome	SRNS, progressive kidney disease	Complex I + III and/or complex II + III deficiency	Lopez et al., 2006
COQ2	Biosynthesis of CoQ_{10}	Infantile-onset multisystem disorder: encephalomyopathy, ataxia	SRNS, FSGS on biopsy, progressive kidney disease	Complex I + III and/or complex II + III deficiency	Salviati et al., 2005; Quinzii et al., 2006; Diomedi-Camassei et al., 2007
COQ6	Biosynthesis of CoQ_{10}	SNHL, seizures, ataxia	SRNS, FSGS on biopsy	Complex I + III and/or complex II + III deficiency	Heeringa et al., 2011
COQ9	Biosynthesis of CoQ_{10}	Infantile-onset multisystem disorder: encephalomyopathy, seizures, HCM, lactic acidosis	Proximal tubulopathy	Complex I + III and/or complex II + III deficiency	Duncan et al., 2009
ADCK4	Biosynthesis of CoQ_{10}	SRNS (goitre, DCM, neurodevelopmental delay in occasional cases)	SRNS	Reduced maximal respiration in fibroblasts (muscle biopsy not performed)	Ashraf et al., 2013
NDUFAF2	Complex I assembly	Leigh syndrome	Renal tubular acidosis	Complex I deficiency	Hoefs et al., 2009
BCS1L	Complex III assembly	Encephalopathy and liver failure; GRACILE syndrome (growth retardation, amino aciduria, cholestasis, iron overload, lactic acidosis, and early death)	Proximal tubulopathy	Complex III deficiency	deLonlay et al., 2001; Visapaa et al., 2002
UQCC2	Complex III assembly	IUGR, neonatal lactic acidosis, renal tubular dysfunction, seizures	Proximal tubulopathy	Complex III deficiency	Tucker et al., 2013
SURF1	Complex IV assembly	Leigh syndrome	Proximal tubulopathy, distal RTA	Complex IV deficiency	Rahman et al., 2001a; Tay et al., 2005
COX10	Haem biosynthesis for complex IV assembly	Encephalomyopathy (leucodystrophy), seizures, ataxia; survival into adulthood reported	Proximal tubulopathy	Complex IV deficiency	Valnot et al., 2000; Pitceathly et al., 2013
COX14	Factor that couples COX I synthesis with COX assembly	Fatal neonatal lactic acidosis	Renal hypoplasia at autopsy	Complex IV deficiency	Weraarpachai et al., 2012
TACO1	Translational activator of cytochrome *c* oxidase 1	Leigh syndrome	Tubulopathy with mild aminoaciduria	Complex IV deficiency	Seeger et al., 2010
TMEM70	Complex V assembly	Multisystem disorder with lactic acidosis, 3-methylglutaconic aciduria and hyperammonaemia	Renal tubular acidosis, hydronephrosis and acute or chronic renal failure	Complex V deficiency	Magner et al., 2015
DGUOK	Nucleoside salvage for mtDNA maintenance	Liver failure, nystagmus, hypotonia and lactic acidosis	Proximal tubulopathy	Multiple RC defects (mtDNA depletion syndrome)	Dimmock et al., 2008
TK2	Nucleoside salvage for mtDNA maintenance	Encephalomyopathy, lactic acidosis	Tubulopathy	Multiple RC defects (mtDNA depletion syndrome)	Carrozzo et al., 2003
SUCLA2	Nucleoside salvage for mtDNA maintenance	Encephalomyopathy, SNHL, lactic acidosis, methylmalonic aciduria	Proximal tubulopathy	Multiple RC defects (mtDNA depletion syndrome)	Carrozzo et al., 2007
SUCLG1	Nucleoside salvage for mtDNA maintenance	Neonatal lactic acidosis, multisystem disease, methyl-malonic aciduria	Horseshoe kidney in one case	Multiple RC defects (mtDNA depletion syndrome)	Rivera et al., 2010

(*continued*)

Table 340.2 Continued

MPV17	Unknown function in mtDNA maintenance	Encephalomyopathy (leucodystrophy), seizures, peripheral neuropathy, liver failure, lactic acidosis	Tubulopathy, hyponatraemia	Multiple RC defects (mtDNA depletion syndrome)	Navarro-Sastre et al., 2008
RRM2B	Nucleotide synthesis for mtDNA maintenance	Encephalomyopathy, seizures, lactic acidosis; Kearns–Sayre syndrome	Proximal tubulopathy, hydronephrosis	Multiple RC defects (mtDNA depletion syndrome ± multiple mtDNA deletions)	Bourdon et al., 2007; Pitceathly et al., 2011
C10orf2	Helicase required for mtDNA replication	Multisystem disorder: cholestatic liver disease, hypotonia, failure to thrive, recurrent vomiting, renal tubulopathy, progressive neurodegeneration	Tubulopathy	Multiple RC defects (mtDNA depletion syndrome ± multiple mtDNA deletions)	Prasad et al., 2013
MRPS7	Protein component of mitochondrial ribosome	SNHL, progressive hepatic and renal failure, lactic acidaemia	Tubulopathy	Combined defect of complexes I, III, and IV in liver but not in muscle	Menezes et al., 2015
MRPS22	Protein component of mitochondrial ribosome	Encephalomyopathy, HCM, lactic acidosis and hyperammonaemia	Tubulopathy	Multiple RC defects (defect of mitochondrial translation)	Saada et al., 2007
SARS2	Seryl aminoacyl-transferase involved in mitochondrial protein synthesis	HUPRA syndrome (hyperuricaemia, pulmonary hypertension, renal failure in infancy and alkalosis)	Distal tubulopathy	Multiple RC defects (defect of mitochondrial translation)	Belostotsky et al., 2011
TSFM	Mitochondrial translation elongation factor EFTs	IUGR, neonatal lactic acidosis, liver dysfunction	Tubulopathy	Multiple RC defects (defect of mitochondrial translation)	Vedrenne et al., 2011
RMND1	Protein involved in mitochondrial translation	Multisystem disorder including myopathy, SNHL, and renal involvement	Renal dysplasia and renal tubular acidosis	Multiple RC defects (defect of mitochondrial translation)	Taylor et al., 2014
CLPB	Heat shock protein/ chaperonin responsible for disaggregating mitochondrial and cytosolic proteins	Multisystem disorder including cataract, neutropenia, epilepsy, and 3-methylglutaconic aciduria	Renal medullary cysts, calculi, nephrocalcinosis, renal failure	Normal	Kanabus et al., 2015; Saunders et al., 2015
TRAP1	Heat shock protein 90-related mitochondrial chaperone	CAKUT and VATERL association	CAKUT	Not investigated	Saisawat et al., 2014
XPNPEP3	X-prolyl amino-peptidase 3	Mild neurological involvement: SNHL and essential tremor	Nephronophthisis-like nephropathy	Not investigated	O'Toole et al., 2010

CAKUT = congenital abnormalities of the kidney and urinary tract; DCM = dilated cardiomyopathy; FSGS = focal segmental glomerulosclerosis; GRACILE = growth (G) retardation (R), amino aciduria (A), cholestasis (C), iron overload (I), lactic acidosis (L), and early death (E); HCM = hypertrophic cardiomyopathy; IUGR = intrauterine growth retardation; mtDNA = mitochondrial DNA; RC = respiratory chain; SNHL = sensorineural hearing loss; SRNS = steroid-resistant nephrotic syndrome; VACTERL = vertebral defects (V), anorectal malformations (A), cardiac defects (C), trachea-oesophageal fistula with or without oesophageal atresia (TE), renal malformations (R), and limb defects (L).

Coenzyme Q_{10} deficiency

Coenzyme Q_{10} (CoQ_{10}), also known as ubiquinone, is responsible for transferring electrons from OXPHOS complexes I and II and the electron transfer flavoproteins to complex III, and also acts as an important anti-oxidant (Rahman et al., 2012). Deficiency of CoQ_{10} can lead to diseases that typically present in childhood and has mainly been associated with glomerular rather than tubular dysfunction (Rotig et al., 2000). Nephrotic syndrome has been described in patients with mutations in the nuclear genes *PDSS2* (Lopez et al., 2006), *COQ2* (Salviati et al., 2005; Quinzii et al., 2006; Diomedi-Camassei et al., 2007), *COQ6* (Heeringa et al., 2011) and *ADCK4* (Ashraf et al., 2013), which all encode proteins in the biosynthetic pathway of CoQ_{10}.

The association of mutations in *COQ2* with kidney disease has led to the usage of the term 'COQ2 nephropathy', the characteristic features of which are strikingly similar to m.3243A>G-related kidney disease, and include steroid resistant nephrotic syndrome, pauci-immune collapsing glomerulopathy/FSGS on light microscopy, and podocyte foot process effacement and proliferation of dysmorphic mitochondria within podocytes on electron microscopy (Diomedi-Camassei et al., 2007). As with the m.3243A>G patients, the reason(s) why CoQ_{10} deficiency should predominantly affect glomerular function remain unclear. One possible explanation is that some of the nuclear genes involved in the CoQ_{10} synthesis pathway may be selectively expressed in different cell types, and indeed recent work on *COQ6* has demonstrated that within the kidney it is expressed almost exclusively in glomerular podocytes rather than tubular cells (Heeringa et al., 2011). However, other mutations associated with CoQ_{10} deficiency and nephrotic syndrome are more widely expressed, which raises the possibility that there are intrinsic properties of the podocyte that render it particularly vulnerable to CoQ_{10} deficiency.

Mice with mutations in *Pdss*2 (the murine form of *PDSS*2) develop collapsing glomerulopathy, tubulointerstitial nephritis, proteinuria, and progressive kidney disease, in a manner analogous to the human condition; the renal phenotype in these animals is reproduced by tissue-specific expression of the mutation in podocytes, but not by expression in tubular cells (Peng et al., 2008), implying that podocytes are more sensitive to the effects of CoQ_{10} deficiency, and that podocyte dysfunction is primarily responsible for the resulting kidney disease. While mutations in *COQ2* can lead to reduced CoQ_{10} levels in both muscle and kidney, the OXPHOS defect in the latter seems to be much more severe (Diomedi-Camassei et al., 2007), suggesting that mitochondrial function in the kidney is more sensitive to CoQ_{10} deficiency.

Tubular dysfunction has also been described in patients with CoQ_{10} deficiency (Rahman et al., 2001b; Duncan et al., 2009).

Animal models of mitochondrial disease

Due to the complexity of mitochondrial genetics, and difficulties in artificially manipulating mtDNA in intact cells, historically it has proven difficult to produce animal models of mitochondrial disease. However, a mouse harbouring a single 4696bp mtDNA deletion encoding six tRNA genes and seven structural genes (the 'mitomouse') was generated by inserting mutant mtDNA that had accumulated in somatic cells into cultured cells lacking mitochondria (so-called cybrids); respiratory deficient cybrids were then isolated and the mutant mtDNA was inserted into fertilized mouse eggs (Inoue et al., 2000). Interestingly, although various organs were affected in the mitomouse, the animals ultimately died of kidney failure, underlining the fact that renal involvement in mitochondrial disease is a significant issue. As discussed above, mitochondrial diseases due to mtDNA deletions have been associated with Fanconi syndrome in humans, and histological examination of the mitomouse kidneys revealed dilated tubules in the cortex. Mouse models of mitochondrial disease due to mutations in nDNA are technically easier to generate, and a variety of models are now available (Tyynismaa and Suomalainen, 2009; Wallace and Fan, 2009); renal involvement has been described in animals with mtDNA depletion syndrome caused by mutations in the nuclear genes *Rrm2b* (which encodes the p53-inducible ribonucleotide reductase (p53R2)) (Bourdon et al., 2007), and *Mpv17*, which is also involved in mtDNA maintenance (Spinazzola et al., 2006), as well as the *Pdss2* mutant mouse mentioned above.

Diagnosis of mitochondrial disease

When to suspect mitochondrial disease

Mitochondrial disease should be considered in the differential diagnosis of unexplained cases of renal dysfunction, and may be suggested by factors such as abnormal mitochondrial morphology on electron microscopy of kidney tissue, subclinical or overt abnormalities in other organs (e.g. impaired glucose tolerance, hearing loss, cardiac hypertrophy), a maternal family history (not necessarily of renal disease due to the heterogeneity of clinical phenotypes associated with mtDNA mutations), and a lack of response to conventional therapy. There are no specific features of underlying mitochondrial disease that can be observed on light microscopy of kidney biopsy tissue; however, it has been suggested recently that the presence of granular swollen epithelial cells in distal tubules and collecting ducts should prompt consideration of a mitochondrial diagnosis (Kobayashi et al., 2010).

Investigations

Current approaches to diagnosing mitochondrial disease have been reviewed in detail in recent articles (Rahman and Hanna, 2009; Tuppen et al., 2010), and a suggested algorithm (designed for neuromuscular disease) is provided in Fig. 340.2. Serum lactate concentration can be raised due to compensatory upregulation of anaerobic glycolysis; however, as filtered lactate is normally reabsorbed in the proximal tubule, and may be wasted in the urine in Fanconi syndrome (Thirumurugan et al., 2004), patients with mitochondrial disease and tubulopathy may not have an elevated serum lactate concentration.

Patients with suspected mitochondrial disease usually proceed to a tissue biopsy for confirmatory tests, ideally from the affected organ. In practice, skeletal muscle is most commonly sampled as it is readily accessible and usually affected. Other organs, including kidney, can also be used and may be more appropriate if they are the primary site of disease; however, the risks of the procedure must be considered and reference ranges for normal OXPHOS complex function tests may be less clearly defined.

Characteristic structural abnormalities in muscle fibres may be noted on light microscopy (e.g. proliferation of abnormal mitochondria—so-called ragged red fibres in the modified Gomori trichrome stain) or on electron microscopy (swollen or misshapen mitochondria). The function of OXPHOS complexes II (succinate dehydrogenase (SDH): subunits entirely encoded by nDNA)

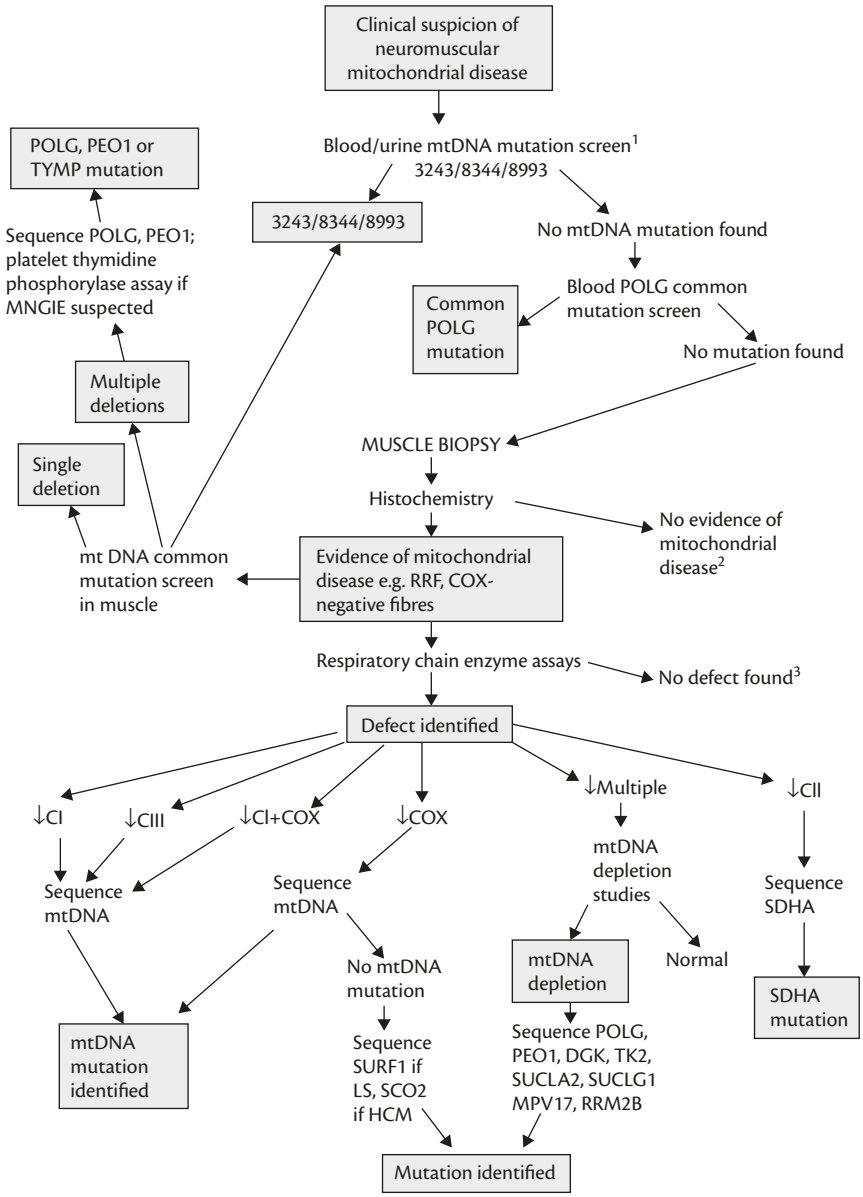

Fig. 340.2 The Queen Square mitochondrial disease investigation pathway.
[1]Mitochondrial DNA (mtDNA) deletion screen can be performed in blood if the patient is < 20 years old.
[2]Perform respiratory chain enzyme assays even if histochemistry normal if strong clinical suspicion.
[3]Sequence mtDNA even if respiratory chain enzyme assays normal if strong clinical suspicion. CI = complex I; CII = complex II; CIII = complex III; HCM = hypertrophic cardiomyopathy; LS = Leigh syndrome; MNGIE = mitochondrial neurogastrointestinal encephalomyopathy; PEO = progressive external ophthalmoplegia; POLG = polymerase gamma; RRF = ragged red fibre; SDHA = gene encoding succinate dehydrogenase subunit A.
Reproduced with permission from Rahman, S. and Hanna, M. G. (2009). Diagnosis and therapy in neuromuscular disorders: diagnosis and new treatments in mitochondrial diseases. *J Neurol Neurosurg Psychiatry*, 80, 943–53.

and IV (cytochrome c oxidase (COX): subunits encoded by both nDNA and mtDNA) can be assessed using histochemical analysis; a mosaic pattern of staining with COX-negative fibres is typically seen in patients with a mtDNA mutation.

Spectrophotometric studies using appropriate substrates can be used to measure the activities of complexes I, II, III, and IV, but this approach requires tissue to be frozen immediately after acquisition, which is rarely the case with kidney tissue unless a mitochondrial diagnosis is already suspected at the time of biopsy. Impairment of combined complex I + III or II + III activity is suggestive of CoQ_{10} deficiency; the diagnosis is confirmed by demonstrating decreased tissue levels of CoQ_{10} and performing genetic testing for known mutations in the biosynthetic pathway (Rahman et al., 2012).

Genetic tests

The entire mitochondrial genome can now be sequenced rapidly to detect point mutations, and a large resource of all known pathogenic mutations and associated phenotypes is freely available (<http://www.mitomap.org>). MtDNA rearrangements were traditionally assessed by Southern blotting, but this is now supplemented with long-range PCR. Multiple deletions in mtDNA or mtDNA depletion (i.e. quantitative reduction of mtDNA copy

number) are suggestive of underlying nDNA mutations encoding proteins important in mtDNA maintenance.

Mutations in 11 genes involved in mtDNA maintenance have now been linked to human mitochondrial disease (Rahman and Poulton, 2009; Pitceathly et al., 2011), and can be tested routinely in the DNA diagnostic laboratory, together with some of the nuclear genes known to cause isolated deficiency of complex I or complex IV (Rahman et al., 2009).

Testing for most of the > 170 nuclear genes so far demonstrated to cause human mitochondrial disease (Rahman, 2015) is only available on a research basis at present; however, the increasing availability of next-generation sequencing techniques is likely to dramatically change the landscape of genetic diagnostics in the near future.

A new approach to the diagnosis of mitochondrial disease has recently been developed, which involves screening for mtDNA abnormalities in epithelial cells spun down from single urine samples. It has been demonstrated that both point mutations and deletions in mtDNA can be detected using this approach; furthermore, in patients with point mutations the mutant load in urinary epithelial cells correlates well both with the level in muscle and overall disease severity (Blackwood et al., 2010).

Magnetic resonance imaging (MRI) of the central nervous system can provide clues as to the existence of underlying mitochondrial disease, including signal change in the basal ganglia, brainstem, or white matter and accumulation of metabolites such as lactate on MR spectroscopy, but MRI changes are not specific in many cases (Bianchi et al., 2007).

Treatment of mitochondrial disease

Treatment in mitochondrial disease is largely supportive as there are very few specific options available (Pfeffer et al., 2012; Kanabus et al., 2014; Rahman, 2015). A multidisciplinary approach is required, given the number of organs that can be affected and the severity of disability that may result. In spite of the association between mitochondrial dysfunction and oxidative stress, antioxidants have been largely unhelpful in mitochondrial disease; a new generation of agents specifically targeted to the mitochondria may turn out to be more successful (Smith et al., 2011), but these are currently only being used experimentally in animal models.

Oral CoQ_{10} supplementation has been reported to improve neuromuscular symptoms in some patients with CoQ_{10} deficiency, although there are relatively few reports to date of improvements in renal function, possibly because in most cases the glomerular damage was irreversible by the time treatment was given. However, both CoQ_{10} supplementation (Saiki et al., 2008) and the antioxidant probucol ameliorate renal complications in *Pdss2* mutant mice (Falk et al., 2011), and improvement in kidney function has been described in a patient with CoQ_{10} deficiency where it was possible to administer CoQ_{10} supplements early in the course of the disease because a diagnosis had previously been made in an elder sibling (Montini et al., 2008). Interestingly, the mechanism of action of CoQ_{10} supplementation remains unclear, as studies have suggested that tissue uptake of exogenous CoQ_{10} is low (Dallner and Sindelar, 2000) and that supplementation does not lead to an increase in intracellular CoQ_{10} content in the kidney (Saiki et al., 2008). In practice, CoQ_{10} is often given to patients with a variety of different kinds of mitochondrial disease other than CoQ_{10} deficiency.

Treatment of Fanconi syndrome and other tubulopathies is primarily directed at replacing fluid and electrolytes lost in the urine, to prevent symptomatic electrolyte deficiencies. In patients with glomerular disease, the blood pressure should be controlled if elevated and inhibitors of the renin–angiotensin system can be used to alter intraglomerular haemodynamics.

Renal transplantation has been performed in patients with mitochondrial disease (Guery et al., 2003), and there are no disease-specific contraindications. However, appropriate care should be taken with general anaesthesia, and given that patients with mitochondrial disease are susceptible to developing diabetes, immunosuppressant agents that can induce abnormalities in blood glucose homeostasis (e.g. steroids and tacrolimus) should be used with caution.

Gene therapy is increasingly being employed in modern medicine to treat hereditary disorders, but this approach has been hampered in mitochondrial disease by technical difficulties in targeting the mitochondrial genome (Rahman, 2015).

Genetic counselling for mothers carrying point mutations in mtDNA mutations is limited by a lack of accurate prediction of either the likelihood of disease transmission or the severity of disease in affected offspring; pre-implantation diagnosis may have a role, but the success of this technique will likely be determined by the precise mutation and the mother's mutant load (Bouchet et al., 2006). Most large-scale deletions in mtDNA are sporadic, and the risk of transmission is generally considered low.

Recently, new strategies have emerged to try to prevent maternal transfer of mutant mtDNA to offspring. One such approach is pro-nuclear transfer, whereby the nucleus from a zygote containing mitochondria with mutant mtDNA is removed and transferred to an enucleated recipient cell containing normal mtDNA (Tachibana et al., 2009). The potential success of this technique has been demonstrated *in vitro* using human embryos, with observed 'carry-over' of mutant mtDNA well below the threshold usually associated with disease (Craven et al., 2010). Further studies are now required to assess the long-term safety of this approach. In the meantime, until reliable techniques are available to accurately predict the risk of disease transmission or prevent it from occurring, the only safe policy is to consider egg donation.

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CHAPTER 341

APOL1 and renal disease

Neil Turner, Teena Tandon, and Rajiv Agarwal

Introduction

Studies in 2008 first identified linkage of markers in the *MYH9* gene with focal segmental glomerulosclerosis (FSGS) in African Americans (Kao et al., 2008; Kopp et al., 2008). This was identified both in HIV nephropathy and in primary FSGS. The association was strong enough to fully account for the increased susceptibility of black races to these diseases. Although *MYH9* (which encodes the myosin, heavy chain 9, non-muscle protein) seemed a plausible candidate gene because it was already known to cause autosomal dominant renal disease (see Chapter 342) and because a non-muscle myosin might be implicated in podocyte diseases (see Chapter 327), a stronger linkage was soon shown with variants in the adjacent apolipoprotein L1 (*APOL1*) gene (Genovese et al., 2010a) (Table 341.1).

Further studies have confirmed that *APOL1* alleles that are common in Africa not only predispose to FSGS, but that they are also implicated in susceptibility to progressive renal disease of other aetiologies. In particular they account for the increased incidence in disease labelled as hypertensive in origin—increasingly, evidence suggests that the hypertension in these patients is secondary to renal disease and not the primary cause of chronic kidney disease (CKD). It suggests that it is genetic kidney disease rather than primary hypertension that predisposes to end-stage renal disease (ESRD) in this population (see Chapter 100).

APOL1 associations with renal disease

People of African descent in the United States have an incidence of ESRD that is 3.5–5 times higher than that of white patients. Environmental factors are likely to contribute to this, but the excess risk attributable to race is at least twofold (Williams and Pollak, 2013).

FSGS

Kopp et al. (2008) (Table 341.1) mapped FSGS to a single prominent peak at chromosome 22q13 close to the *MYH9* gene. This genetic association was retested by Kao et al. (2008) by a genome-wide admixture scan and a highly significant association was found between *MYH9* and non-diabetic ESRD with lod score of 5.7. Freedman et al. (2009) detected a strong association between the *MYH9* gene and hypertensive ESRD (odds ratio (OR) 3.4). Findings remained significant even after adjusting for age, gender, and adjustments for multiple testing.

However, Genovese et al. (2010a, 2010b) on analysis of novel single nucleotide polymorphisms (SNPs) in 205 African Americans with biopsy-proven FSGS and 180 African American control subjects without nephropathy found that the strongest association was with variants in a 10 kb region of the last exon of *APOL1*, adjacent to *MYH9*. Two variants in this region of *APOL1*, named G1 and G2, are found exclusively in individuals of African descent and are known to provide protection from sleeping sickness caused by *Trypanosoma brucei rhodesiense*. The same mutations were shown to predispose to kidney disease at a younger age (Freedman et al., 2010; Friedman and Pollak, 2011; Kanji et al., 2011; Freedman and Langefeld, 2012; Lipkowitz et al., 2013). In most studies the risk is recessive, that is, increased risk is only seen if an individual has a risk allele on each chromosome. Some studies have identified a single copy of G1 as conveying some risk (Tzur et al., 2012; Skorecki and Wasser, 2013).

Kopp et al. (2011) identified astonishingly high ORs for those carrying two risk alleles: of 29 for HIV nephropathy, and 17 for FSGS. FSGS in those with two risk alleles progressed faster to ESRD. They estimated those with two risk alleles had an approximately 4% chance of developing FSGS and if infected with HIV, a 50% risk of developing HIV nephropathy.

Other renal diseases

In addition to demonstrating the FSGS association, Kopp et al. (2011) also found that homozygotes were at increased risk of other renal diseases.

Lipkowitz et al. (2013) studied 675 participants from the African American Study of Kidney Disease and Hypertension (AASK) study in which iothalamate-determined glomerular filtration rate (GFR) was measured. They confirmed the primacy of the association of (recessive) *APOL1* G1 and G2 alleles with poor outcomes, and found that the risk conveyed by the alleles became greater according to worse function and higher proteinuria at study entry, and that patients with two risk alleles did not appear to benefit from angiotensin-converting enzyme inhibition or blood pressure control.

Parsa et al. (2013) (Table 341.1) found that 23% of the AASK population had two copies of the G1 or G2 risk variants of *APOL1*. They had lower GFR and higher proteinuria than other patients in the study. They were twice as likely (58%) to reach ESRD or doubled creatinine than patients with one or no copies of the risk variants. Neither blood pressure control nor proteinuria seemed to influence outcome. In the CRIC study, primary outcomes were halving of estimated GFR or reaching ESRD. Kidney function declined more rapidly in those with two *APOL1* risk alleles, both in those with and in those without diabetes.

In studies using eGFR estimations mixed genetic backgrounds may confound eGFR equation-based associations betwen race and outome. Udler et al. (2015) discuss this and find that adjusting for

Table 341.1 Details of studies demonstrating an association of genetic mutations with chronic kidney disease

Study	Method	Results
Kao et al. (2008)	Genome-wide admixture scan in 1372 ESRD cases (703 diabetic) and 806 controls Participants recruited as FIND and CHOICE study	Multiple common SNP in gene encoding *MYH9* associated with 2–4-fold greater risk of non-diabetic ESRD Highly significant association between African ancestry and non-diabetic ESRD (lod score 5.7)
Kopp et al. (2008)	Admixture mapping linkage disequilibrium genome scan on 190 African American patients with FSGS and 222 controls Extension study analysed 14 SNPs for hypertension ESRD (n = 241) or type 2 diabetes mellitus-associated ESRD (n = 284) with matched controls (n = 192)	Single prominent peak at chromosome 22q 13 Apex close to *MYH9* gene. Multiple *MYH9* haplotypes and SNPs associated with FSGS Strong association of African ancestry with FSGS in African American and with hypertensive ESRD (OR 2.2; 95% confidence interval 1.5–3.4)
Freedman et al. (2009)	15 *MYH9* SNPs evaluated in 175 African American patients with chronic glomerulonephritis -associated ESRD, 696 with hypertension-associated ESRD and 948 controls without kidney disease	Significant association detected with 14/15 SNP in all 871 non-diabetic ESRD (OR 3.4 for associated SNP) Suggested two-hit model. Gene–gene and gene–environment interaction leads to kidney disease in susceptible individuals with *MYH9* gene
Genovese et al. (2010a)	Evaluated 205 African American patients with FSGS and 180 African American controls participating in 1000 genome project using novel SNPs	Strongest association between 10 kb region of last exon of *APOL1* not *MYH9*, most robust in G1 allele *APOL1* association more specific than *MYH9* in hypertension-associated end-stage kidney disease in African Americans *APOL1* confers protection from trypanosome
Kanji et al. (2011)	Evaluated association of *APOL1* gene variant with age of initiation of dialysis in 407 non-diabetic African Americans with ESRD. Patients were participants in ArMORR study, prospective cohort study	African Americans carrying two copies of G1 risk allele initiated haemodialysis at 49.0 ± 14.9 years compared to 55.9 ± 16.7 years for subjects with one copy of G1 allele and 61.8 ± 17.1 years in subjects without either risk allele
Kopp et al. (2011)	Determined *APOL1* genotypes for 271 African American cases, 168 European American cases, and 939 control subjects.	Identified an OR for those carrying two risk alleles of 29 for HIV nephropathy, and 17 for FSGS. FSGS in those with two risk alleles progressed faster to ESRD
Lipowitz et al. (2012)	675 participants from the AASK study	More rapid deterioration in those with two risk alleles
Tzur et al. (2012)	Examined *APOL1* genotype in 995 African and Hispanic American dialysis patients with diabetic and non-diabetic ESKD	African American patients with two *APOL1* risk alleles initiated dialysis > 9 years earlier than those without *APOL1* risk allele (P = 0.0003). Similar results in non-diabetic Hispanic cohort G1 heterozygotes showed 5.3 years lower mean age of dialysis initiation (P = 0.045)
Parsa et al. (2013)	693 black individuals in the AASK cohort in which renal impairment was attributed to hypertension; and 2955 patients (48% black) from the Chronic Renal Insufficiency Cohort (CRIC) study in which diagnoses were varied, but 46% had diabetes	Two risk alleles associated with more rapid progression and poorer outcomes in both studies, in disease attributed to hypertension, diabetes and other conditions. Treatment type or blood pressure did not seem to alter outcome
Foster et al. (2013)	3067 African Americans in the Atherosclerosis Risk in Communities Study who did not have CKD at baseline	Two risk alleles associated with 1.5-fold increased risk of CKD and 1.9-fold increased risk of ESRD.

'genetic' as opposed to self-declared race improves apparent outcomes for black race, but confirmed that *APOL1* alleles drove the risk of CKD.

The association with renal disease attributed to hypertension is discussed further in Chapter 100.

Transplantation

In a study of 22 kidneys transplanted from donors with two *APOL1* risk alleles, graft survival was found to be significantly poorer (Reeves-Daniel et al., 2011). An extended multicentre, registry-based retrospective survey by the same group, involving 99 kidneys carrying two *APOL1* risk alleles versus 576 carrying 0 or 1 (Freedman et al., 2015) found the same observation again. It is important that despite a hazard ratio for graft failure of about 2, 55% of these kidneys were functioning beyond 10 years. Most graft failures occurred within the first 2 years.

By contrast, a study of genotypes of kidney recipients failed to find an associaton between recipient *APOL1* genotype and outcome (Lee et al. 2012). In other words, the kidney confers the risk, not its new host.

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CHAPTER 342

MYH9 and renal disease

Neil Turner and Bertrand Knebelmann

Introduction

Mutations in the *MYH9* gene encoding the heavy chain of non-muscle myosin IIA are associated with a characteristic cluster of manifestations that has attracted a number of eponyms (Table 342.1). As the separation between these distinct eponyms is incomplete, and individuals with a common mutation can display different features, the term MYH9-associated diseases is more appropriate. The haematological abnormalities were first identified in 1909 and 1945 and the associated renal disease first recognized in 1972 (Epstein et al., 1972).

Clinical features

Patients with renal disease generally have either Epstein or Fechtner syndrome by historic definitions (Table 342.1). Essentially those with Fechtner syndrome have all the abnormalities listed, while those with Epstein syndrome lack the leucocyte inclusions and cataracts. However, renal variants seem always to have the platelet abnormalities. This is helpful as these are usually quickly picked up on autoanalysers.

Leucocyte inclusions can be identified in granulocytes on Giemsa stains, but may be missed unless looking specifically for them. They are described to as Döhle-like as they resemble the inclusions seen in 'toxic' neutrophils when the rate of production is high, though their composition is different.

Renal impairment is generally progressive and associated with proteinuria, but not usually with haematuria. Proteinuria is often low level or absent early; later it may be significant but seems rarely to reach nephrotic range. Deterioration may be rapid and Sekine et al. (2010) described nine patients with mutations at one location, R702, in whom several declined to end-stage renal failure during adolescence and all developed progressive hearing impairment which was evident in several by the age of 5 years, and in all by age 30. Only one developed a cataract, but this was a young series.

Mild or moderate bleeding tendency has been described in some patients but usually there is no apparent bleeding diathesis despite exceptionally low platelet counts. Presumably the few giant platelets generally function well.

Diagnosis

The characteristic platelet abnormalities, when recognized, distinguish the condition from most alternative diagnoses. Idiopathic thrombocytopenic purpura is a common initial diagnosis in children following the identification of thrombocytopenia. Although there are other causes of giant platelets they are not generally associated with renal disease.

Renal biopsies are not widely described, probably because platelet counts discourage biopsy, but changes are not specific. Kopp (2010) gives an excellent detailed summary. Light microscopy is most often described as showing segmental and global glomerulosclerosis, or sometimes mesangial expansion or proliferation. Interestingly electron microscopy sometimes shows glomerular basement membrane changes that have further confused the relationship of these diseases to Alport syndrome (see Chapter 323), with irregular thickening and very occasionally focal splitting, but these are probably rarely extensive.

Gene sequencing is rarely required to make the diagnosis but it is increasingly available.

Molecular basis of MYH9 diseases

The association of these conditions with mutations in the coding region of *MYH9* was described in 2000 (Seri et al., 2000). Non-muscle myosin II is expressed in nearly all cells and knockout studies in mice show that it is essential for normal development. *MYH9* is expressed at high levels in the podocyte, but also in mesangial, endothelial, and tubular cells. It is also found in the inner ear and in platelets (Arrondel et al., 2002; Sekine et al., 2010).

MYH9 encodes one of three possible heavy chains (the others encoded by *MYH10* and *MYH14*) for the NM II molecule, determining its isoform as NM IIA, NM IIB, or NM IIC respectively. The non-muscle myosins are motors that have roles in maintaining and changing cell structure and shape, and motility. The function of NM II and consequence of mutations are reviewed by Vicente-Manzanares et al. (2009). There is some correlation between genotype and phenotype, with mutations in the motor domain being most strongly associated with kidney disease (Pecci et al., 2008).

The platelet defect has been shown to be related to haploinsufficiency (reduced gene dosage). The mechanism of renal disease is not clear. While it seems likely to involve podocytes, it is phenotypically not closely similar to the defects of podocyte structural proteins that typically cause severe nephrotic syndrome and focal segmental glomerulosclerosis (FSGS) (see Chapter 327).

The inclusion bodies in Sebastian syndrome differ slightly from those of May–Hegglin anomaly but staining with monoclonal antibodies shows that they contain maldistributed aggregated myosin.

Differential diagnosis

The disease was described initially as an autosomal dominant form of Alport syndrome (see Chapter 323) with macrothombocytopenia. However, in some families, male carriers may present with thrombocytopenia and/or deafness in the absence of any clinical

Table 342.1 Manifestations of MYH9-associated diseases. Originally described syndromes are listed in the left-hand column. In clinical practice there is little value in separating them. ?* indicates that cataracts are described but not common. May–Hegglin and Sebastian syndromes differ only in the characteristics of the leucocyte inclusions

Syndrome and OMIN identifier	Renal disease	Leucocyte inclusions	Giant platelets, thrombocytopaenia	Deafness	Cataract
May–Hegglin (Saito and Kunishima, 2008) OMIM 155100		+	+		?*
Sebastian (Saito and Kunishima, 2008) OMIM 606249		+	+		?*
Epstein (1972) OMIM 153650	+		+	+	
Fechtner (Peterson et al., 1985) OMIM 153640	+	+	+	+	+

renal involvement, a pattern that is never observed in Alport syndrome (Knebelmann et al., 2001). Molecular genetics has clarified the situation, but the phenotype is superficially similar as patients have characteristically progressive loss of renal function with proteinuria (but not usually much haematuria) and progressive sensorineural deafness.

Focal segmental glomerulosclerosis and chronic kidney disease

MYH9 was originally highlighted in genomic studies of racial susceptibility to FSGS and hypertensive renal disease, but later a stronger association was found with the adjacent gene *APOL1* (see Chapter 341), and it is *APOL1* that appears responsible for a large part of the excess end-stage renal disease seen in those of African ancestry in the United States. Some subsequent analyses in Caucasian populations have suggested that there might be weaker linkage to *MYH9* itself in chronic kidney disease and diabetic nephropathy (O'Seaghdha et al., 2011; Cooke et al., 2012), but these need to be verified.

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