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PERSPECTIVE

Mouse models of ciliopathies: the state of the art

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The ciliopathies are an apparently disparate group of human diseases that all result from defects in the formation and/or function of cilia. They include disorders such as Meckel-Grüber syndrome (MKS), Joubert syndrome (JBTS), Bardet-Biedl syndrome (BBS) and Alström syndrome (ALS). Reflecting the manifold requirements for cilia in signalling, sensation and motility, different ciliopathies exhibit common elements. The mouse has been used widely as a model organism for the study of ciliopathies. Although many mutant alleles have proved lethal, continued investigations have led to the development of better models. Here, we review current mouse models of a core set of ciliopathies, their utility and future prospects.

Introduction

Over the past decade it has emerged that a diverse and overlapping spectrum of human diseases share a common origin in the cilium, a microtubule-based organelle templated from the centriole (Satir and Christensen, 2007). These diseases have collectively become known as the ciliopathies (Table 1) and have been the subject of many recent reviews (Badano et al., 2006; Fliegauf et al., 2007; Baker and Beales, 2009; Oh and Katsanis, 2012). In addition, the complex biology of making the cilium and its resulting structure are topics that have been well reviewed by others (Rosenbaum and Witman, 2002; Satir and Christensen, 2007; Pedersen et al., 2008). In brief, the cilium comprises nine microtubule doublets, describing a ring, surrounded by a membrane; this 9+0 structure is seen in all primary cilia (Fig. 1). Motile cilia, such as in the trachea, have an additional central pair of microtubules, giving a 9+2 structure (Fig. 1). As the cilium grows, proteins are added at the tip, requiring active transport along the cilium through a process known as intraflagellar transport (IFT). A kinesin-based anterograde IFT motor carries cargo outwards, whereas a cytoplasmic dynein 2 retrograde motor transports cargo back towards the cell body (Fig. 1). These cargos are associated with IFT proteins that facilitate this translocation. Defects in any of these processes impact ciliogenesis (Rosenbaum and Witman, 2002; Satir and Christensen, 2007; Pedersen et al., 2008).

Given the limitations of studying patients, research with mice has proved invaluable for understanding the roles of cilia in health and disease. The designation 'mouse model' has, in recent times, become a catch-all term used to describe any genetic variant, whether or not it truly models a human disorder. These so-called models include mice with spontaneous or chemically derived mutations, or carrying engineered alleles; current technology allows almost any mutation that can be envisaged to be incorporated into the mouse genome. Furthermore, conditional mutations, whereby gene function can be abrogated in a spatially and temporally controlled manner, are also being used widely. Although we discuss all kinds of mouse mutants in this review, for the purpose of lucidity we refer to changes in DNA sequence as 'mutations' (resulting in mutant mice), and reserve the term 'model' to refer to a mouse that closely reflects at least some aspects of human disease. By contrast, we use the term 'tool' to refer to mutations that do not model a disease, but still provide biological insight (Sive, 2011).

In this Perspective, we provide a brief overview of ciliary function, and then set out to address how well the ciliopathies have been modelled in the mouse, reflecting areas in which better models are required and how existing models can be and are being applied. We limit our central discussion to a core subset of ciliopathies, excluding primary ciliary dyskinesia (PCD) and polycystic kidney disease (PKD), which are briefly described in Boxes 1 and 2, respectively.

Roles of cilia: signalling and sensory

For many decades it was thought that the primary cilium was a purely vestigial organelle without function (Bloodgood, 2009). However, in parallel with the discovery of ciliopathies, crucial roles for primary cilia in development and physiology have emerged. Indeed, the numerous requirements for cilia are reflected in the broad spectrum of phenotypes associated with ciliopathies (Fig. 2; Table 1). The immotile 9+0 primary cilium is argued to be primarily a sensory and signalling organelle that is required for the 'outside-in' transduction of external stimuli (Marshall and Nonaka, 2006; Christensen et al., 2007; Berbari et al., 2009). By contrast, motile cilia are required for lung clearance, establishment of left-right (L-R) patterning during development, male and female fertility, and cerebrospinal fluid circulation (see Box 1). Recently, it has emerged that motile cilia might also have sensory functions (Bloodgood, 2010).

The role of cilia in cell-cell signalling, although initially controversial, has become increasingly evident. Primary amongst cilia-associated signalling pathway is Hedgehog (Hh); pathway elements are shuttled into and out of the cilium in response to ligand, resulting in changes in the levels of processed and un-

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Table 1. Hallmarks of human ciliopathies

	Alström	BBS	JBTS	Jeune	MKS	NPHP	OFD
Cleft palate					✓		
Cognitive defects		✓	✓			✓	✓
Deafness	✓						
Encephalocele					✓		
Liver disease		✓	✓	✓	✓	✓	✓
Obesity	✓	✓	✓				
Polydactyly		✓	✓	✓	✓		✓
Renal cysts		✓	✓	✓	✓	✓	✓
Retinopathy	✓	✓	✓	✓		✓	
Situs inversus		✓	✓		✓		✓
Skeletal defects				✓	✓		✓

Tick marks indicate presence of a hallmark feature in human ciliopathy patients. BBS, Bardet-Biedl syndrome; JBTS, Joubert syndrome; MKS, Meckel-Grüber syndrome; NPHP, nephronophtisis; OFD, orofaciodigital syndrome.

processed Gli signal-transducing proteins (Eggenschwiler and Anderson, 2007; Goetz and Anderson, 2010; Murdoch and Copp, 2010). Individual cilial mutants exhibit phenotypes that apparently reflect both loss and gain of Hh signalling, owing to the fact that cilia are involved in the processing of both activator and repressor Gli proteins. The role of cilia in both canonical Wnt and non-canonical Wnt/planar cell polarity (PCP) signalling is controversial (Wallingford and Mitchell, 2011). Although some studies suggest that embryos lacking cilia demonstrate neither canonical nor non-canonical Wnt defects, other studies strongly argue that there is a requirement for cilia in Wnt signalling (reviewed in Wallingford and Mitchell, 2011). Indeed, Wnt defects have been specifically linked to ciliopathies. Other signalling pathways have also been linked with cilia, although the full significance of these associations remains to be elucidated. Receptors for both fibroblast growth factor (FGF) and leptin localise to the cilium (Tanaka et al., 2005; Baly et al., 2007), and cilia have also been strongly implicated in platelet-derived growth factor (PDGF) and Notch signalling (Schneider et al., 2005; Ezratty et al., 2011).

Primary cilia also play various sensory roles, including in mechanosensation, thermosensation, photoreception and osmosensation (Berbari et al., 2009). In the kidney, primary cilia have been implicated as stress or fluid flow sensors, using a mechanism that involves the polycystin proteins (Box 2). The 'two cilia hypothesis' argues that a similar mechanism is required for L-R patterning of the embryo (McGrath et al., 2003; Tabin and Vogan, 2003); motile cilia generate a unidirectional flow while immotile cilia respond to it (Box 2). In addition, the connecting cilium in photoreceptor cells of the eye joins the cell bodies to the opsin-filled outer segments; these are replaced every few days, requiring marked IFT activity. Defects in connecting cilia lead to retinal dystrophy and blindness (Insinna and Besharse, 2008). In summary, cilia are important across multiple body systems: in addition to the functions mentioned above, cilia are involved in olfaction, hearing, behaviour and bone growth (Berbari et al., 2009; Waters and Beales, 2011).

Mechanistic insights from global and conditional cilia loss

Given the numerous requirements for cilia in diverse cellular processes, it is unsurprising that complete loss of cilia is devastating to a mouse or a human being. It was through analysis of the most severe ciliogenesis mouse mutants that we gained initial insight into the molecular roles of cilia. Such mutants fulfill Sive's definition of a tool and cannot be considered models (Sive, 2011).

Mutants lacking the anterograde IFT motor components *Kif3a* and *Kif3b* were initially reported to lack nodal cilia (resulting in defective L-R patterning) and to arrest by mid-gestation during development; cilia outside of the node were not examined (Nonaka et al., 1998; Marszalek et al., 1999; Takeda et al., 1999). Only subsequently did it become evident that this lethality resulted from a requirement for IFT in Hh signalling, and that this was impacting tissues beyond the node (Huangfu et al., 2003). In the same analysis Huangfu, Anderson and colleagues demonstrated similar early-lethal phenotypes for the N-ethyl N-nitrosourea (ENU)-derived IFT mutants *Ift172*^{wim} and *Ift88*^{fxo} (Huangfu et al., 2003). Mice carrying mutations in *Dyn2ch1*, which encodes a dynein molecule required for retrograde IFT, also demonstrate early embryonic lethality resulting from morphologically abnormal cilia (Huangfu and Anderson, 2005). These catastrophic abnormalities in embryogenesis have been attributed to defective Hh signalling: cilia-derived defects in other pathways simply do not get the opportunity to manifest. As such, major defects in ciliogenesis appear as Hh signalling defects. The existence of such abnormalities in humans is more difficult to assess than is immediately obvious. The equivalent developmental stage at which these defects are lethal in mice is only a few weeks of age in humans; the normal frequency of early miscarriage makes assessing such embryonic deaths challenging, so we cannot completely rule out that these mutants model human defects. Nevertheless, weaker alleles must be used to model the human ciliopathies that we do see.

Owing to the essential requirement for cilia during development, it has been problematic to assess the function of cilia postnatally using conventional mouse mutants. However, when cilia were globally deleted postnatally (>8 weeks of age) by Cre-mediated methods, the resulting mice showed only very slow-onset PKD (Davenport et al., 2007). Strikingly, postnatal cilia loss was shown to result in hyperphagia-induced obesity; this was linked to hypothalamic cilia function (Davenport et al., 2007). Deleting the *Pkd1* locus postnatally shed light on the reason why a strong cystic kidney phenotype was not observed following Cre-mediated deletion of cilia. Severe cystic kidney disease was evident with *Pkd1*

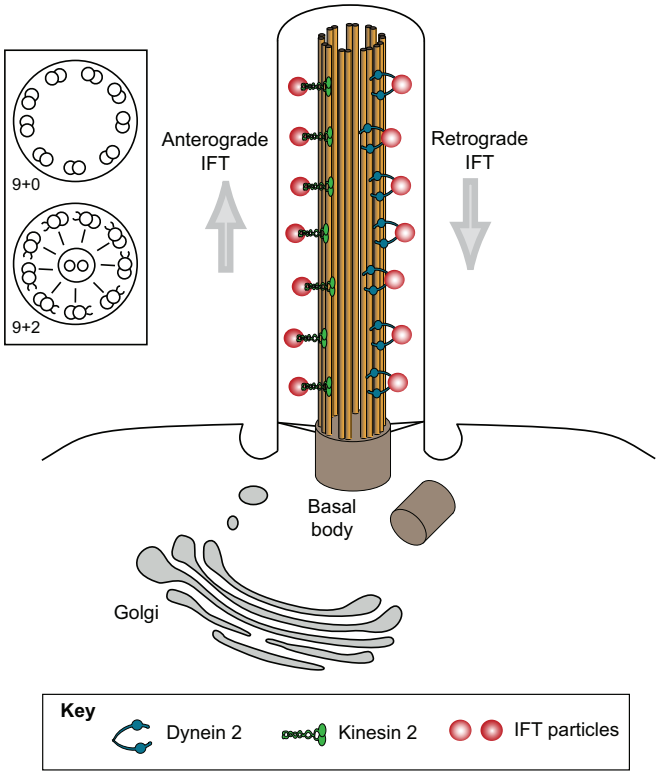

Fig. 1. The primary cilium. Primary cilia are small microtubule-based outgrowths of the plasma membrane that extend from a modified centriole, called the basal body, which serves as a microtubule-organising centre. The axoneme of primary cilia consists of nine peripheral microtubule doublets (9+0); motile cilia contain an extra pair of microtubules in the centre (9+2) (see inset). Extension and maintenance of the cilium requires IFT, a mechanism by which cargo is transported along the ciliary axonemes. Anterograde IFT is mediated by IFT-B particles and the kinesin 2 motor (composed of KIF3A, KIF3B and KAP). Retrograde IFT requires IFT-A complexes and the cytoplasmic dynein 2 motor, which is relatively poorly defined, but includes DYNC2H1 and DYNC2LI1. Trafficking of proteins from the Golgi to the cilium requires the action of small GTPases and some members of the IFT-B complex (IFT54 and IFT20).

deletion before 13 days of age; by contrast, *Pkd1* deletion after 14 days of age caused a mild, slow-onset cystic kidney disease, demonstrating a developmental or possibly growth-related switch (Piontek et al., 2007). Postnatal cilia deletion in bone growth plates, mediated by conditional *Kif3a* deletion, resulted in dwarfism, underscoring a role for cilia in long bone growth; again, this ciliary function clearly occurs prior to adulthood (Song et al., 2007). Although the full spectrum of postnatal cilia function is perhaps not yet fully appreciated, it is becoming increasingly evident that cilia perform a number of crucial tasks in the adult.

Tools and models for understanding human ciliopathies

As for most human diseases, researchers have sought to specifically model the ciliopathies. Although studies have been conducted in various model organisms, the mouse has provided an excellent model system owing to the similarity of its anatomy, physiology and genetics to the human. To date, a large number of mutant ciliopathy alleles have been created and analysed (Table 2), whereas others exist as mutant embryonic stem (ES) cell lines. Given that they are primarily recessive genetic disorders, it might seem that modelling ciliopathies would be relatively simple. However, the hypomorphic nature of many human mutations, probably in combination with copy number variation, impacts human disease phenotypes and therefore makes the generation of accurate models a challenge. The current progress of modelling individual ciliopathies is discussed below.

Box 1. Primary ciliary dyskinesia

The most widely known function of cilia is that of motile cilia in the trachea; cilia on multiciliated cells beat in a coordinated manner and with a whip-like motion, driving mucus and any associated debris upwards from the lungs. Tracheal cilia have a 9+2 structure (with a central pair of microtubules), spokes, and both inner and outer dynein arms (Fig. 1) (Satir and Christensen, 2007). Similarly, multiciliated cells in the ependyma of the brain and the female reproductive tract circulate cerebrospinal fluid or help to propel the egg, respectively. The sperm flagellum is also a motile 9+2 cilium. Motile 9+0 monocilia are present in the embryonic node, where they drive fluid leftwards in the developing embryo (Nonaka et al., 1998), thus establishing situs (L-R axis). Controversy about whether the motile nodal cilia are truly 9+0 or 9+2 was raised by a study in which rapid fixation was used, revealing a central pair in a proportion of cilia (Caspary et al., 2007). Unfortunately, directly linking motility to cilial ultrastructure in the node is beyond current experimental approaches. However, the rotational nature of tracheal cilia motility in individuals with central pair defects is surprisingly similar to that of nodal cilia (Chilvers et al., 2003). In combination, these facts raise the question of whether additional cilial beating patterns might exist in the node.

Defective cilia motility leads to primary ciliary dyskinesia (PCD; OMIM: 244400), resulting in lung clearance defects that can develop into bronchiectasis, sinusitis and rhinitis, incidence of both male and female infertility, and abnormal situs; hydrocephalus incidence is increased in individuals with PCD, although it remains rare (Fliegauf et al., 2007). Eleven genes underlying these defects have been identified in humans, explaining about half of known cases (Zariwala et al., 2011). Various mouse models have been developed that carry mutations in these loci and that demonstrate aspects of the disorder, particularly rhinitis, sinusitis and situs defects (Livraghi and Randell, 2007). The primary cellular defect (abnormal cilia motility) is reproducibly seen in these models as immotile cilia. Hypermotile cilia have not been reported in models, in contrast to observations in a significant minority of individuals with PCD. Strikingly, most PCD models show additional defects to those routinely seen in PCD patients. Hydrocephalus is highly prevalent and it has been argued that this relates to differences in the scale of mouse and human anatomy (Ibanez-Tallon et al., 2002). Similarly, cardiac defects have been reported (Tan et al., 2007), which probably result from situs specification defects. Both of these problems have been overcome by conditional deletion of *Dnaic1* (a known human PCD locus) in adult mice (Ostrowski et al., 2010); obviously this approach limits the opportunity to model disease progression in juveniles. Recently, an adult viable model has been published that does not demonstrate hydrocephalus or significant postnatal cardiac defects. The *Dnahc11*^{iv} mouse shows immotile cilia, situs defects, rhinitis and sinusitis, but no significant male or female fertility problems (Lucas et al., 2011); this should allow progression of PCD to be followed from birth into old age for the first time. Strikingly, no mouse model has recreated the bronchiectasis seen in individuals with PCD, arguably owing to innate differences in anatomy, physiology, scale and/or lifespan between humans and mice. No attempt to examine the effect of infection on PCD models has been published, raising the possibility that this might influence bronchiectasis progression in the models.

Meckel-Grüber syndrome

Meckel-Grüber syndrome (MKS; OMIM: 249000) is one of the most severe human ciliopathies, consistently resulting in neonatal

Box 2. The polycystin proteins: ADPKD and links to L-R asymmetry

Autosomal dominant polycystic kidney disease (ADPKD; OMIM: 173900) is characterised by the development of fluid-filled cysts in the kidney and by defects in other tissues (Harris and Torres, 2009), including cystic liver and pancreas, cerebral aneurysms, and cardiac abnormalities. The causative genes were identified as *PKD1* and *PKD2*, which encode polycystin-1 (PC1; also known as PKD1) and polycystin-2 (PC2; also known as PKD2), respectively. Both PC1 and PC2 are large multi-pass transmembrane proteins. Several mouse *Pkd1* and *Pkd2* mutants have been used for the study of ADPKD. Both homozygous *Pkd1*- and *Pkd2*-null mice die between 15.5 dpc and birth, exhibiting severe kidney cysts and vascular defects (Wilson, 2008). Heterozygotes are adult viable and demonstrate a mild phenotype, with low level cysts appearing with age; $Pkd1^{-/+}$, $Pkd2^{-/+}$ double heterozygotes are also viable but exhibit accelerated renal cyst appearance compared with single heterozygotes of either gene (Wu et al., 2002).

A model explaining the roles of PKD1 and PKD2 during renal development posits that these proteins are components of a stress-sensing cation channel complex localised to sensory cilia that project into the kidney lumen. The ectodomain of PKD1 is thought to sense membrane stress caused by fluid flow (Nauli et al., 2003; Praetorius and Spring, 2003). PKD1 contains 15 extracellular PKD domains that have been specifically implicated in stress sensing (Forman et al., 2005; Ma et al., 2009); indeed, PKD domains are 'hotspots' for human ADPKD mutations (http://pkdb.mayo.edu). However, to our knowledge, no mouse line harbouring point mutations in any PKD domains of PKD1 has been generated. PKD2 is known to be a nonselective cation channel that binds, via intracellular coiled coil domains, to PKD1 (Hanaoka et al., 2000). Some models posit that stress sensing by PKD1 elicits an intracellular Ca^{2+} spike via PKD2 channel activity, resulting in the regulation of intracellular pathways that have anti-growth, and thereby anti-cystic, effects (Harris and Torres, 2009). The polycystin family of proteins also seems to have a role in the breaking of L-R symmetry during embryogenesis (Drummond, 2012). Situs defects and ectopic or absent expression of key L-R genes are evident in mice harbouring mutations in *Pkd2* or the *Pkd1*-related gene *Pkd1l1* (Pennekamp et al., 2002; Ermakov et al., 2009; Field et al., 2011); *Pkd1* itself seems to have no role in the L-R pathway (Karcher et al., 2005). It could be that a similar mechanism as in the kidney is also in operation in situs determination, namely the sensation of fluid flow by polycystin complexes. Indeed, an intracellular Ca^{2+} spike on the left side of the embryo occurs in a flow- and PKD2-dependent manner (McGrath et al., 2003). These observations support the 'two cilia hypothesis', which argues that embryo symmetry is broken by motile nodal cilia generating a leftward fluid flow that is then perceived by PKD1l1-PKD2 complexes in peripheral sensory cilia, thereby eliciting downstream chemical asymmetries.

lethality. Common MKS symptoms and signs include cystic kidneys, liver fibrosis and occipital encephalocele (Table 1). Other ciliopathy-associated defects are also occasionally reported, including neural tube (NT) defects, polydactyly, hydrocephalus, laterality defects, congenital heart malformations, cleft lip and palate, and underdeveloped male genitalia (reviewed in Logan et al., 2011). Ten human MKS loci have been identified (Table 2) and the proteins that they encode have all been associated functionally with the cilium. Notably, phenotypic (Table 1) as well as genotypic overlap exists between MKS and other ciliopathies, particularly Joubert syndrome (JBTS). For example, *TMEM216* mutations cause both MKS and JBTS (Valente et al., 2010), whereas mutations in *MKS1*, *TMEM67* and *CEP290* also underlie Bardet-Biedl syndrome (BBS). Although it is tempting to speculate that variation in allele strength underpins the syndromic outcome, clear evidence also exists for genetic modification of syndromes (Khanna et al., 2009). Mice carrying mutations in *Mks1*, *Tmem67*, *Cep290*, *Cc2d2a*, *Nphp3*, *Tctn2*, *B9d1* and *B9d2* now exist (Table 2). Some of these mutants seem to closely mirror human MKS. However, in most cases, more phenotypic analysis is required to fully assess whether they model the human syndrome.

To date, two of the best mouse models for human MKS are those carrying mutations in the *Mks1* gene. The ENU-derived $Mks1^{krc}$ allele, a splice-site mutation, results in the loss of detectable *Mks1* transcript and presumably the protein (Weatherbee et al., 2009); intriguingly, an equivalent homologous mutation has been reported in humans with MKS (Frank et al., 2007). On a mixed genetic background, homozygotes arrested between 18.5 days post coitum (dpc) and shortly after birth, displaying a plethora of phenotypes highly similar to those observed in individuals with MKS: polydactyly, multiple bile duct formation, neural defects (including exencephaly), defects in supraoccipital bone formation, cystic kidneys, L-R patterning defects, pulmonary hypoplasia, hydrocephalus, genitourinary defects and, in one case, cleft palate (Weatherbee et al., 2009). $Mks1^{krc}$ mutants also displayed ovarian defects and a reduced presphenoid bone; for this reason, the authors of that study suggest that a detailed analysis of $Mks1^{krc}$ might define novel features of MKS. A second ENU-derived mutation ($Mks1^{del64\text{-}323}$), which deletes the probably important B9 domain of Mks1, prevents the protein from localising to its normal domain at the basal body and centrosome (Cui et al., 2011). Homozygous mutants demonstrated cleft lip, eye defects, polydactyly, heart malformations, L-R patterning defects including polysplenia or asplenia, kidney enlargement with cysts, liver cysts, and several skeletal abnormalities, which are all hallmarks of human MKS. The defects demonstrated by $Mks1^{del64\text{-}323}$ were stronger than those of $Mks1^{krc}$ mutants, perhaps implying that $Mks1^{del64\text{-}323}$ is a dominant-negative allele (Cui et al., 2011). Recently, a *B9d1*-null mouse has been described that exhibits MKS-like phenotypes, including large cystic kidneys, ductal plate malformations, polydactyly, occasional exencephaly and other hallmarks of ciliopathies, including dextrocardia (indicative of L-R patterning defects), holoprosencephaly and eye defects (Dowdle et al., 2011). Phenotypically, these mutants are all relatively good models of human MKS.

$Mks1^{krc}$, $Mks1^{del64\text{-}323}$ and *B9d1*-null mutants were all found to exhibit very few cilia on the embryonic node, NT and limb bud mesenchyme, whereas tracheal and bile duct cilia numbers were unaffected (Weatherbee et al., 2009; Cui et al., 2011; Dowdle et al., 2011). The node cilia defects probably underpin the L-R patterning abnormalities, whereas disrupted Hh signal transduction probably underlies both NT and limb patterning defects. Analysis in human cells showed that MKS1 is required for docking the basal body to the apical actin cytoskeleton (Dawe et al., 2007). However, basal body docking proved to be normal in $Mks1^{krc}$ mutants (Weatherbee et al., 2009). The reason for this discrepancy is not clear, but might reflect differences between mouse and human cells, or perhaps differences in the cell types assessed. Embryonic fibroblasts isolated from *B9d1*-null mice possess structurally normal cilia but still demonstrate reduced Hh signalling capacity, and a number of proteins that normally localise to cilia failed to localise to *B9d1*-deficient cilia (Dowdle et al., 2011). Thus, B9d1 is not only required for ciliogenesis in some contexts but also for proper protein localisation to the ciliary compartment. Similar findings have recently been made for Tctn2 (also known as MKS8), Tmem67

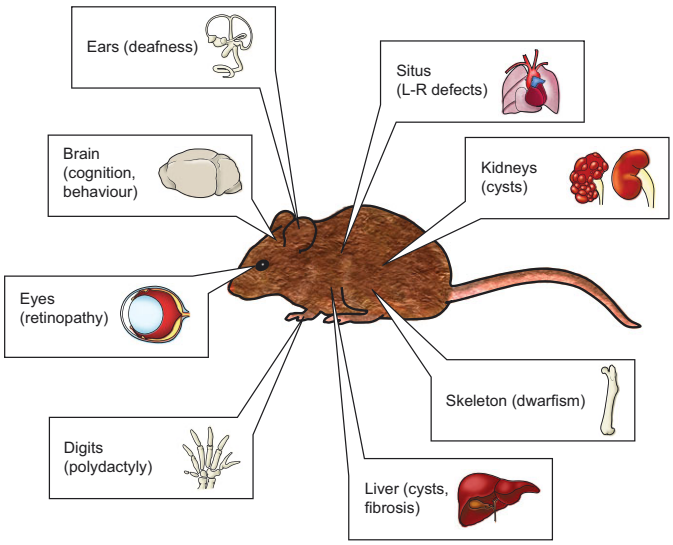

Fig. 2. Phenotypes of ciliopathies. Postnatal manifestations of ciliopathies reflect the pleiotropic nature of these disorders, which influence multiple tissues and functions. Sensory defects are evident in both hearing and sight; cilia are required for correct polarisation of sensory hair cells in the organ of Corti and for making and regenerating the outer segment of photoreceptor cells. Coloboma and microphthalmia are seen in certain ciliopathies, probably reflecting defective Hh signalling. Hh signalling also impacts limb patterning, and ciliopathy-associated defects give rise to polydactyly and shortening of the limbs, leading to dwarfisms. Human ciliopathies are known to result in cognitive impairment, a symptom that is most likely to manifest as behavioural changes in the mouse. Anatomical changes to brain structure are evident both pre- and postnatally, reflecting alterations in neurodevelopment. Hypothalamic cilia in the brain play a role in appetite and obesity. Kidney cilia defects result in polycystic kidney disease (PKD; Box 2), a phenotype that is most severe when the defects are present embryonically or immediately postnatally. Similar cystic phenotypes are reported in both the pancreas and liver, often seen together with fibrosis. PKD is believed to result from a requirement for cilia in flow detection within kidney tubules. A similar mechanism of cilia-dependent flow detection is argued to act during embryogenesis, during which it influences situs (L-R patterning); defective situs is a common indicator of ciliopathies.

(MKS3) and Cc2d2a (MKS6) (Garcia-Gonzalo et al., 2011), implying that MKS-associated proteins might have roles in ciliary protein import as well as in promoting ciliogenesis.

Orofaciodigital syndrome

Orofaciodigital syndrome (OFDS; OMIM: 311200), as its name suggests, encompasses oral, facial and digital defects. Oral and facial malformations include lingual hamartomas, accessory oral frenula, cleft palate, bifid or trifid tongue, telecanthus or hypertelorism, cleft lip, and alar hypoplasia or notching. Poly- and syndactyly, as well as camptodactyly, make up the digital component of OFDS (Toriello, 2009). Up to 13 OFDS loci have been argued to exist (Toriello, 2009), but of these, only the *OFD1* locus has been identified (Ferrante et al., 2001). *OFD1* is an X-linked gene and mutations are usually lethal in males; only heterozygous females exhibit the spectrum of OFDS defects. There is highly variable expressivity, even within single families, the cause of which is unknown. Notably, the human *OFD1* locus is reported to escape X-inactivation (de Conciliis et al., 1998), meaning that variability cannot be attributed to the randomness of X-inactivation. As well as the standard triad of defects, brain malformation and polycystic kidneys are also commonly seen in OFDS (Toriello, 2009).

An *Ofd1*-null mouse has been engineered. However, both hemizygous male and heterozygous female embryos or pups fail to survive, meaning that it cannot be bred. This has been overcome by use of a conditional allele that is deleted preimplantation, permitting analysis of both heterozygous females and hemizygous males (Ferrante et al., 2006). Male embryos (completely lacking gene function) were small, did not turn and died by ~11.5 dpc, although development had arrested 1-2 days earlier. A striking pericardial oedema was evident, accompanied by randomisation or failure of heart looping, suggesting defective L-R patterning. These effects were mediated in part through defective Hh signalling, as assayed by NT, bone and limb patterning; absence of nodal cilia underlies the L-R patterning defects (Ferrante et al., 2006; Bimonte et al., 2011). In contrast to the situation in humans, the mouse *Ofd1* locus undergoes random X-inactivation, resulting in clones of cells with activity and others lacking activity (Ferrante et al., 2003). Heterozygous $Ofd1^{-/+}$ females died at birth, demonstrating what might be argued to be an extreme version of the human syndrome. The pups were small, with craniofacial defects including cleft palate, which possibly underlies the lethality. Brain disorganisation, reduced lung size, cardiac great vessel defects and cystic kidneys were also reported (Ferrante et al., 2006). In female mutants, cilia were missing from the cells within kidney cysts, whereas adjacent cells were ciliated; it seems likely that this reflects X-inactivation clones (Ferrante et al., 2006). However, this loss of cilia phenotype is not shared by all cell types, because the node remained ciliated in mutant embryos; close inspection of the published images did suggest that some of these cilia might be dysmorphic. However, the incidence of many aspects of the human syndrome have not been explicitly investigated, meaning that the quality of this mouse as a model of OFDS remains unclear.

Joubert syndrome

First described by Marie Joubert, Joubert syndrome (JBTS; OMIM: 213300) is an autosomal recessive disorder that comprises mental retardation, abnormal breathing, atypical eye movements and ataxia in association with agenesis of the cerebellar vermis (Sattar and Gleeson, 2011). Central to its identification has become the 'molar tooth sign', identifiable through brain imaging (Parisi, 2009). This reflects the neurodevelopmental defects that underlie this condition. Both retinal dystrophy and renal anomalies are also associated with a proportion of JBTS cases (Sattar and Gleeson, 2011). Genetically, 15 loci have been identified that are argued to cause JBTS (Table 2); as for MKS, many of these have also been identified in other ciliopathies.

In the mouse, homozygous loss of *Inpp5e*, the *JBTS1* locus, resulted in lethality late in development, associated with polydactyly, cystic kidneys, anophthalmia, decreased bone ossification and exencephaly (Jacoby et al., 2009); all are indications of a severe ciliopathy. This was accompanied not by cilia loss but by deformation. Postnatal deletion of an *Inpp5e* conditional allele resulted in obesity and low-level cystic kidneys (Jacoby et al., 2009), similar to the effects of postnatal cilia loss through other loci (Davenport et al., 2007). Although it is possible that a very specific

Table 2. Mouse mutants of ciliopathy genes

Locus (protein)	Mutants	Type	Viability	Key references
Alström				
Alms1 (Alms1)	*Alms1*^{foz}	Spontaneous	AV	Arsov et al., 2006b
	Alms1^{L2131X}	ENU – truncation at exon 10	AV	Li et al., 2007
	Alms1$^{Gt(XH152)Byg}$	Gene trap – hypomorphic	AV	Collin et al., 2005
BBS				
Bbs1 (Bbs1)	*Bbs1*^{GT1NK}	Gene trap – probably null	EL, AV	Kulaga et al., 2004
	Bbs1^{tm1Vcs} (M390R)	Targeted knock-in	AV	Davis et al., 2007
Bbs2 (Bbs2)	*Bbs2*^{tm1Vcs}	Targeted – null	AV	Nishimura et al., 2004
Bbs3 (Arl6)	*Arl6*^{tm1Vcs}	Targeted deletion – transcript specific	AV	Pretorius et al., 2010
Bbs4 (Bbs4)	*Bbs4*^{Gt1NK}	Gene trap	EL, birth, AV	Kulaga et al., 2004
	Bbs4^{tm1Vsc}	Targeted – null	EL, birth, AV	Mykytyn et al., 2004
Bbs5 (Bbs5)	None			
Bbs6 (Mkks)	*Mkks*$^{Gt(0ST367255)Lex}$	Gene trap	EL, AV	Ross et al., 2005
	Mkks^{tm1Vcs}	Targeted – null	EL, AV	Fath et al., 2005
Bbs7 (Bbs7)	*Bbs7* null[a]	Targeted	ND	Zhang et al., 2012
Bbs8 (Tct8)	*Tct8*^{tm1Reed}	Targeted reporter – null	AV	Tadenev et al., 2011
Bbs9 (Bbs9)	None			
Bbs10 (Bbs10)	None			
Bbs11 (Trim32)	*Trim32*$^{Gt(BGA355)Byg}$	Gene trap – probably null	AV	Kudryashova et al., 2009
	Trim32^{tm1Spc}	Targeted knock-in – hyopmorphic	AV	Kudryashova et al., 2011
Bbs12 (Bbs12)	None			
Bbs13 (Mks1)	*Mks1*^{krc}	ENU – probably null	EL	Weatherbee et al., 2009
	Mks1$^{del64-323}$	ENU – possible dominant negative	EL	Cui et al., 2011
Bbs14 (*Cep290*)	*Cep290*^{rd16}	Spontaneous	AV	Chang et al., 2006
	Cep290^{tm1Jgg}	Targeted – null	AV	Lancaster et al., 2011
Bbs15 (Wdpcp)	None			
Bbs16 (Sdccag8)	None			
JBTS				
Jbts1 (Inpp5e)	*Inpp5e*$^{tm1.1Ssch}$	Targeted conditional – null	EL (global KO)	Jacoby et al., 2009
Jbts2 (Tmem216 and Tmem138)	None			
Jbts3 (Jouberin)	*Ahi1*^{tm1Jgg}	Targeted – null	PNL (80%)	Lancaster et al., 2009; Lancaster et al., 2011
	Ahi1^{tm1Rujf}	Targeted reporter – null	ND	Hsiao et al., 2009; Westfall et al., 2010
Jbts4 (Nphp1)	*Nphp1*$^{tm1.1Hung}$	Targeted – null	AV	Jiang et al., 2008
	Nphp1^{tm1Jgg}	Targeted – null	AV	Louie et al., 2010
Jbts5 (Cep290)	*Cep290*^{rd16}	Spontaneous	AV	Chang et al., 2006
	Cep290^{tm1Jgg}	Targeted – null	AV	Lancaster et al., 2011
Jbts6 (Tmem67)	*Tmem67*^{tm1Dgen}	Targeted reporter	PNL	Garcia-Gonzalo et al., 2011
Jbts7 (Rpgrip1l)	*Rpgrip1l*^{tmUrt}	Targeted – null	Birth	Vierkotten et al., 2007
Jbts8 (Arl13b)	*Arl13b*^{hnn}	ENU – null	EL	Caspary et al., 2007
Jbts9 (Cc2d2a)	*Cc2d2a*$^{Gt(AA0274)Wtsi}$	Gene trap – null	EL	Garcia-Gonzalo et al., 2011
Jbts10 (Ofd1)	*Ofd1*$^{tm2.1Bfra}$	Targeted conditional – null	Male: EL; female: birth	Ferrante et al., 2006
Jbts11 (Ttc21b)	*Ttc21b*^{aln}	ENU – probably null	EL	Tran et al., 2008
Jbts12 (Kif7)	*Kif7*$^{tm1.2Hui}$	Targeted – null	Birth	Cheung et al., 2009
	Kif7^{maki}	ENU	EL (late)	Liem et al., 2009
Jbts13 (Tctn1)	*Tctn1*$^{Gt(KST296)Byg}$	Gene trap – null	EL	Reiter and Skarnes, 2006
Jbts14 (Tmem237)	None			
Jbts15 (Cep41)	*Tsga14*$^{Gt(AW0157)Wtsi}$	Gene trap – null	EL	Lee et al., 2012

Table 2 continued on next page.

Table 2. Continued

Locus (protein)	Mutants	Type	Viability	Key references
Jeune				
Atd1 (Atd1)	None			
Atd2 (IFT180)	*Ift80*$^{Gt(ANO245)Wtsi}$	Gene trap – strong hypomorph	Mostly EL; some AV	Rix et al., 2011
Atd3 (Dync2h1)	*Dync2h1*$^{Gt(RRM278)Byg}$	Gene trap	EL	Garcia-Garcia et al., 2005
	Dync2h1^{lln}	ENU	EL	Huangfu and Anderson, 2005
Atd4 (Ttc21b)	*Ttc21b*^{aln}	ENU – probably null	EL	Tran et al., 2008
Atd5 (Wdr19)	*Ift144*^{twt}	ENU – hypomorph	EL	Ashe et al., 2012
MKS				
Mks1 (Mks1)	*Mks1*$^{del64-323}$	ENU – dominant negative	EL	Cui et al., 2011
	Mks1^{krc}	ENU – probably null	EL	Weatherbee et al., 2009
Mks2 (Tmem216)	None			
Mks3 (Tmem67)	*Tmem67*^{tm1Dgen}	Targeted reporter	PNL	Garcia-Gonzalo et al., 2011
Mks4 (Cep290)	*Cep290*^{rd16}	Spontaneous	AV	Chang et al., 2006
	Cep290^{tm1Jgg}	Targeted – null	AV	Lancaster et al., 2011
Mks5 (Rpgrip1l)	*Rpgrip1l*^{tmUrt}	Targeted – null	Birth	Vierkotten et al., 2007
Mks6 (Cc2d2a)	*Cc2d2a*$^{Gt(AA0274)Wtsi}$	Gene trap – null	EL	Garcia-Gonzalo et al., 2011
Mks7 (Nphp3)	*Nphp3*^{pcy}	Hypomorphic	AV	Olbrich et al., 2003
	Nphp3^{tm1Cbe}	Targeted – null	EL	Bergmann et al., 2008
Mks8 (Tctn2)	*Tctn2*$^{tm1.1Reit}$	Targeted – null	EL	Garcia-Gonzalo et al., 2011; Sang et al., 2011
Mks9 (B9d1)	*B9d1*$^{tm1a(EUCOMM)Wtsi}$	Gene trap – null	EL	Dowdle et al., 2011
Mks10 (B9d2)	*B9d2/Tgfb1*^{tm1Flv} *(Stumpy)*	Targeted deletion	ND	Town et al., 2008
NPHP				
Nphp1 (Nphp1)	*Nphp1*$^{tm1.1Hung}$	Targeted – null	AV	Jiang et al., 2008
	Nphp1^{tm1Jgg}	Targeted – null	AV	Louie et al., 2010
Nphp2 (Invs)	*Invs*^{Inv}	Random insertion	AV	Yokoyama et al., 1993
Nphp3 (Nphp3)	*Nphp3*^{pcy}	Hypomorphic	AV	Olbrich et al., 2003
	Nphp3^{tm1Cbe}	Targeted – null	EL	Bergmann et al., 2008
Nphp4 (Nphp4)	*Nphp4*^{nmf192}	ENU	AV	Won et al., 2011
Nphp5 (Iqcb1)	None			
Nphp6 (Cep290)	*Cep290*^{rd16}	Spontaneous	AV	Chang et al., 2006
	Cep290^{tm1Jgg}	Targeted – null	AV	Lancaster et al., 2011
Nphp7 (Glis2)	*Glis2*^{tm1Amj}	Targeted – null	AV	Kim et al., 2008
	Glis2^{tm1Tre}	Targeted reporter	AV	Attanasio et al., 2007
Nphp8 (Rpgrip1l)	*Rpgrip1l*^{tmUrt}	Targeted – null	Birth	Vierkotten et al., 2007
Nphp9 (Nek8)	*Nek8*^{jck}	Possible hypomorph	AV	Liu et al., 2002
Nphp10 (Sdccag8)	None			
Nphp11 (Tmem67)	*Tmem67*^{tm1Dgen}	Targeted reporter	PNL	Garcia-Gonzalo et al., 2011
Nphp12 (Ttc21b)	*Ttc21b*^{aln}	ENU – probably null	EL	Tran et al., 2008
OFD				
Ofd1 (Ofd1)	*Ofd1*$^{tm2.1Bfra}$	Targeted conditional – null	Male: EL Female: birth	Ferrante et al., 2006

[a]*Bbs7*$^{-/-}$ mutants are reported in Zhang et al., 2012; the nature of the allele is not clear.

BBS, Bardet-Biedl syndrome; JBTS, Joubert syndrome; MKS, Meckel-Grüber syndrome; NPHP, nephronophtisis; OFD, orofaciodigital syndrome; EL, embryonic lethal; AV, adult viable; PNL, postnatal lethal; ND, not determined; KO, knock out; blue, gene traps and targeted alleles; red, chemical mutagenesis; green, spontaneous.

deletion at a certain time or in certain tissues might accurately model JBTS, it seems more likely that a hypomorphic allele would be required. Quite strikingly, most null alleles for JBTS loci follow this same pattern: they are embryonic lethal and result in classic, severe ciliopathy phenotypes (Table 2).

By contrast, targeted deletion of the *JBTS3* locus in mice (*Ahi1*; encoding the Jouberin protein) more closely models aspects of the human syndrome. Importantly, homozygous null mice survive to birth, although they show runting and the majority (80%) do not survive to adulthood. Those that do survive develop

nephronophthisis (NPHP) (Lancaster et al., 2009). In addition, between 2 to 4 weeks of age they demonstrate defects in retinal outer segment development and photoreceptor apoptosis, resulting from abnormal opsin distribution (Louie et al., 2010), a phenotype associated with NPHP. This is in part explained by the fact that Jouberin and Nphp1 proteins interact (Eley et al., 2008), a finding supported by evidence of genetic interactions in the eye (Louie et al., 2010). A second targeted *Ahi1*-null allele has been studied and is reported to exhibit reduced levels of Rab8a in photoreceptors (Hsiao et al., 2009; Westfall et al., 2010); Rab8a is required for vesicle trafficking from post-Golgi membranes to the plasma membrane near the ciliary transition zone. Some outer segment proteins are also reduced in *Ahi1*-null mice, suggesting that the retinal degeneration phenotype results from defective transport and protein stabilisation in photoreceptors (Westfall et al., 2010). Perhaps the most significant element of JBTS is the neural defects. Re-analysis of the *Ahi1*-null mouse revealed a reduced cerebellum and underdeveloped vermis, which is similar but much less severe than observed in individuals with JBTS (Lancaster et al., 2011). Cell-based assays supported a role for Jouberin in Wnt signalling (Lancaster et al., 2009). Subsequent analysis in the *Ahi1*-null using an in vivo reporter revealed reduced midline Wnt signalling. Reduced cell proliferation, consistent with the known role of Wnt proteins as mitogens, was also detected. Rescue of both the morphological and proliferative defects, using a Wnt agonist, provided strong evidence for the role of Wnt signalling in JBTS (Lancaster et al., 2011).

Two mouse mutants for *Cep290* (*Jbts5*) have been characterised. The first carries the retinal degeneration 16 (*Cep290*^{rd16}) allele, an in-frame deletion of 298 residues of the 2472 amino acid protein (Chang et al., 2006). The second mutant, *Cep290*^{tm1jgg}, carries an engineered null allele (Lancaster et al., 2011). Both mutations are adult viable, but demonstrate early-onset retinal degeneration in addition to some degree of runting. Anosmia is reported for *Cep290*^{rd16} mice (McEwen et al., 2007), a phenotype not discussed in the *Cep290*^{tm1jgg} analysis. Strikingly, mice carrying the null but not the *Cep290*^{rd16} allele also demonstrate cerebellar hypoplasia, with midline-vermis fusion, highly similar to the *Ahi1*-null phenotype (Lancaster et al., 2011).

The degree to which all aspects of JBTS have or even can be modelled in mouse is difficult to assess. The molar tooth sign itself might prove specific to humans, but of course this is a diagnostic indicator, so its absence might not be significant in modelling the syndrome. Differences in the nature of the normal human and mouse cerebellum could be more significant, with the far more complex nature in humans providing greater scope for defects and phenotypes. Further phenotypic analysis of behavioural phenotypes could prove fruitful in beginning to answer such questions.

Jeune syndrome

Jeune syndrome (OMIM: 208500), also known as asphyxiating thoracic dysplasia (ATD), is a chondrodysplasia that results in a constricted rib cage, polydactyly, short limbs and short stature. A high level of infant mortality is seen, resulting from respiratory insufficiency. Children that survive usually develop kidney cysts and liver defects including fibrosis (Oberklaid et al., 1977). Retinal degeneration and pancreatic cysts are also seen (Keppler-Noreuil et al., 2011). Five human ATD loci have been identified (Table 2). Hypermorphic alleles of the IFT-associated genes *IFT80*, *TTC21B*, *DYNC2H1* and *WDR19* (also known as *IFT144*) underlie ATD2, 3, 4 and 5, respectively, whereas the locus mutated in ATD1 remains to be identified. Notably, *TTC21B* mutations also underlie some NPHP cases (Table 2).

Mouse mutants exist for *Ift80*, *Ttc21b*, *Dync2h1* and *Wdr19*. A gene-trap allele of *Ift80* is argued to be a strong hypomorph, showing low levels of wild-type transcript (Rix et al., 2011). This allele results in a high level of embryonic lethality, but on rare occasions mice survive past birth. These survivors demonstrate shortening of the long bones, rib-cage constriction and polydactyly; situs inversus, kidney cysts and retinal degeneration were explicitly screened for but not detected. Examination of ciliation and Hh signalling in fibroblasts revealed apparently normal cilia but reduced signalling (Rix et al., 2011). This mutant models many aspects of ATD, but is difficult to analyse owing to high rates of lethality. The presence of some survivors raises the question of whether random segregation of genetic background might contribute to the variability, but these details are not published.

Mice carrying ENU-derived point-mutant and gene trap *Dync2h1* alleles have been analysed (Table 2). Both mutants exhibit a very severe ciliopathy phenotype with early lethality resulting from abnormal ciliogenesis and the associated defects in Hh signalling (Garcia-Garcia et al., 2005; Huangfu and Anderson, 2005). Similarly, the ENU-derived *Ttc21b*^{aln} point mutant shows a severe ciliopathy, dying mid-gestation (Tran et al., 2008). This is a presumed null allele, because protein is undetectable in mutants. *Ttc21b*^{aln} embryos exhibit cilial abnormalities caused by defective retrograde IFT that results in faulty Hh signalling. The cilia themselves show a bulb-like thickening at the tip and accumulation of IFT88 protein (Tran et al., 2008). Clearly, these mutants do not model Jeune syndrome, and it seems reasonable to speculate that a first step towards generating a model would be to engineer weaker alleles – global hypomorphs, patient-associated point mutations or specific conditional deletions.

Bardet-Biedl syndrome

Bardet-Biedl syndrome (BBS; OMIM: 209900) is characterised by features that include cognitive impairment, obesity, retinal degeneration, anosmia, cystic kidneys and polydactyly; abnormal situs is also occasionally seen (Tobin and Beales, 2007; Zaghloul and Katsanis, 2009). BBS is genetically heterogeneous, with 16 loci having been identified in humans (Table 2). Initially argued to be genetically recessive, evidence of triallelic inheritance or strong genetic modification of penetrance has been reported (Burghes et al., 2001; Katsanis et al., 2001). However, this remains controversial, as evidenced by a recent study of 29 families that found no evidence of complex genetic inheritance (Abu-Safieh et al., 2012). Similarly to loci associated with MKS and JBTS, BBS loci can underlie multiple ciliopathies (Table 2). Proteins encoded by the BBS loci localise primarily to the ciliary basal body and axoneme (Zaghloul and Katsanis, 2009); many of them are components of the so-called BBSome, a protein complex argued to be involved in cilial targeting (Jin and Nachury, 2009; Jin et al., 2010).

Mouse mutants exist for a number of BBS loci (Table 2). Two *Bbs1* alleles have been analysed. The gene-trap *Bbs1*^{GT1Nk} results in loss of detectable mRNA (Kulaga et al., 2004). Approximately half of the homozygotes die in utero, with the remainder demonstrating runting at birth. By 10 weeks of age, 10% are obese

and 30% exhibit retinal degeneration, clearly modelling aspects of the human syndrome. However, polydactyly, and renal, liver and situs defects were not reported for *Bbs1*^{GT1Nk} mutants (Kulaga et al., 2004). Defects were evident in olfactory protein localisation (Kulaga et al., 2004), but olfaction itself has not been tested in these mice. Subsequently, it has emerged that there are also defects in neuronal migration in *Bbs1*^{GT1Nk} mice (Ishizuka et al., 2011). The second allele (*Bbs1*^{M390R}) specifically models the common M390R human mutation (Davis et al., 2007). Aspects of the human syndrome exhibited by this mutant include retinal degeneration, male infertility and obesity resulting from hyperphagia; obese mice were hyperleptinaemic and demonstrated reduced locomotor activity. Ependymal cell cilia maintained a normal 9+2 ultrastructure, but some were elongated and abnormally swollen at the distal ends (Davis et al., 2007).

An engineered null allele of *Bbs2* (*Bbs2*^{tm1Vcs}) is adult viable, but also results in small pups at birth, and retinal degeneration and obesity (with hyperphagia) by a few weeks of age (Nishimura et al., 2004). In addition, cystic kidneys were evident in *Bbs2*^{tm1Vcs} mice, a BBS phenotype not reported in *Bbs1* mutants. Defective sperm flagellation, resulting in male infertility, was also reported. Olfaction, when examined, was found to be defective (Nishimura et al., 2004).

Two *Bbs4* alleles have been analysed (Table 2). Mice carrying a gene-trap (*Bbs4*^{Gt1Nk}) or an engineered null (*Bbs4*^{tm1Vcs}) allele both showed pre- or perinatal lethality (Kulaga et al., 2004; Mykytyn et al., 2004). Pups were runted at weaning, but became obese with age, demonstrating aspects of metabolic disease including fatty liver in the engineered null (Kulaga et al., 2004; Mykytyn et al., 2004). Retinal degeneration was also evident in mutants, with greater loss of cone than rod function (Mykytyn et al., 2004; Eichers et al., 2006). Furthermore, *Bbs4*^{Gt1Nk} and *Bbs1*^{M390R} exhibited reduced social dominance (Eichers et al., 2006; Davis et al., 2007). Although no renal defects were initially reported, subsequent analysis has revealed cystic kidneys in *Bbs4*^{tm1Vcs} mice (Guo et al., 2011), but polydactyly was not exhibited (Mykytyn et al., 2004; Eichers et al., 2006).

Recently, various studies have examined multiple Bbs mutants in parallel for additional phenotypes. It has thus emerged that mice null for *Bbs2*, *Bbs4* or *Bbs6* (Table 2) are resistant to leptin activity, suggesting a putative mechanism underlying obesity in BBS (Seo et al., 2009). When tracheal cilia were examined in *Bbs1*, *Bbs2*, *Bbs4* and *Bbs6* mutant mice, they exhibited terminal bulges and abnormal beat frequencies (Shah et al., 2008); it remains unknown to what extent this models a phenotype seen in humans with BBS. Similarly, morphological evaluation of brain neuroanatomy revealed ventriculomegaly of the lateral and third ventricles, thinning of the cerebral cortex, and reduced volume of the corpus striatum and hippocampus (Davis et al., 2007). Again, the relevance of this phenotype to individuals with BBS is presently unclear.

Alström syndrome

Alström syndrome (ALMS; OMIM: 203800) is a rare ciliopathy (~50 diagnosed cases in the UK) characterised by cone-rod dystrophy leading to blindness, childhood obesity, hearing loss, hyperinsulinaemia, type 2 diabetes and dilated cardiomyopathy, together with pulmonary, renal, urological and hepatic dysfunction and chronic respiratory tract infections (reviewed in Marshall et al., 2011a). This autosomal recessive disorder is caused by *ALMS1* mutations, of which ~100 are known (Marshall et al., 2011b). *ALMS1* is a 23 exon gene that is thought to be transcribed as multiple splice variants that might have different cellular functions. The gene is expressed widely in the tissues that are affected in ALMS, including photoreceptors, liver, pancreatic islet cells, organ of Corti, renal tubules and the hypothalamus. Disease-causing *ALMS1* mutations are typically nonsense and frameshift mutations.

There are a multitude of *Alms1* mouse mutants, including ENU mutants, gene-trapped alleles and spontaneous mutations (Table 2). The *Alms1*^{foz} (fat aussie) mutant, harbouring a spontaneous 11 bp deletion in exon 8, displays impaired hearing in aged mice, obesity (probably caused by hyperphagia), type 2 diabetes, dilated renal tubules and fibrotic liver (Arsov et al., 2006a; Arsov et al., 2006b; Larter et al., 2009). Male *Alms1*^{foz} mice are sterile owing to germ cell loss and spermatid flagellation deficiencies. Furthermore, cilia in renal tubules are lost in aged *Alms1*^{foz} mice. An ENU-induced mutation (*Alms1*^{L2131X}) causing a nonsense mutation in exon 10 is a weaker allele but still causes renal cilia loss with aging, as well as obesity, type 2 diabetes, defective sperm formation and steatosis. Hearing was not assessed in *Alms1*^{L2131X} mutants, but vision was found to be impaired, with defects in rhodopsin transport (Li et al., 2007). An *Alms1* gene-trapped mouse model, *Alms1*$^{Gt(XH152)Byg}$ (Table 2), also exhibits some classical ALMS features (Collin et al., 2005; Jagger et al., 2011), including progressive photoreceptor degeneration, obesity, hyperglycaemia, hypogonadism in males, enlarged kidneys and hepatic fibrosis (Collin et al., 2005). ALMS1 localises to the basal body of the kinocilium in the cochlea, and *Alms1*$^{Gt(XH152)Byg}$ mutants exhibit abnormalities of the stereociliary bundles as well as lesions in the stria vascularis, and progressive loss of sensory and intermediary cells (Jagger et al., 2011). Intriguingly, ALMS1 remains localised to the centrosome in cells lacking cilia, perhaps indicating non-ciliary roles for the protein (Jagger et al., 2011).

Nephronophthisis

Nephronophthisis (NPHP; OMIM: 256100) is an autosomal recessive nephropathy and the most prevalent genetic disorder causing end-stage renal disease (ESRD) in juveniles. Individuals with NPHP type 1 develop polyuria, polydipsia, anaemia and secondary enuresis; ESRD manifests at an average age of 13 years, although symptoms rarely become apparent before 6 years. A rare infantile form of the disease causes ESRD by 5 years. Many individuals with NPHP exhibit extra-renal symptoms, including retinitis pigmentosa, oculomotor apraxia, coloboma, polydactyly, skeletal dysplasia, short ribs, encephalocele and liver fibrosis, as well as occasional situs inversus, cardiac malformations and bronchiectasis (Wolf and Hildebrandt, 2011). To date, twelve loci have been implicated in NPHP, many of which also underlie other ciliopathies (Table 2). Mouse mutants are available for ten of the loci; only *Iqcb1* and *Sdccag8* have not been mutated, although mutant ES cell lines are available.

The *Nphp1* locus has been mutated (Jiang et al., 2008; Jiang et al., 2009; Louie et al., 2010), although mice carrying the alleles produced (null alleles) exhibit retinitis and defects in spermatogenesis, but not cystic kidneys. This difference in phenotype might be due to more complex genetics in human NPHP (Louie et al., 2010) or to species-specific differences in gene

function. The *NPHP9* locus encodes the serine-threonine kinase gene *NEK8*. The *Nek8*^{jck} mouse, a putative hypomorph (Liu et al., 2002), develops cystic kidney disease, progressively worsening to renal failure and death at ~6 months of age. The missense mutation encoded by *Nek8*^{jck} is a glycine-to-valine change in an RCC repeat; missense mutations in *NEK8* RCC repeats have also been found in humans with NPHP (Otto et al., 2008), suggesting that *Nek8*^{jck} might model some cases of human NPHP at the sequence level as well as phenotypically.

An *Nphp3* loss-of-function mutation in mouse (*Nphp3*^{tm1Cbe}; Table 2) results in congenital heart defects, situs inversus and mid-gestation embryonic lethality; this probably models severe truncating *NPHP3* mutations, which cause a spectrum of early patterning defects in humans that resemble those seen in MKS (Bergmann et al., 2008). The phenotype of the *Nphp3*^{pcy} cystic kidney mouse model (probably a hypomorphic *Nphp3* allele) closely resembles the renal pathology of adolescent NPHP patients (Olbrich et al., 2003; Bergmann et al., 2008). Interestingly, the *Nphp3*^{pcy} mouse has been used in cystic kidney treatment studies; it is responsive to treatment by a vasopressin V2 receptor antagonist (Gattone et al., 2003).

Infantile NPHP, caused by mutations in *NPHP2* (*INVS*), is characterised by ESRD by 5 years, and has been associated with situs inversus and cardiac malformations (Salomon et al., 2009). Retinitis pigmentosa is also occasionally associated with *NPHP2* mutations (O'Toole et al., 2006). The *Invs*^{inv} mutation, generated by random insertional mutagenesis, causes situs inversus (Yokoyama et al., 1993) owing to defective fluid flow at the embryonic node (Okada et al., 1999). Additional roles in cardiac morphogenesis, independent of global L-R asymmetry determination, have also been argued (McQuinn et al., 2001). Furthermore, kidneys of *Invs*^{inv} mutant mice exhibit defects including cyst formation and a delay in tubular maturation (Yokoyama et al., 1993; Sugiyama and Yokoyama, 2006). The early lethality of this mouse mutant detracts from its utility as a model, although it has proven to be an excellent tool.

The ENU-derived mutant *Nphp4*^{nmf192}, containing a nonsense mutation in exon 4, has proven a useful tool in understanding the eye defects of individuals with NPHP. Homozygous mutants develop photoreceptor degeneration by 9 weeks of age; outersegments fail to develop properly, whereas synaptic ribbons seem to degenerate at postnatal day 14 (Won et al., 2011). These defects manifest in the presence of a structurally normal connecting cilium in mutants. However, homozygous *Nphp4*^{nmf192} mutants exhibit no renal phenotypes; thus, this mouse should be viewed as a valuable tool for elucidating the role of *Nphp4* in the retina, but not as a model of human NPHP.

Translational prospects

The obvious utility of mouse ciliopathy models is in understanding the cellular and molecular basis of the defects. Moreover, models allow the progression of diseases to be systematically followed in a highly controlled manner, both pre- and postnatally, an analysis that is not possible to assess in humans. It is hoped that applying knowledge gained through the study of mouse models will reveal targeted approaches to treatment using existing or novel therapies. Where human defects can be rescued postnatally through gene therapy, treating models will present an important stepping stone in therapy development. Human mutations that introduce premature stop codons, leading to protein truncation, could in theory be treatable using readthrough-inducing drugs (Zingman et al., 2007), a strategy that can also be successfully explored in models.

The search for novel therapies to treat ciliopathies will also progress through more standard drug discovery methods. For example, high-throughput assays using cultured cells or simpler organisms maintained in multi-well systems allow libraries of putative drugs to be screened. Although it is possible that cell lines derived from mouse models of ciliopathies could be used for such approaches, this is by no means required for drug development. The main use of mouse models will be in subsequent whole animal testing of putative drugs that emerge from any approach. Such models represent an important stage in therapy development, providing a proof of principle of true efficacy as well as safety in vivo.

Perspectives

It is clear from the mutants we have discussed that a null allele is not necessarily a good model of a disease. Exceptions do exist, however; for example, the *B9d1*-null mouse models many aspects of human MKS (Dowdle et al., 2011). However, in most cases, complete loss of a ciliopathy-associated gene leads to early lethality in the mouse. Human ciliopathy symptoms lie on a spectrum related to the strength and nature of the genetic mutation. The fact that mutations in *CEP290* underlie BBS, MKS and NPHP underscores the fact that different mutations at the same locus can result in distinctive syndromic outcomes (Table 2). Thus, to model most ciliopathies, we will need hypomorphic alleles with a range of strengths (although a minority will also require the development of dominant-negative alleles). A range of alleles from various sources is now available to the community or can be readily generated. Many gene traps are simple null alleles, but a proportion have proved to be hypomorphic (Lee et al., 2007). The engineering of human point mutations in the mouse [e.g. *Bbs1*^{M390R} (Davis et al., 2007)] is technically demanding but has proved successful and has the advantage of explicitly modelling specific patient mutations. The use of ENU mutagenesis, which randomly incorporates point mutations into the genome, has been successful in generating various allele types (Acevedo-Arozena et al., 2008). Largely, identification of these mutants has resulted from phenotype-driven screens, although the systematic screening of banks of ENU-mutagenised DNA linked to parallel frozen sperm archives presents the opportunity to screen large numbers of mutant mice in potentia (Quwailid et al., 2004; Acevedo-Arozena et al., 2008). The systematic sequencing and databasing of such mutagenised genomes will ultimately make this approach simpler and quicker (Gondo, 2008). In combination, these tools will allow isolation of a series of mutations of differing strengths and types. However, it must be remembered that mice and humans have anatomical, physiological and genetic differences, and that simple models might not always be achievable. The molar tooth sign of individuals with JBTS, for instance, seems unlikely to be recreated in the mouse.

Conditional mutagenesis is used more often as a tool to understand gene function than for the development of human disease models. Deleting a locus postnatally, or in a particular tissue, allows severe defects that are otherwise not survivable to be overcome. In the case of the *Ofd1* mouse, this approach has permitted transmission of the mutation (Ferrante et al., 2006) and

therefore its analysis. In other studies, conditional mutagenesis has been used to assess the role of ciliopathy loci postnatally (Davenport et al., 2007; Haycraft et al., 2007; Song et al., 2007; Jacoby et al., 2009). Although it is not currently obvious whether and how such techniques might elicit a disease model, conditional gene inactivation can nevertheless provide significant insight into molecular mechanisms and disease aetiology through the production of tools.

The importance of genetic background on the expressivity of ciliopathy mutant alleles is becoming increasingly evident. For example, homozygous embryos of the ENU mutant *Mks1*^{krc} die at around 13.5 dpc on a C3H background but survive up to 18.5 dpc on a mixed CD1/C3H background (Weatherbee et al., 2009). In our own analyses, we discovered that the gasping mutants (Ermakov et al., 2009) show differing timing of embryonic death on different background strains, with earlier death on B6J than C3H (D.P.N., Alexander Ermakov and Paraskevi Goggolidou, unpublished data). However, high-impact studies continue to be published that fail to reveal information on genetic background, a situation that needs to be rectified. With full sharing of background strain data on publication, together with greater systematic phenotypic analysis, it might be the case that particular genetic backgrounds will emerge as ciliopathic or anti-ciliopathic, either globally or for certain phenotypes. Such insight might allow targeted breeding to weaken or strengthen a phenotype. Indeed, it has already emerged that certain ciliopathic phenotypes manifest specifically on certain background strains, as illustrated by the *Tctn2*$^{tm1.1Reit}$ mutant (Sang et al., 2011).

The systematic broad-spectrum phenotyping of mouse mutants is becoming increasingly important as geneticists seek to fully characterise gene function. This is epitomised by projects including the German Mouse Clinic, the European Mouse Disease Clinic (EUMODIC) and the International Mouse Phenotyping Consortium (IMPC) (Fuchs et al., 2011; Gates et al., 2011), which are examples of large international endeavours aimed at high-throughput phenotyping of mouse mutants. Hand in hand with phenotyping, databases have been established that catalogue results as well as the specific assays performed; importantly, negative data are also made available (Morgan et al., 2010). Complete broad-spectrum phenotyping for every mutant is practically and financially beyond the capability of most individual labs. However, in smaller projects, there is a clear group of phenotypes that should be scored for putative ciliopathies (Fig. 2); whether or not such phenotypes have been analysed needs to be made clear to the community when studies are published. Examples provided in this review illustrate how multiple papers have slowly built complex phenotypes over time, aspects of which were either initially missed or not reported. Similarly, negative data are often not mentioned in published studies, leading others to question whether a phenotype was absent, missed or not assessed. Increased transparency and disclosure in the literature are imperative in more clearly defining the phenotypes of ciliopathy models and their similarity to the human diseases.

In conclusion, the continued improvement in modelling ciliopathies seems most likely to come from the combination of novel hypomorphic mutations and the careful control of genetic background. Systematic phenotyping, in combination with making all results available – be it in published studies or from large-scale phenotyping projects – will allow for an increasingly nuanced understanding of the contributions of different mutations to syndromic outcomes.

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COMPETING INTERESTS

The authors declare that they do not have any competing or financial interests.

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