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Unraveling the genetics of Joubert and Meckel-Gruber syndromes

Katarzyna Szymanska, Verity L. Hartill and Colin A. Johnson*
Department of Ophthalmology and Neuroscience, University of Leeds, Leeds, UK

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Abstract. Joubert syndrome (JBTS) and Meckel-Gruber syndrome (MKS) are recessive neurodevelopmental conditions caused by mutations in proteins that are structural or functional components of the primary cilium. In this review, we provide an overview of their clinical diagnosis, management and molecular genetics. Both have variable phenotypes, extreme genetic heterogeneity, and display allelism both with each other and other ciliopathies. Recent advances in genetic technology have significantly improved diagnosis and clinical management of ciliopathy patients, with the delineation of some general genotype-phenotype correlations. We highlight those that are most relevant for clinical practice, including the correlation between *TMEM67* mutations and the JBTS variant phenotype of COACH syndrome. The subcellular localization of the known MKS and JBTS proteins is now well-described, and we discuss some of the contemporary ideas about ciliopathy disease pathogenesis. Most JBTS and MKS proteins localize to a discrete ciliary compartment called the transition zone, and act as structural components of the so-called "ciliary gate" to regulate the ciliary trafficking of cargo proteins or lipids. Cargo proteins include enzymes and transmembrane proteins that mediate intracellular signaling. The disruption of transition zone function may contribute to the ciliopathy phenotype by altering the composition of the ciliary membrane or axoneme, with impacts on essential developmental signaling including the Wnt and Shh pathways as well as the regulation of secondary messengers such as inositol-1,4,5-trisphosphate (InsP3) and cyclic adenosine monophosphate (cAMP). However, challenges remain in the interpretation of the pathogenic potential of genetic variants of unknown significance, and in the elucidation of the molecular mechanisms of phenotypic variability in JBTS and MKS. The further genetic and functional characterization of these conditions is essential to prioritize patients for new targeted therapies.

Keywords: Joubert syndrome, Meckel-Gruber syndrome, primary cilium, transition zone

1. Introduction

The primary cilium is a microtubule-based organelle present as a single longitudinal protrusion on the apical surface of most vertebrate cells, where it acts as an "antenna" to receive and transduce chemo- and mechanosensory signals. Cilia are derived from the eldest of two centrioles of the centrosome, the cell's microtubule organizing centre. During quiescence, the microtubule organizing centre is redundant and the "mother centriole" can act at the cell surface to organize ciliogenesis, rendering this a cell cycle-dependent process. Primary cilia are regarded as cellular signaling hubs, regulating diverse signaling pathways, including Wnt, Shh, and Notch with particularly important roles during embryonic development and the patterning of the developing neural tube [1]. Defects in primary cilia are associated with a heterogeneous grouping of inherited developmental conditions known as the

*Corresponding author: Colin A. Johnson, PhD, Section of Ophthalmology and Neuroscience, Leeds Institute of Molecular Medicine, Wellcome Trust Brenner Building, St. James's University Hospital, Beckett Street, Leeds LS9 7TF, UK. Tel.: +44 (0) 113 343 8443; Fax: +44 (0) 113 343 8603; E-mail: c.johnson@leeds.ac.uk.

ciliopathies that often present with cystic kidney disease, and other diverse multi-organ phenotypes that affect the central nervous system, eye and skeleton [2]. As a group, ciliopathies, including autosomal dominant polycystic kidney disease, are comparatively common Mendelian inherited conditions with an overall estimated prevalence of 1 in 2,000 [3]. The importance of the primary cilium in health and genetic disease has only become apparent in the last decade, and the processes involved in cilia formation and maintenance remain poorly characterized.

Primary cilia have a complex ultrastructure with compartmentalization of molecular components that combine in functional modules. Components that are required for both the formation and function of the cilium have to be transported from the cytoplasm of the cell by the process of intraflagellar transport (IFT). Ciliopathies are caused by mutations in proteins that are structural or functional components of the primary cilium [2]. The loss of these components can disrupt ciliary functions such as the control of protein entry and exit from the cilium, the possible trafficking of essential ciliary components, and the regulation of signaling cascades and control of the cell cycle. Many proteins that are mutated in ciliopathies are localized to the transition zone (TZ), a compartment of the proximal region of the cilium [4, 5]. The TZ anchors the cilium to the plasma membrane, and is thought to act as a "ciliary gate" that both restricts and facilitates the movement of proteins and lipids in and out of the cilium. In particular, a protein complex at the TZ, known as the "MKS-JBTS module", contains many of the proteins mutated in Meckel-Gruber syndrome (MKS) and Joubert syndrome (JBTS) [6, 7].

In this review, we focus on JBTS and MKS as exemplar ciliopathies to provide an overview of their clinical diagnosis, management and molecular genetics. To date, mutations in 24 genes have been described as a cause of JBTS, and 13 for MKS. Both conditions therefore have extreme genetic heterogeneity, display allelism both with each other and other ciliopathies, and have marked variability of phenotypes. This has complicated the delineation of general genotype-phenotype correlations for JBTS and MKS, but we highlight the most relevant for clinical practice and those that provide insights into protein function. Finally, we describe some contemporary insights into the normal function of selected JBTS and MKS ciliary proteins, and discuss how their dysfunction impacts on pathogenic mechanisms in the ciliopathy disease state.

2. Diagnosis and management

JBTS is a rare autosomal recessive (AR) neurodevelopmental condition with a prevalence of 1:100,000 [8] and was first described by Marie Joubert in 1969 [9]. Typical cranial magnetic resonance imaging findings, hypotonia, and developmental delay or mental retardation are necessary for the diagnosis [9–11]. Other suggestive features include an irregular breathing pattern (apnea or tachypnea) and abnormal eye movements or oculomotor apraxia [12]. The typical cranial magnetic resonance imaging finding is of a pathognomonic "molar tooth sign" (MTS), which describes cerebellar vermis hypoplasia or aplasia, a fourth ventricle abnormality, elongated superior cerebellar peduncles and a deep interpeduncular fossa [12, 13] (Figs. 1A and 1B). Concurrent central nervous system (CNS) abnormalities may also be present and approximately 10% of patients have a Dandy-Walker malformation or enlarged posterior fossa [14]. Many affected individuals have dysmorphic facial features and some have polydactyly [8].

A number of variant forms of JBTS have been described (also known as JBTS related disorders or Joubert syndrome related disorders (JSRDs) in older literature) that have additional features such as polydactyly, coloboma, retinal dystrophy, renal cysts, oral frenulae, and hepatic fibrosis [12]. When ocular and renal anomalies occur, this has been described as CORS (cerebellar-ocular-renal syndrome) [15]. COACH syndrome describes the combination of coloboma, oligophrenia (mental retardation), ataxia, cerebellar vermis hypoplasia and hepatic fibrosis [16].

The prognosis in JBTS is highly variable, with some children dying in infancy, but others surviving with variable developmental outcomes. Around 50% of children will learn to walk independently, often with a broad-based gait due to truncal ataxia [17]. Recommendations for the appropriate management of JBTS and JSRD have been set out previously [8, 18]. The key features include initiation of a comprehensive, multidisciplinary approach for support, education and physical and occupational therapy. CNS abnormalities may require neurosurgical intervention. Neurological input is required for the management of seizures and regular ophthalmology review is recommended for the diagnosis and correction of eye abnormalities. Monitoring is required for potential complications and includes annual assessment of both renal and hepatic function [18].

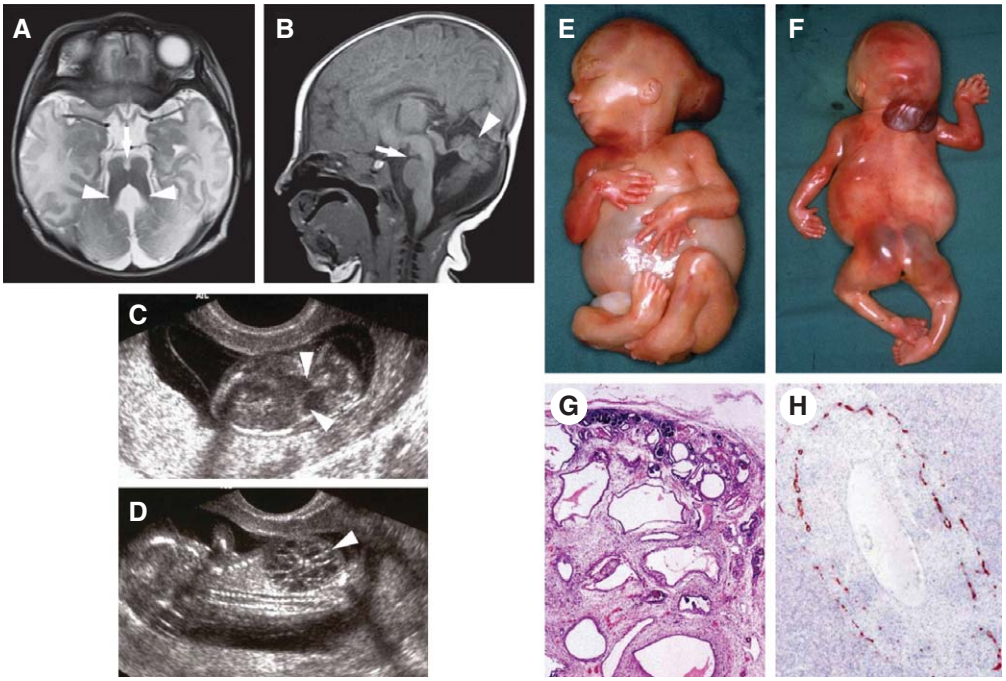

Fig. 1. Clinical features of Joubert syndrome (JBTS) and Meckel-Gruber syndrome (MKS). (A) Axial T2-weighted magnetic resonance imaging scan in a 5-year-old girl showing typical brain anomalies of JBTS, including a deepened interpeduncular fossa (arrow) and elongated superior cerebellar peduncles (arrowheads) that comprise the pathognomonic "molar tooth sign", as well as cerebellar vermis hypoplasis. (B) A sagittal T1-weighted magnetic resonance imaging scan showing the deepened interpeduncular fossa (arrow) and cerebellar vermis hypoplasis (arrowhead). The cerebellar hemispheres and brainstem are also hypoplastic. (C-D) Ultrasound findings at 14+/40 weeks of gestation for MKS showing (C) encephalocele (arrowheads) and (D) large cystic kidneys (arrowhead). (E-F) typical external features for a fetus with MKS at 16 wk of gestation showing massive flank masses due to bilateral renal cystic dysplasia, encephalocele and post-axial polydactyly of all limbs. (G) Hematoxylin-eosin staining of MKS fetal kidney at gestation age 18+/40 showing cystic dysplasia, comprising large, fluid-filled cysts, small cysts and cystic swelling of the proximal tubules and glomeruli, with the absence of normal renal parenchyma. (H) Immunohistochemical staining of MKS fetal liver at gestation age 18+/40 for cytokeratin-19, showing the retention of embryonic bile duct structures (the ductal plate malformation) without the formation of patent bile ducts. Images (A) and (B) are used by kind permission of Dr. Daniel Doherty (Seattle Children's Hospital, University of Washington, USA). Images (C) to (F) are used by kind permission of Dr. Riitta Salonen (Rinnekoti Foundation, Helsinki, Finland) from the Robert J. Gorlin Slide Collection.

MKS is a lethal, AR, congenital anomaly syndrome characterized by posterior fossa abnormalities (most often occipital encephalocele) (Figs. 1C–1F), bilateral enlarged cystic kidneys (Figs. 1D and 1G), polydactyly (Figs. 1E and 1F) and hepatic ductal plate malformation leading to hepatic fibrosis and hepatic cysts (Fig. 1H) [19]. The incidence is estimated to be between 1:13,250 and 1:140,000 live births, although increased incidence is recorded in certain ethnic groups including the Finnish and Gujarati-Indian populations [20].

This condition was first reported by Johann F. Meckel in 1822 and subsequently by C.B. Gruber in 1934 [19]. Although the CNS defects are considered to be obligatory features of MKS they have a more variable presentation. CNS anomalies can vary between total craniorachischisis to a partial defect of the corpus callosum, and can include optic nerve hypoplasia [21]. The renal parenchymal histology is often of a typical nature with cystic dysplasia [22] (Fig. 1G). The typical histology of the liver demonstrates a proliferation and dilation of the bile ducts (Fig. 1H), alongside an excess of collagenous connective tissues [22]. These findings are thought to represent arrested development of the hepatic biliary system [23, 24]. Abnormalities of the intrahepatic bile ducts and the presence of cystic kidneys are considered to be consistent features and of important diagnostic value in MKS [22, 24]. Salonen [22] proposed that bilateral multicystic kidneys, hepatic fibrotic changes and a CNS malformation are minimal diagnostic criteria. Other occasional features include post-axial polydactyly (Figs. 1E and 1F),

shortening and bowing of the long bones, retinal colobomata, pulmonary hypoplasia and situs defects [19, 22]. Rare features include cystic changes in other organs, such as the lungs and thyroid, abnormalities of the genitalia in male fetuses, microcephaly, congenital heart defects and cleft palate [19, 25–29].

MKS may be associated with an elevated maternal alpha-fetoprotein level during antenatal screening [30]. Antenatal ultrasonography can identify the classic CNS, renal and digital anomalies and the diagnosis can be made before 14 wk gestation in the classical presentation of MKS [30, 31]. Ultrasound diagnosis may be more difficult later in pregnancy due to the presence of oligohydramnios [30]. MKS is invariably lethal either *in utero* or in the first hour or days of life, usually due to pulmonary hypoplasia. The longest recorded survivor was 28 mo of age [32]. In view of the AR inheritance pattern of this condition, couples with a previously affected child should be offered appropriate genetic counseling with discussion of prenatal diagnosis by chorionic villus sampling if the genetic cause is known, or prenatal ultrasound scan within the first trimester [20, 33].

The differential diagnosis for MKS and JBTS is extensive and includes AR polycystic kidney disease, trisomy 13, Smith-Lemli-Opitz syndrome, hydrolethalus syndrome, Bardet-Biedl syndrome and oral-facial-digital syndrome type 1 (OFD1). The number of differential diagnoses, as well as the extensive allelism between ciliopathies, emphasizes the need for accurate clinical and molecular diagnosis in these conditions [20].

3. Progress in gene identification

JBTS and MKS have extreme genetic heterogeneity, and display allelism both with each other and other ciliopathies such as oral-facial-digital syndrome (OFD) and BBS. To date, mutations in 24 genes have been identified as causative for JBTS and 13 as causative for MKS (Table 1). In the following section, we provide a brief overview of historical and contemporary gene discovery studies for these conditions.

3.1. JBTS

The first patient affected with JBTS was reported in 1969 [9] and the disease was proposed to be AR in 1977 [34]. The first JBTS locus was reported by Natacci et al. [35], who identified a patient affected with JBTS and a deletion at chromosome 17p11.2. Subsequently, loci were identified to chromosome 9q [36], 6q23 [37] and 3q24 [38] using the specialized linkage analysis technique of homozygosity mapping in extended consanguineous families. The locus for a variant form of JBTS phenotype that included retinal dysplasia and cystic kidneys (known as "JBTS type B", or "cerebello-oculo-renal syndrome"; CORS2) was initially mapped to 11p12-q13.3 [39]. The breakthroughs in gene identification came in 2004, when Parisi et al. [40] identified a deletion involving *NPHP1* in a patient affected with JBTS as well as nephronophthisis (a hereditary kidney disease now also described as a ciliopathy) (Table 1, Fig. 1). In the same year, Ferland et al. [41], investigated JBTS patients with the classical phenotype and linkage to 6q23.2-q23.3, and identified pathogenic mutations in the *AHI1* gene encoding the protein jouberin.

Subsequent genetic studies have provided many seminal insights into the JBTS phenotype and ciliopathies. In 2006, Valente et al. [42] and Sayer et al. [43] identified mutations in *CEP290* as a cause of JBTS and substantiated the importance of primary cilia dysfunction. Allelism between JBTS and MKS was demonstrated in 2007 with the identification of mutations in *TMEM67* as a cause of JBTS [44], as well as the identification of mutations in *RPGRIP1L* to be a cause of both JBTS and MKS [45, 46] (Table 1). Mutations in the *ARL13B*, *CC2D2A* and *INPP5E* genes were identified to all cause classical JBTS [47–49]. ARL13B is a small Arf-family GTPase and *INPP5E* encodes an inositol 1,4,5-trisphosphate (InsP3) 5-phosphatase. InsP3 5-phosphatases hydrolyze InsP3, which acts as a secondary messenger to mobilize calcium from intracellular stores. These findings clearly implicate the dysregulation of embryonic signaling pathways as a cause of the ciliopathy phenotype (Table 1). Further allelism between an unusual X-linked form of JBTS [50] and OFD was demonstrated by mutations in *OFD1* [51] (Table 1). Affected males presented with a typical JBTS phenotype, but with additional features, including coloboma, and without a typical MTS.

In recent years, advances in genetic technology (principally, whole-exome sequencing; WES) have enabled a renaissance in gene discovery, often without initial linkage analysis, that has now enabled the study of smaller, non-consanguineous families. A key biological insight from these studies has been the importance of the Tectonic (TCTN) family of

Table 1
Summary of known JBTS and MKS loci and identified genes

Locus	Gene	Entrez gene ID	Aliases	Cytogenic location	Founder mutation(s)	Reference
JBTS1	*INPP5E*	56623	CORS1, MORMS	9q34.3		48,49
JBTS2	*TMEM216*	51259		11q12.2	Ashkenazi p.R73L	52,53
JBTS3	*AHI1*	54806		6q23.3		41
JBTS4	*NPHP1*	4867	NPH1, SLSN1	2q13		40
JBTS5	*CEP290*	80184	BBS14, LCA10, MKS4, NPHP6, POC3, SLSN6	12q21.32		43
JBTS6	*TMEM67*	91147	MECKELIN, MKS3, NPHP11	8q22.1		44
JBTS7	*RPGRIP1L*	23322	CORS3, FTM, MKS5, NPHP8	16q12.2		46
JBTS8	*ARL13B*	200894	ARL2L1	3q11.1		47
JBTS9	*CC2D2A*	57545	MKS6	4p15.32		48
JBTS10	*OFD1*	8481	JBTS10, CXorf5	Xp22.2		51
JBTS11	*TTC21B*	79809	ATD4, IFT139, JBTS11, NPHP12, THM1	2q24.3		120
JBTS12	*KIF7*	374654		15q26.1		54
JBTS13	*TCTN1*	79600		12q24.11		55
JBTS14	*TMEM237*	65062	ALS2CR4	2q33.1		59
JBTS15	*CEP41*	95681	TSGA14	7q32.2		57
JBTS16	*TMEM138*	51524		11q12.2		58
JBTS17	*C5orf42*	65250		5p13.2	French-Canadian p.Arg1336Trp, p.Ala1564Thr, c.7400+1G>A	60
JBTS18	*TCTN3*	26123	OFD4, C10orf61	10q24.1		62
JBTS19	*ZNF423*	23090	NPHP14, OAZ	6q21		121
JBTS20	*TMEM231*	79583	MKS11	16q23.1		63
JBTS21	*CSPP1*	79848	CSPP	8q13.1-q13.2		65–67
JBTS22	*PDE6D*	5147	PDED	2q37.1		64
NA	*TCTN2*	79867	C12orf38, MKS8	12q24.31	68	
NA	*EXOC8*	149371	EXO84, SEC84	1q42.2	61	
MKS1	*MKS1*	54903	BBS13, POC12	17q22	Finnish c.1408-35_1408-7del29	71
MKS2	*TMEM216*	51259		11q12.2	Ashkenazi p.R73L	53
MKS3	*TMEM67*	91147	MECKELIN, JBTS6, NPHP11	8q22.1	Pakistani c.1575+1G>A	72
MKS4	*CEP290*	80184	BBS14, JBTS5, LCA10, NPHP6, POC3, SLSN6	12q21.32		73
MKS5	*RPGRIP1L*	23322	CORS3, FTM, JBTS7, NPHP8	16q12.2	European p.T625P	46
MKS6	*CC2D2A*	57545	JBTS9	4p15.32	Finnish c.1762C>T	75
MKS7	*NPHP3*	27031	SLSN3	3q22.1		122
MKS8	*TCTN2*	79867	C12orf38	12q24.31		76
MKS9	*B9D1*	27077	MKSR1	17p11.2		77
MKS10	*B9D2*	80776	MKSR2	19q13.2		78
MKS11	*TMEM231*	79583	JBTS20	16q23.1		79
NA	*EXOC4*	60412	SEC8, SEC8L1	7q33		
NA	*CSPP1*	79848	JBTS21, CSPP	8q13.1-q13.2	66	

Alias names of genes summarize alternative gene symbols and any reported allelism with other ciliopathies (indicated by locus name; NA = not applicable). Any reported founder mutations and references for the primary gene discovery paper(s) are listed. ATD = Asphyxiating thoracic dystrophy; CORS = Cerebellar-ocular-renal syndrome; JBTS = Joubert syndrome; MKS = Meckel-Gruber syndrome; NPHP = Nephronophthisis; OFD = Oral-facial-digital syndrome; SNLS = Senior-Løken syndrome.

transmembrane proteins and small tetraspanin-like transmembrane proteins (TMEMs) in the pathogenesis of the JBTS phenotype (Table 1). Initial WES studies identified the JBTS genes *TMEM216* [52, 53], *KIF7* [54], *TCTN1* [55, 56], *CEP41* [57] and *TMEM138* [58]. More recent studies have used WES to identify

mutations in *TMEM237* [59] and *C5orf42* as a common cause in French-Canadian JBTS patients [60]. *C5ORF42* was also reported to be mutated in a cohort of Saudi-Arabian JBTS patients [56]. The most recently identified JBTS genes include *EXOC8* [61], *TCTN3* [62], *TMEM231* [63], *PDE6D* [64], *CSPP1* [65–67] and *TCTN2* [68].

3.2. MKS

MKS is a Finnish heritage disease, which describes a group of 40 recessive conditions that due to founder effects and genetic isolation are more common in Finns. The first MKS locus, MKS1, was mapped to chromosome 17q21-q24 [29] in endogamous Finnish families using a combination of homozygosity mapping and haplotype analysis to identify regions of linkage disequilibrium. Homozygosity mapping also identified loci (MKS2 and MKS3) at chromosomes 11q13 [69] and 8q24 [70] respectively, in consanguineous families from the Middle East and the Indian sub-continent. In 2006, Kyttälä et al. [71] identified mutations in the *MKS1* gene as a cause of MKS in the Finnish population, whilst Smith et al. [72] identified mutations in *TMEM67* in the MKS3 locus that encodes the TMEM67/meckelin orphan receptor (Table 1). Subsequently, homozygosity mapping identified point mutations in *CEP290* [73] and *RPGRIP1L* [46] as causes of MKS, as well as microdeletions in *CEP290* [74]. The identification of mutations in *CC2D2A* for Finnish MKS families (excluded for mutations in *MKS1*), provided an unusual example of a second major cause for a Finnish heritage disease [75]. In contrast, microheterogeneity at the MKS2 locus for two adjacent genes that both encode tetraspanin-like transmembrane proteins (TMEM138 and TMEM216), prevented the identification of mutations in *TMEM216* as a cause of MKS until 2010 [53]. Interestingly, although mutations in *TMEM216* are allelic for both JBTS and MKS, mutations in *TMEM138* have not been described as a cause of MKS (Table 1). Conversely, *MKS1* truncating mutations have only been described as a cause of MKS, and it remains highly likely that missense or hypomorphic mutations are a cause of JBTS.

Subsequent gene discovery studies for MKS have used WES to prioritize functional candidate genes, often based on sequence homology to known MKS or JBTS genes. For example, *TCTN1*, a known JBTS gene, was used to prioritize screening and then identify a pathogenic private mutation in *TCTN2*, a paralogue and member of the Tectonic family of genes [76]. Furthermore, the *MKS1* protein contains a B9 domain of unknown function that is also present in only two other proteins in the human genome (B9D1 and B9D2). Mutations in *B9D1* were identified as a cause of MKS [77], followed by the description of a family with a pathogenic private mutation in *B9D2* [78]. The most recently identified MKS genes include *TMEM231* [79] and *CSPP1* [66].

4. Genotype-phenotype correlations

JBTS and MKS both have unusually broad phenotypic variability, with many occasional clinical features that have been reported in addition to the obligate features. In principle, genotype-phenotype correlations would improve the prioritization of variants in diagnostic screens that use WES or targeted capture strategies. However, the correlations have proven to be broad and are difficult to apply in practice. In the next section, we provide an overview of the general genotype-phenotype correlations for JBTS and MKS, highlighting the most relevant for clinical practice and those that provide insights into protein function.

Marked genotype-phenotype correlations for JBTS include those for mutations in the *NPHP1* and *AHI1* genes. These have an overall prevalence of 2% and 11% respectively, based on the screening of an unselected cohort of 117 JBTS patients [80], making *AHI1* mutations a major cause of JBTS. JBTS caused by a deletion in *NPHP1* is associated with additional features that include nephronophthisis, with retinal and cerebellar involvement. However, the MTS was atypical in these patients and was characterized by moderate cerebellar vermis hypoplasia and elongated, but not thickened superior cerebellar peduncles [40, 81]. Patients with JBTS caused by *AHI1* mutations have a high prevalence of retinal dystrophy, in the absence of occasional features such as polydactyly, liver fibrosis, coloboma or encephalocele. In one Dutch cohort, 75% of patients with *AHI1* mutations had a retinal phenotype compared to 10% for a renal phenotype [82], although nephronophthisis and/or cortical polymicrogyria were also occasional features [83–85].

The most striking genotype-phenotype correlation is between *TMEM67* mutations and the JBTS variant phenotype of COACH syndrome (Table 1). *TMEM67*

mutations account for 57% to 83% [86–88] of cases of COACH syndrome, demonstrating the strongest association between *TMEM67* mutations and liver involvement or coloboma [87, 89]. Due to this strong correlation, JBTS patients with liver involvement should be prioritized for *TMEM67* screening [88]. Altogether mutations in *TMEM67* have been found to account for 9% of JSRD patients, with or without liver disease [88]. Mutations in *TMEM67* also cause 16% of all MKS cases [87], making *TMEM67* mutations a major cause of MKS. Interestingly, both polydactyly and occipital encephalocele are less frequent in patients with *TMEM67* mutations than those with *MKS1* mutations [90].

Several studies have identified correlations between the type of mutation, or location within a protein domain, with ciliopathy patient phenotype. For example, missense mutations in *CC2D2A* have been associated with JBTS, whereas null alleles cause MKS [91]. Characteristic features of JBTS patients with *CC2D2A* mutations include ventriculomegaly and seizures [92]. Another example is that missense mutations in exons 8 to 15 of *TMEM67,* especially in combination with a truncating mutation, are correlated with the MKS phenotype [87]. These exons encode the cysteine-rich domain of the TMEM67/meckelin orphan receptor, and missense mutations would presumably alter or prevent the binding of the uncharacterized ligands to this receptor. Similarly, mutations in *AHI1* appear to cluster in the predicted WD40 domain of the jouberin/AHI1 protein [80, 83] which is thought to mediate protein-protein interactions. Clustering of *RPGRIP1L* missense mutations (including p.T516P, the most common missense mutation) also occurs in exon 15 for patients with JBTS and renal involvement [93, 94]. *RPGRIP1L* exon 15 encodes a C2 protein domain in this protein, which is a calcium-dependent membrane-targeting region that binds to phospholipids and inositol polyphosphates. C2 protein domains are exclusively found in signal transduction enzymes such as protein kinase C or membrane trafficking proteins. Interestingly, CC2D2A also contains a C2 domain, and these findings clearly implicate the JBTS and MKS ciliary proteins in the transduction and regulation of multiple signaling pathways during embryonic development.

5. Insights into molecular mechanisms from gene discovery studies

Following the initial gene discovery studies, contemporary research has focused on the delineation of possible cellular functions for JBTS and MKS ciliary proteins. Several recent studies have used biochemical assays and proteomic studies to delineate networks of protein-protein interactions (Fig. 2) and in some instances infer possible functions from those of other better characterized members of a complex.

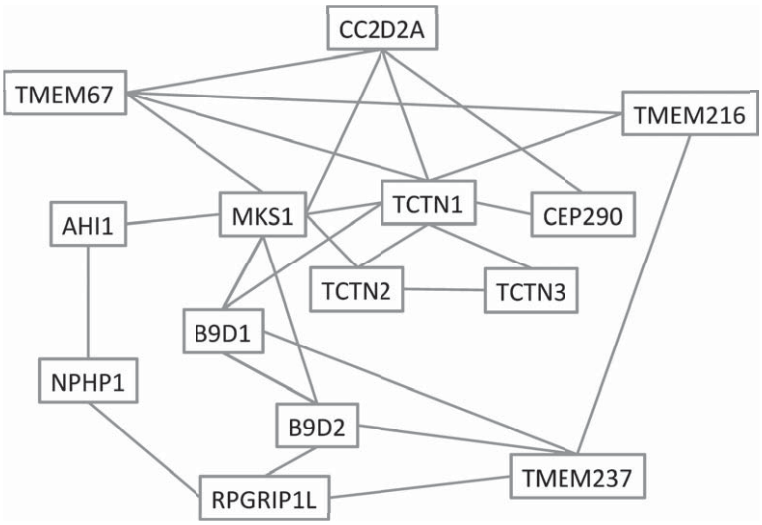

Fig. 2. Schematic representation of experimentally verified Joubert syndrome (JBTS) and Meckel-Gruber syndrome (MKS) protein-protein interactions. The network summarizes the interactions (gray lines) that form the "MKS-JBTS module" at the ciliary transition zone. Protein names are indicated in black boxes. Biochemical methods to determine interactions included yeast two-hybrid assays and proteomics studies. See main text for further details.

Elegant transgenesis and localization studies in animal models such as zebrafish (*Danio rerio*) and the nematode *Caenorhabditis elegans* have inferred genetic interactions between JBTS and MKS genes, although these do not always support the existence of biochemical interactions. However, in most of these studies the target ciliary protein is over-expressed either with a convenient epitope tag for biochemical purification, or a fluorescent protein reporter in genetic interaction experiments, and is not at physiological levels of expression. It remains problematic if over-expressed proteins correctly model the localization and interactions of the *in vivo* cognate protein, and these studies should be interpreted with some caution.

Despite these limitations, several studies have identified the protein complex known as the "MKS-JBTS module" at the ciliary TZ that contains many JBTS and MKS proteins [6, 7]. These include all of the known small tetraspanin-like TMEMs mutated in ciliopathies (TMEM216, TMEM138, TMEM237 and TMEM231), as well as others (TMEM17 and TMEM107) that have yet to be implicated in a human ciliopathy. The Tectonic transmembrane proteins (TCTN1, TCTN2 and TCTN3) and the orphan receptor TMEM67/meckelin also localize to the TZ. All of these transmembrane proteins are thought to be inserted into the local plasma membrane at the TZ. This, in turn, may mediate interactions or regulate the function of membrane-targeting proteins with C2 domains such as RPGRIP1L and CC2D2A [6, 7]. For example, a proteomics study of TCTN1 showed that this protein forms a complex at the TZ with MKS1, TMEM216, TMEM67, CEP290, B9D1, TCTN2 and CC2D2A [55] (Fig. 2). Confirmation of the direct interaction between CC2D2A and CEP290 has come from yeast two-hybrid studies and glutathionine S-transferase (GST) pull-downs, with genetic interactions between these genes also demonstrated in zebrafish models [48].

The role of CEP290 and the B9 proteins at the TZ remains unclear, but they presumably act as linkers or mediators between the TMEMs and either the vesicular cargo that is targeted to the TZ during ciliogenesis, or the subsequent transport of cargo proteins within the cilium by IFT. The former model is supported by analogy from studies on AHI1/jouberin, a JBTS ciliary protein that contains WD40 and Src Homology 3 (SH3) protein interaction domains. AHI1/jouberin interacts with RAB8A, a small GTP/GDP-binding protein that mediates the vesicular transport of proteins from the endoplasmic reticulum to the Golgi and the plasma membrane [95]. Loss of AHI1/jouberin causes defects in ciliogenesis and also defects in vesicle transport [95].

The elaborate machinery of the TZ appears to be in place to maintain the integrity of the ciliary gate, and the disruption of this function may contribute to the ciliopathy phenotype by altering the composition of the ciliary membrane or axoneme [96]. Several recent studies have confirmed that disruption of the TZ results in the incorrect movement of proteins in and out of the cilium, with particular emphasis on the transport of enzymes and transmembrane proteins that mediate intracellular signaling. For example, TMEM231 and TMEM17 localize to the TZ, with the localization of TMEM231 dependent on other TZ proteins (CC2D2A and B9D1) [97]. In turn, this regulated the transport of G-protein-coupled signaling receptors (GPCRs; specifically, somatostatin and serotonin receptors SSTR3 and HTR6) into the ciliary membrane [97]. Loss of either *Tmem231* or *B9d2* in mice caused defects in ciliogenesis and Shh signaling [97]. Sang et al. [68] showed that loss of *Tctn2* caused defects in ciliogenesis. In a separate study, loss of *Tctn1*, *Tctn2*, *Tmem67* or *Cc2d2a* caused tissue-specific defects in ciliogenesis and ciliary membrane composition. In addition, defects were observed in the ciliary localization of adenylyl cyclase 3 (ACIII, an enzyme that catalyzes the formation of the secondary messenger cAMP), the transmembrane signaling proteins smoothened and Pkd2/polycystin-2, and Arl13b (a small Arf-family GTPase) [55]. Interestingly, Arl13b regulates the migration of interneurons in the developing brain, and this may provide a partial explanation for the neurological defects observed in JBTS patients [98].

In contrast to other JBTS and MKS proteins that predominantly localize to the ciliary TZ, both ARL13B and INPP5E localize to the ciliary axoneme. The ciliary localization of ARL13B depends on TZ function, as described above, but the axonemal localization of INPP5E depends on the functions of both ARL13B and PDE6D (a phosphodiesterase that appears to act as a chaperone for prenylated ciliary and retinal proteins) [55, 64, 99]. An attractive hypothesis is that INPP5E dysfunction (due to either mutation or mislocalization) causes alterations in ciliary signaling through changes in the levels of the secondary messenger InsP3. It is probable that this is one of the fundamental pathogenic mechanisms in both JBTS and MKS, but it has yet to be formally tested. The JBTS phenotype has also

been associated with alterations in the tubulin post-translational modifications of the ciliary axoneme, and this would presumably affect both the stability of the cilium and the trafficking of ciliary proteins. Specifically, the JBTS protein CEP41, encoding a centrosomal and microtubule-binding protein, regulates the entry of TTLL6 (a tubulin polyglutamylase enzyme) to the ciliary axoneme [57]. This thus implicates tubulin post-translational modification and therefore the composition of the axoneme in the ciliopathy phenotype [57, 100].

Finally, the loss or mislocalization of many JBTS and MKS proteins cause defects in Shh signaling, as demonstrated in a number of mouse ciliopathy models [97, 101–103]. One explanation is that TZ disruption prevents the correct trafficking of KIF7, a ciliary-associated kinesin motor protein that regulates Shh signaling through altering the relative levels of the activator and repressor isoforms of GLI transcription factors [54, 104]. GLI proteins are the downstream effectors of the Shh signaling pathway, and KIF7 appears to act as both a negative regulator by preventing the inappropriate activation of GLI2 in the absence of ligand, and as a positive regulator by preventing the processing of GLI3 into its repressor form. Animal models with loss of TZ-associated JBTS and MKS proteins also have dysregulation of the "canonical" and "non-canonical" branches of the Wnt signaling pathway [53, 101, 103, 105, 106], but the mechanistic detail of how the ciliary TZ regulates this pathway is less clear than for the Shh pathway. One possibility, at least for the canonical β-catenin-mediated branch, is that AHI/jouberin directly interacts and sequestrates the downstream effector β-catenin at the cilium [107, 108]. In turn, this would limit the nuclear entry of β-catenin and its availability to act as a transcription factor for Wnt-responsive genes.

6. Future perspectives

To date, mutations in the known JBTS and MKS genes appear to account for no more than 60% cases. The spectrum of causative genes for these conditions is therefore incomplete, but the remaining genes are likely to be uncommon or even harbor mutations that are private to a single family. However, with the widespread availability and affordability of WES and targeted clonal sequencing techniques such as molecular inversion probe sequencing, many researchers are re-investigating known genes in patients that have been previously mutation negative. In addition, whole-genome sequencing (WGS) at low coverage depth now allows rapid copy number analysis. These studies are likely to uncover copy number variations and intronic mutations, as well as changes in the promoter sequence or in *cis* regulatory elements as potential pathogenic causes. This will improve JBTS and MKS patient diagnosis, and with the emerging genotype-phenotype correlations for JBTS variants such as COACH, prognostic testing may be improved as well. However, as described above, the efforts to describe most of these correlations in anything but broad terms are confounded by both the allelism and unusual phenotypic variability for these conditions. Furthermore, the range of phenotypes associated with the ciliopathies continues to be broadened [109–114]. The molecular mechanisms that cause such phenotypic variability remain largely unknown, but are likely to include the effect of modifier alleles in other ciliary-related genes and stochastic effects in signaling pathways during embryonic development. Two examples that support the former hypothesis include the association of common missense coding variants in the *RPGRIP1L* and *AHI1* genes with the expressivity of retinal degeneration in the ciliopathy phenotype. Thr229 in the common variant p.A229T in *RPGRIP1L* is associated with retinal degeneration in a range of ciliopathies including JBTS and MKS [115], and Trp830 in the variant p.R830W in AHI1/jouberin is associated with a more than sevenfold increase in relative risk of retinal degeneration within a cohort of individuals with the kidney ciliopathy nephronophthisis [116].

These findings and recent developments in WES and WGS emphasize the difficulties in assessing the pathogenic potential of variants of unknown significance in both basic research and clinical diagnosis of not only the ciliopathies but, more generally, for AR conditions [117]. This is a key challenge that needs to be addressed to prevent false positive results from hindering the translation of research findings into clinical diagnostic testing and to enable the further biological understanding of disease mechanisms. For JBST and MKS, the major causative genes are now known and there are good insights into the function for some of the encoded proteins. In interpreting variants of unknown significance in the ciliopathies, researchers and clinical scientists can now take full advantage of public datasets of genomic variation, functional genomic data and model-organism phenotypes. However, it remains important that variants identified from the many

targeted screening and WES experiments for JBTS and MKS are reported as public datasets with a standard nomenclature that follows published guidelines. For this purpose, we would suggest that the Leiden Open Variation Database v3.0 is used since this provides both gene- and patient-specific data storage, including datasets from WES and WGS [118].

In the near future, we envisage the further convergence of genetic data with other independent lines of evidence that assess the pathogenic potential of a variant. These will include comparative genomic approaches and bioinformatic datasets, although the experimental validation of the damaging impact of a candidate variant still provides the most definitive proof. Future studies should use assays of patient-derived cells or tissues, as well as well-established cell or animal models of gene function. Not only will these lead to improvements in the diagnosis and clinical management of ciliopathy patients, but they will also provide pre-clinical models to test future therapeutic interventions. In JBTS, these could modify or treat cystic kidney disease or ciliopathy disease progression, and perhaps the long-term outlook of patients with these conditions. Since JBTS and MKS are predominantly AR, they are caused by the absence of normal protein (rather than the presence of an abnormal protein) so they can, in principle, be corrected by gene-replacement. In the first demonstration of this strategy in a ciliopathy mouse model, McIntyre et al. [119] used the well-established *Ift88*^{Tg737Rpw} mouse mutant with many typical phenotypic features including anosmia. Remarkably, the adenoviral-mediated expression of Ift88 (a protein essential for IFT in cilia) in fully-differentiated olfactory sensory neurons of mutant mice was sufficient to restore both ciliary structures and rescue olfactory function [119]. In the future, targeted therapies such as antisense oligonucleotide therapy and stop codon read-through therapy may be beneficial for patients with suitable splice-site or nonsense mutations. This makes the JBTS and MKS group of ciliopathies a top priority for further genetic and functional characterization in order to prioritize patients for these potential treatments.

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