

# Ciliopathies: Genetics in Pediatric Medicine

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## Abstract

*Ciliary disorders*, which are also referred to as *ciliopathies*, are a group of hereditary disorders that result from dysfunctional cilia. The latter are cellular organelles that stick up from the apical plasma membrane. Cilia have important roles in signal transduction and facilitate communications between cells and their surroundings. Ciliary disruption can result in a wide variety of clinically and genetically heterogeneous disorders with overlapping phenotypes. Because cilia occur widespread in our bodies many organs and sensory systems can be affected when they are dysfunctional. Ciliary disorders may be isolated or syndromic, and common features are cystic liver and/or kidney disease, blindness, neural tube defects, brain anomalies and intellectual disability, skeletal abnormalities ranging from polydactyly to abnormally short ribs and limbs, ectodermal defects, obesity, *situs inversus*, infertility, and recurrent respiratory tract infections. In this review, we summarize the features, frequency, morbidity, and mortality of each of the different ciliopathies that occur in pediatrics. The importance of genetics and the occurrence of genotype-phenotype correlations are indicated, and advances in gene identification are discussed. The use of next-generation sequencing by which a gene panel or all genes can be screened in a single experiment is highlighted as this technology significantly lowered costs and time of the mutation detection process in the past. We discuss the challenges of this new technology and briefly touch upon the use of whole-exome sequencing as a diagnostic test for ciliary disorders. Finally, a perspective on the future of genetics in the context of ciliary disorders is provided.

## Keywords

- ▶ ciliopathy
- ▶ cilia
- ▶ next-generation sequencing
- ▶ diagnostics
- ▶ genotype-phenotype

## Cilia

### Structure of Cilia

Cilia are cellular organelles with an antenna-like structure that protrude from the cellular membrane of almost all cell types.<sup>1</sup> Some cells have bundles with 200 to 300 cilia, whereas most cells only form a single cilium.<sup>1</sup> Cilia can be divided in two types: motile cilia, which often occur as groups of cilia on the cell surface and are capable of beating in a constant frequency whereby a fluid flow is generated, and primary cilia that occur one per cell and are immotile and involved in the communication of the cell with its surroundings.<sup>2</sup> Besides

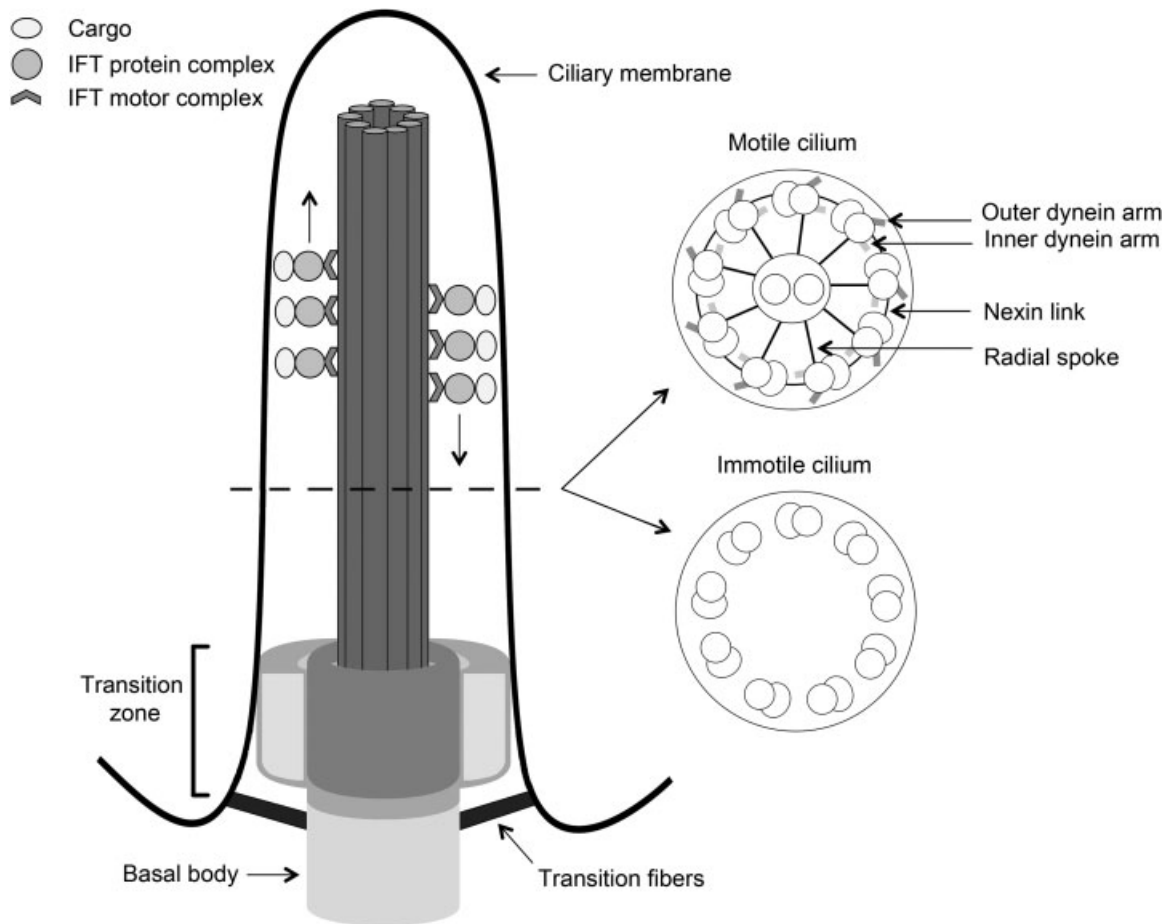
their difference in capacity to actively move, motile and immotile cilia also differ structurally. Both cilia types erect from a basal body, a centriolar-based structure that is used as a foundation to build the ciliary microtubular skeleton; however, the number of microtubule pairs in motile and immotile cilia is unequal and components that drive active movement are only present on motile cilia (▶ **Fig. 1**).<sup>1,3,4</sup> Formation of motile and immotile cilia also slightly differs as centriole replication precedes cilia formation in multiciliated cells, whereas this step is not required in monociliated cells.<sup>2</sup> Generally, when a cell starts to assemble a cilium,

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**Fig. 1** Schematic structure of motile and immotile cilia. Cilia are composed of a basal body and a ciliary axoneme that protrudes from the apical plasma membrane. These two structures are linked via the transition zone (TZ) that consists of three compartments, that is, a base and two compartments filling the space between the microtubules and the axonemal membrane, which are thought to act as a diffusion barrier for regulation of ciliogenesis and signaling. Cilia contain microtubule skeletons that are composed of nine microtubule doublets that organize in a ring. A central microtubule pair is usually also present in motile cilia but not in immotile cilia. Inner and outer dynein arms, radial spokes, and nexin links are also solely present in motile cilia and actively regulate ciliary movement. During this process, dynein heavy chains of one doublet slide against microtubules of a neighboring doublet, thereby orchestrating the beating of the cilium in an ATP-dependent fashion. In both cilium types, the ciliary microtubules also function as rails for intraflagellar transport (IFT) trains that control bidirectional ciliary transport. During this process, IFT-B particles are associated with kinesin-2 motors and regulate transportation of cargo from the ciliary base to the tip, while IFT-A particles and ciliary dynein motors transport cargo in the opposite direction.

the centrosome that is normally involved in separating the chromosomes during mitosis is relocated and anchors just below the surface of the apical membrane.<sup>5</sup> During the conversion of a centriole to a basal body, distal and subdistal centriolar appendages transform into transition fibers and basal feet, which act as physical attachments to the plasma membrane (→Fig. 1). These elements are also required to establish the orientation of the basal body in the plasma membrane.<sup>6</sup> Distal to the transition fibers resides the ciliary transition zone, which functions as a gate and regulates the entry and exit of proteins into and out of the cilium.<sup>7,8</sup> Vesicles with membrane proteins are targeted here to be integrated into the ciliary membrane, a process that is coordinated by the BBSome, a complex of proteins that are mutated in Bardet-Biedl syndrome (BBS).<sup>9,10</sup> Vesicle delivery to the cilium contributes to the establishment of a highly specialized ciliary membrane that contains various receptors and signaling proteins.<sup>11</sup> Within the cilium proteins are

transported from base to tip and back over the axonemal microtubules (→Fig. 1). This transport, first described in unicellular algae, is performed by the intraflagellar transport (IFT) machinery.<sup>12,13</sup> IFT is essential for ciliary functionality as protein synthesis does not occur within the cilium.<sup>14</sup>

#### Function and Occurrence of Motile and Immotile Cilia

Motile cilia are found in only a few tissues where they transport extracellular fluid over the epithelial surface. For example, they occur on respiratory epithelial cells in the trachea where they are essential for mucus clearance, and on the apical surface of epithelial cells that line the fallopian tubes where they facilitate transportation of the (fertilized) ovum.<sup>1</sup> Cells with a single motile cilium also exist; sperm cells have a specialized motile cilium (flagellum) that provides their motility, and single motile cilia are also found on the apical surface of nodal cells that regulate left-right asymmetry during embryogenesis.<sup>15</sup> Nonmotile cilia are found on

almost all quiescent cells and function differently.<sup>1</sup> They generally act as a sensory signaling hub of the cell, and are key players in embryonic development and tissue morphogenesis and homeostasis by regulating Wnt, Hedgehog, and many other signaling pathways.<sup>2,16,17</sup> They initiate or regulate signaling by sensing their environment and ensuring delivery and/or removal of signaling proteins to or from the cilium.<sup>16</sup> Taken together, over the last decades, cilia demonstrated to be essential organelles that regulate a wide array of developmental, cellular, and physiologic processes wherein their motile and/or sensory capacities play a major role.

## Ciliopathies

Ciliopathies are disorders that are caused by defects in motile or immotile cilia. As cilia occur on virtually all cells of the human body, their dysfunction can lead to a broad spectrum of features.<sup>1</sup> Common features are renal cystic disease, blindness, neural tube defects, intellectual disability, skeletal abnormalities ranging from polydactyly to abnormally short ribs and limbs, ectodermal defects, obesity, *situs inversus*, infertility, and respiratory anomalies. These features can occur isolated or may be part of a recognizable syndrome. Here, we describe the different types of ciliopathies, their characteristics, and to what extent they genetically and phenotypically overlap.

### Neurodevelopmental Ciliopathies

Joubert syndrome (JBTS) and Meckel-Gruber syndrome (MKS) are rare neurodevelopmental disorders that genetically and phenotypically overlap. JBTS was first described by Marie Joubert in 1969 and is diagnosed when patients have hypotonia, ataxia, developmental delay, and distinctive cerebellar and brain stem malformations that can be recognized as a “molar tooth sign” (MTS; ▶ **Fig. 2A**) on magnetic resonance imaging (MRI).<sup>18–20</sup> JBTS patients also often present with kidney cysts, polydactyly, coloboma, retinal dystrophy, respiratory abnormalities (episodic tachypnea or apnea), oral frenula, and hepatic fibrosis.<sup>21</sup> The health of JBTS patients largely depends on the occurrence and severity of liver and kidney disease that require appropriate management and treatment.<sup>19,22</sup> MKS shows marked clinical overlap with JBTS but is more severe and perinatal lethal. This disorder is characterized by occipital encephalocele, polycystic kidneys, polydactyly, and hepatic fibrosis (▶ **Fig. 2B–D**).<sup>23</sup> MKS is diagnosed when two of these four features are present or when one is present in combination with two other anomalies, including cleft lip/palate, cardiac septal defects, holoprosencephaly, agenesis of corpus callosum, Dandy-Walker malformation, microphthalmia, *situs inversus*, ambiguous genitalia, and shortening and bowing of long bones.<sup>24,25</sup> The incidence of JBTS ranges from 1:80,000 to 1:100,000 live births,<sup>22</sup> whereas the prevalence of MKS is less frequent at 1:140,000.<sup>26</sup> Exceptions exist as the occurrence of MKS in the Finnish population is as high as 1:9,000 births.<sup>27,28</sup>

Both JBTS and MKS are usually autosomal recessive, but a few JBTS cases with X-linked inheritance have been reported.<sup>29</sup> JBTS and MKS overlap genetically; 25% of the 25

known JBTS genes have also been found mutated in MKS (▶ **Table 1**).<sup>20,30</sup> It remains elusive why certain mutations in a gene lead to JBTS, while other mutations in the same gene cause MKS. In some cases mutation type may determine the phenotypic outcome. For example, missense mutations in *CC2D2A* cause JBTS, whereas null alleles are associated with MKS.<sup>20</sup> Modifier genes have also been proposed to play a role in phenotypic outcome and could explain intrafamilial phenotypic differences.<sup>20,30</sup> Genotype-phenotype correlations have been reported and can facilitate choices for specific genetic testing. For example, *AH11* mutations often occur in JBTS patients with retinal dystrophy, whereas *NPHP1* and *RPGRIP1L* mutations are most frequent in JBTS with renal dysplasia. Moreover, in 50% of JBTS patients with both retinal and renal phenotypes *CEP290* mutations are detected.<sup>19,22</sup> When considering the protein localization, it is evident that most JBTS and MKS proteins reside in the ciliary transition zone (▶ **Fig. 1**). When they are mutated, the structure of the ciliary transition zone is perturbed causing disruption of its function as a ciliary gate, thereby affecting a variety of signaling pathways including Wnt/ $\beta$ -catenin and Hedgehog signaling.<sup>20,30</sup>

### Ciliopathies with Major Skeletal Involvement

Many ciliopathies are characterized by skeletal abnormalities such as dwarfism, short limbs and ribs, and brachydactyly/polydactyly. Ciliopathies with major skeletal involvement can be divided into four groups: cranioectodermal dysplasia (CED), short-rib thoracic dysplasia (SRTD), Ellis-van Creveld syndrome (EVC), and oral-facial-digital syndrome (OFDS). CED is rare and autosomal recessive. Typical features are sagittal craniosynostosis, narrow thorax with pectus excavatum, rhizomelia, brachydactyly, and ectodermal dysplasia (▶ **Fig. 2F**).<sup>31</sup> Vital organ dysfunction often occurs and causes death in approximately 20% of CED patients before the age of 7.<sup>31</sup> SRTD is clinically and genetically related to CED. This family of disorders includes the following syndromes; short-rib polydactyly syndrome (SRPS), Jeune asphyxiating thoracic dysplasia (JATD), and Mainzer-Saldino syndrome (MZSDS). These disorders are autosomal recessive and frequencies range from 1:100,000 to 1:1,000,000.<sup>32</sup> The most prominent SRTD features are limb shortening and a small rib cage. These features are most pronounced in perinatal lethal SRPS (▶ **Fig. 2E**). Additional SRPS features may include polydactyly, cleft lip/palate, and anomalies of a variety of organs and tissues including the brain, heart, eyes, kidneys, liver, pancreas, intestines, and genitalia.<sup>33</sup> Patients with JATD present with similar, but milder skeletal anomalies compared with SRPS. The main complication in JATD patients is the narrow thorax phenotype that causes severe respiratory insufficiency; this phenotype causes lethality in 20 to 60% of JATD infants (▶ **Fig. 2G**). Besides skeletal abnormalities, JATD patients also occasionally present with nephronophthisis (NPHP), blindness, liver fibrosis, and intellectual disability.<sup>32</sup> Similar as in CED, MZSDS is also featured by a relatively mild narrow thorax phenotype. Other features of MZSDS are shortened limbs, blindness, NPHP, liver fibrosis, and pancreatic abnormalities.<sup>34</sup> EVC is another ciliopathy that is featured

**Table 1** Genes mutated in ciliopathies

Gene	OMIM	Description
Neurodevelopmental ciliopathies		
AHI1	608894	JBTS
ARL13B	608922	JBTS
ATXN10	611150	JBTS-like
B9D1	614144	MKS
B9D2	611951	MKS
C5orf42	614571	JBTS
CC2D2A	612013	JBTS, MKS
CEP104	NA	JBTS
CEP290	610142	JBTS, SLSN, LCA, MKS, BBS
CEP41	610523	JBTS
CSPP1	611654	JBTS
EXOC4	608185	MKS
EXOC8	615283	JBTS
HYLS1	610693	MKS-like
ICK	612325	ECO
IFT81	605489	JBTS-like
INPP5E	613037	JBTS
KIAA0586	610178	JBTS, SRTD
KIF14	611279	MKS
KIF7	611254	JBTS, MKS-like, ACLS
MKS1	609883	MKS, BBS
NPHP1	607100	JBTS, NPHP, SLSN
NPHP3	608002	MKS, NPHP
PDE6D	602676	JBTS
PIBF1	607532	JBTS
RPGRIP1L	610937	JBTS, MKS
TCTN1	609863	JBTS
TCTN2	613846	JBTS
TCTN3	613847	JBTS, OFDS
TMEM107	616183	MKS, OFDS
TMEM138	614459	JBTS
TMEM216	613277	JBTS, MKS
TMEM231	614949	JBTS, MKS
TMEM237	614423	JBTS
TMEM67	609884	MKS, JBTS, NPHP, BBS
TTBK2	611695	JBTS-like
ZNF423	604557	JBTS, NPHP
Ciliopathies with major skeletal involvement		
C2CD3	615944	OFDS
CEP120	613446	SRTD
C21ORF2	603191	JATD
DDX59	615464	OFDS
DYNC2H1	603297	JATD, SRTD
EVC	604831	EVC, WAD
EVC2	607261	EVC, WAD
IFT122	606045	CED
IFT140	614620	SRTD
IFT172	607386	JATD, MZSDS, SRTD, RD

(Continued)

**Table 1** (Continued)

Gene	OMIM	Description
IFT43	614068	CED
IFT80	611177	SRTD
NEK1	604588	SRTD
OFD1	300170	OFDS, SGBS, JBTS
SCLT1	611399	OFDS
TBC1D32	615867	OFDS
TTC21B	612014	JATD, NPHP
WDR19	608151	JATD, CED, NPHP
WDR34	613363	SRTD
WDR35	613602	CED, SRTD
WDR60	615462	SRTD
Isolated and syndromic obesity		
ALMS1	606844	ALMS
ARL6	608845	BBS, RP
BBIP1	613605	BBS
BBS1	209901	BBS
BBS10	610148	BBS
BBS12	610683	BBS
BBS2	606151	BBS
BBS4	600374	BBS
BBS5	603650	BBS
BBS7	607590	BBS
BBS9	607968	BBS
CCDC28B	610162	BBS
CEP19	615586	MOSPGF
IFT27	615870	BBS
LZTFL1	606568	BBS
MKKS	604896	BBS, MKKS
TRIM32	602290	BBS
TTC8	608132	BBS, RP
WDPCP	613580	BBS
Renal ciliopathies		
ANKS6	615370	NPHP
CEP164	614848	NPHP
CEP83	615847	NPHP
DCDC2	605755	NPHP
GLIS2	608539	NPHP
INVS	243305	NPHP
IQCB1	609237	SLSN
NEK8	609799	NPHP
NPHP4	607215	NPHP, SLSN
PKD1	601313	ADPKD
PKD2	173910	ADPKD
PKHD1	606702	ARPKD
SDCCAG8	613524	SLSN
VHL	608537	VHL
XPNPEP3	613553	NPHP-like
Primary ciliary dyskinesia		
ARMC4	615408	PCD

(Continued)

**Table 1** (Continued)

Gene	OMIM	Description
<i>C21orf59</i>	615494	PCD
<i>CCDC103</i>	614677	PCD
<i>CCDC114</i>	615038	PCD
<i>CCDC151</i>	615956	PCD
<i>CCDC39</i>	613798	PCD
<i>CCDC40</i>	613799	PCD
<i>CCDC65</i>	611088	PCD
<i>CCNO</i>	607752	PCD
<i>CENPF</i>	600236	PCD
<i>DNAAF1</i>	613190	PCD
<i>DNAAF2</i>	612517	PCD
<i>DNAAF3</i>	614566	PCD
<i>DNAAF5</i>	614864	PCD
<i>DNAH11</i>	603339	PCD
<i>DNAH5</i>	603335	PCD
<i>DNAI1</i>	604366	PCD
<i>DNAI2</i>	605483	PCD
<i>DNAL1</i>	610062	PCD
<i>DRC1</i>	615288	PCD
<i>DYX1C1</i>	608706	PCD
<i>HYDIN</i>	610812	PCD
<i>LRRC6</i>	614930	PCD
<i>NME8</i>	607421	PCD
<i>RSPH1</i>	609314	PCD
<i>RSPH4A</i>	612647	PCD
<i>RSPH9</i>	612648	PCD
<i>SPAG1</i>	603395	PCD
<i>ZMYND10</i>	607070	PCD

Abbreviations: ACLS, acrocallosal syndrome; ADKPD, autosomal dominant polycystic kidney disease; ALMS, Alström syndrome; ARKPD, autosomal recessive polycystic kidney disease; BBS, Bardet-Biedl syndrome; CED, cranioectodermal dysplasia syndrome; ECO, endocrine-cerebro-osteodysplasia syndrome; EVC, Ellis-van Creveld syndrome; JATD, Jeune asphyxiating thoracic dysplasia; JBTS, Joubert syndrome; LCA, Leber congenital amaurosis; MKKS, McKusick-Kaufman syndrome; MKS, Meckel-Gruber syndrome; MOSPGF, morbid obesity and spermatogenic failure; NPHP, nephronophthisis; OFDS, oral-facial-digital syndrome; PCD, primary ciliary dyskinesia; RP, retinitis pigmentosa; SGBS, Simpson-Golabi-Behmel syndrome; SLSN, Senior-Løken syndrome; SRTD, short-rib thoracic dysplasia; VHL, von Hippel-Lindau syndrome; WAD, Weyers acrodistal dysostosis.

Note: This overview of genes mutated in ciliopathies shows the heterogeneity of certain ciliopathies.

Corresponding OMIM identifiers are shown.

NA: OMIM number is not available.

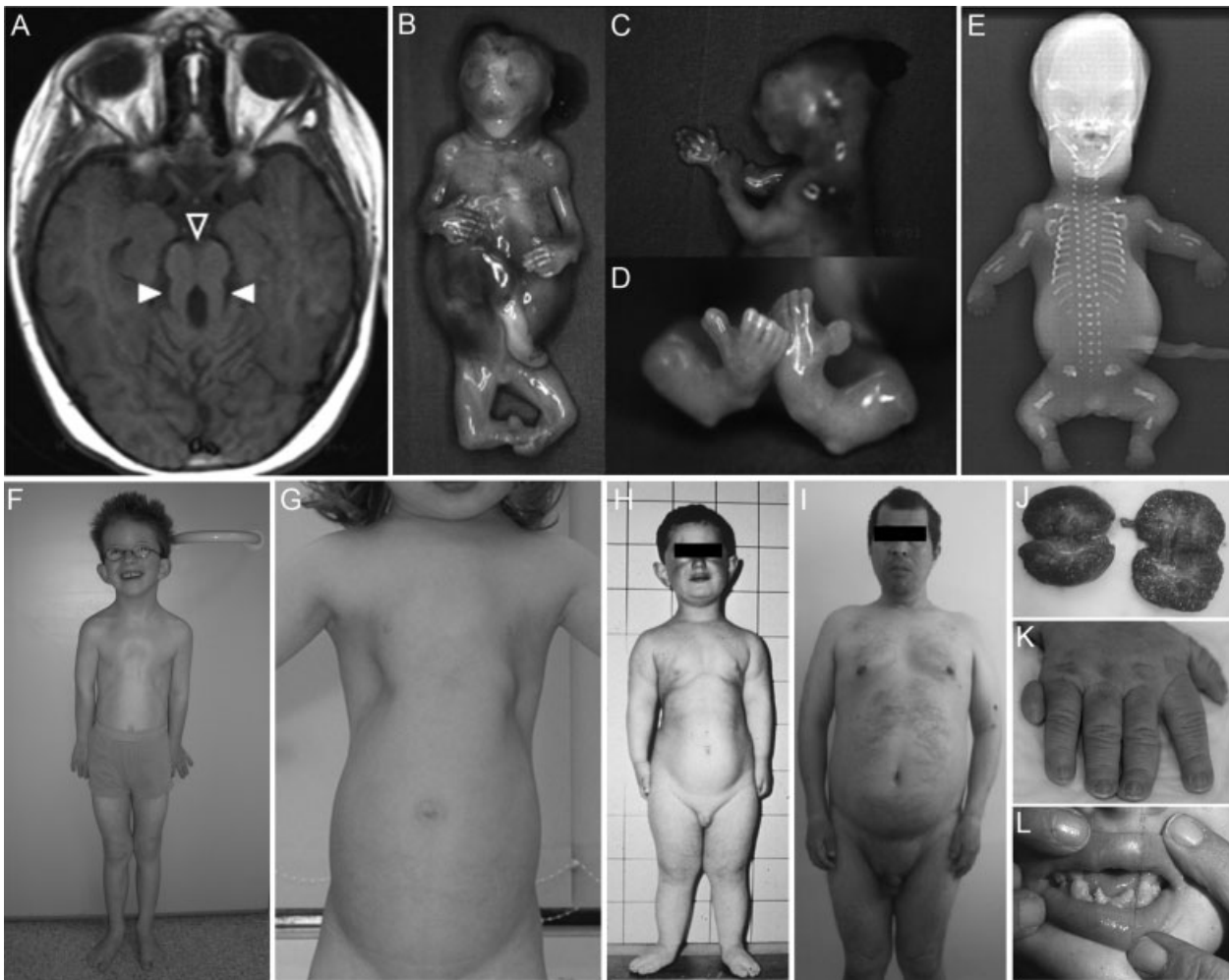
by major skeletal anomalies. Besides dwarfism, polydactyly, and ectodermal dysplasia, congenital heart malformations are commonly seen in EVC patients (►Fig. 2H, L). The latter have a major influence on the prognosis for individual patients.<sup>35</sup> Finally, a variety of OFDSs have been reported; however, many are based on single or few cases that are not molecularly tested.<sup>36</sup> The best known OFD type is OFD1,

which is X-linked and characterized by bifid tongue, oral frenula, cleft lip/palate, dental anomalies, syndactyly, polydactyly, polycystic kidney disease, and central nervous system abnormalities (►Fig. 2K).

Ciliary chondrodysplasias are genetically heterogeneous. EVC is caused by mutations in *EVC* and *EVC2*. *EVC* mutations have also been reported in patients with autosomal dominant Weyers acrodistal dysostosis, which presents with a milder phenotype than EVC.<sup>32</sup> Genetic overlap is mostly seen between CED, JATD, SRPS, and MZSDS (►Table 1), but it also occurs between OFDS and JBTS (►Table 1).<sup>29,36–38</sup> Most CED and SRTD mutations have been found in genes encoding IFT and associated motor proteins (►Fig. 1 and ►Table 1). OFDS is also genetically heterogeneous and recent data suggest that a proportion of OFDS proteins reside in centriolar appendages where they regulate ciliogenesis in an antagonistic manner; *C2CD3* promotes ciliary growth, whereas *OFD1* is a repressor.<sup>39,40</sup>

### Isolated and Syndromic Obesity

Nonsyndromic obesity is a growing problem in the general population and mainly caused by multifactorial factors. Yet in some cases isolated obesity is a mendelian disorder that results from ciliary anomalies.<sup>41</sup> Previously, ciliary defects had already been described in obesity syndromes such as in BBS and Alström syndrome (ALMS). Both syndromes display an autosomal recessive inheritance; however, oligogenic inheritance has also been described in BBS.<sup>42,43</sup> The prevalence of BBS varies markedly between populations—from 1:160,000 in northern Europe to 1:13,500 births in isolated communities in Kuwait.<sup>44</sup> Cardinal BBS features are rod-cone dystrophy, obesity, polydactyly, intellectual disability, hypogonadism, and renal dysfunction (►Fig. 2I). Secondary features include cardiovascular abnormalities, craniofacial anomalies, psychomotor delay, type 2 diabetes mellitus (T2DM), and hearing loss. The clinical diagnosis of BBS is based on the presence of at least four primary features or a combination of three primary plus two secondary features. In general, the BBS phenotype evolves during the first decade of life, except for polydactyly that is present at birth; generating an accurate clinical diagnosis may therefore be challenging in infancy and early childhood.<sup>45</sup> BBS has an adverse prognosis, with renal impairment being a frequent and important cause of death.<sup>46</sup> The phenotypically related ALMS is characterized by cone-rod dystrophy, obesity, hearing impairment, cardiomyopathy, T2DM diabetes, and hepatic and renal disease.<sup>47</sup> A clinical diagnosis is based on the aforementioned cardinal features and differs from BBS by the presence of hearing impairment and absence of polydactyly and learning difficulties.<sup>44</sup> While it may be difficult to make a clinical diagnosis of BBS and ALMS in early childhood, molecular testing clearly distinguishes both disorders. To date, only one gene, *ALMS1*, is known to be mutated in ALMS (►Table 1).<sup>48</sup> In BBS mutations in 19 genes are reported to be causative (►Table 1), explaining more than 80% of the patients.<sup>44</sup> The protein encoded by *ALMS1* and most of the proteins encoded by BBS-associated genes localize to the base of the primary cilium. *ALMS1* is also found in endosomal structures and is suggested to play a role



**Fig. 2 Phenotypic features of ciliopathies.** The following features are commonly seen in ciliopathies. (A) Distinctive cerebellar and brain stem malformation that is known as the “molar tooth sign” in a JBTS patient. Open and filled arrowheads point out a deepened interpeduncular fossa and elongated superior cerebellar peduncles, respectively. (B–D) MKS fetus at the 14th week of gestation showing swollen stomach due to enlarged kidneys (B), occipital encephalocele (C), and polydactyly of hands (B) and feet (D). (E) Radiograph of an SRTD fetus displaying a short and narrow thorax, horizontally oriented ribs, short tubular bones with smooth ends, short and ovoid tibiae, and postaxial polysyndactyly. (F) The patient with CED showing a narrow thorax, pectus excavatum, and rhizomelic shortening of limbs. (G) Narrow thorax in a JATD patient. (H, L) Images of an EVC patient with a long narrow chest and shortness of the limbs (H), and hypodontia, that is, absence of upper and lower conical incisors (L). (I) BBS patient with truncal obesity, micropenis, and apathetic facial features. (J) Cystic kidneys of a patient with infantile NPHP. (K) Postaxial polydactyly of the right hand in a patient with OFDS. Reprinted from Brancati et al. Joubert syndrome and related disorders. *Orphanet J Rare Dis* 2010 Jul 8;5:20, Copyright (2010), with permission from BioMed Central Ltd. (A); Tallila et al. Identification of *CC2D2A* as a Meckel syndrome gene adds an important piece to the ciliopathy puzzle. *Am J Hum Genet* 2008 Jun;82(6):1361–1367, Copyright (2008) with permission from Elsevier (B–D); El Hokayem et al. *NEK1* and *DYNC2H1* are both involved in short rib polydactyly Majewski type but not in Beemer Langer cases. *J Med Genet* 2012 Apr;49(4):227–233, Copyright (2012) with permission from BMJ Publishing Group Ltd. (E); Gilissen et al. Exome sequencing identifies *WDR35* variants involved in Sensenbrenner syndrome. *Am J Hum Genet* 2010 Sep 10;87(3):418–423, Copyright (2010) with permission from Elsevier (F); Schmidts. Clinical genetics and pathobiology of ciliary chondrodysplasias. *J Pediatr Genet* 2014 Nov;3(2):46–94, Copyright (2014) Thieme Publishers (G); Baujat and Le Merrer. Ellis-van Creveld syndrome. *Orphanet J Rare Dis* 2007 Jun; 4;2:27, Copyright (2007) with permission from Central Ltd. (H, L); Sahin et al. Two brothers with Bardet-Biedl syndrome presenting with chronic renal failure. *Case Rep Nephrol* 2015 Apr 3;2015:764973, Copyright (2015) (I); Oud et al. Early presentation of cystic kidneys in a family with a homozygous *INVS* mutation. *Am J Med Genet A* 2014 Jul;164A(7):1627–1634, Copyright (2014) with permission from John Wiley and Sons Inc. (J); Poretti et al. Delineation and diagnostic criteria of oral-facial-digital syndrome type VI. *Orphanet J Rare Dis* 2012 Jan 11;7:4, Copyright (2012) with permission from BioMed Central Ltd (K).

in the recycling endosome pathway.<sup>49</sup> The BBSome, composed of BBS1–2, 4–5, 7–9, and BBIP10, plays an important role in trafficking proteins into and out of the cilium, whereas the chaperonin complex that consists of BBS6 and BBS10–12 is required for assembly of the BBSome.<sup>44,50,51</sup> The remaining BBS proteins have variable functions. Some are thought to act

as GTPases or E3 ubiquitin ligases; others have been shown to regulate centriole migration and IFT.<sup>44</sup>

### Renal Ciliopathies

Polycystic kidneys are commonly seen in ciliopathies. The best-known renal ciliopathies are NPHP, and autosomal

dominant and autosomal recessive polycystic kidney disease (ADPKD and ARPKD, respectively). ADPKD is most common with an incidence of approximately 1:1,000 and is characterized by enlarged polycystic kidneys.<sup>52</sup> Liver cysts and abnormalities of the vasculature and heart may also be present.<sup>52</sup> *PKD1* and *PKD2* mutations explain 85% and 15% of ADPKD patients, respectively. Most individuals with ADPKD have a normal renal function during childhood; however, renal insufficiency progresses during life and results in end-stage renal disease (ESRD) in 50% of affected individuals.<sup>52</sup> In contrast, ARPKD is usually diagnosed in late pregnancy or at birth by the detection of enlarged cystic kidneys by ultrasound. Grossly enlarged kidneys cause pulmonary hypoplasia and thoracic compression leading to respiratory distress and death in approximately 30 to 50% of affected neonates. In the majority of remaining patients ESRD evolves in adulthood. In addition, virtually all ARPKD patients show hepatic involvement, that is, hepatomegaly and liver fibrosis.<sup>53</sup> To date, only mutations in *PKHD1* have been reported and these explain at least 80 to 87% of ARPKD patients.<sup>54</sup> *PKHD1* encodes the fibrocystin protein that forms a ciliary protein complex together with *PKD1* and *PKD2*.<sup>55</sup> Another monogenetic cause of ESRD in children is NPHP. Three types have been described based on age of onset of ESRD; infantile (< 2 years), juvenile (~13 years), and adolescent (~19 years) NPHP.<sup>56</sup> Juvenile NPHP is most common and affects 5 to 10% of the children with ESRD.<sup>57</sup> It is estimated to occur in 1:50,000 births in Canada and 1:900,000 in the United States.<sup>57</sup> Typical histopathologic characteristics are renal cysts, tubular basement membrane disruption, and tubulointerstitial fibrosis. Most features are shared between the different NPHP types; however, tubular basement membrane disruption is usually absent in infantile NPHP. Also, kidneys of patients with infantile NPHP are usually enlarged, whereas normal-sized or smaller kidneys are seen in patients with other forms of NPHP (→Fig. 2J).<sup>57</sup> NPHP can both be isolated and syndromic. Twenty NPHP genes are known so far and explain roughly one-third of the patients. All NPHP proteins reside in the basal body, transition zone, or axonemal base of primary cilia; however, they have also been found in other cellular sites such as cell junctions and nuclei.<sup>58,59</sup> Mutations in NPHP genes perturb a variety of signaling cascades including Wnt, Hedgehog, Hippo, and DNA damage response signaling, which in turn disrupt renal development and tissue homeostasis.<sup>59</sup>

### Primary Ciliary Dyskinesia

Most phenotypes associated by dysfunction of motile cilia result in primary ciliary dyskinesia (PCD). Cardinal symptoms are neonatal respiratory abnormalities and airway infections during childhood, which eventually develop into bronchiectasis. Patients also often present with *situs inversus*, dextrocardia, and infertility.<sup>60,61</sup> Diagnosis of PCD is challenging due to the phenotypic overlap with other respiratory diseases such as asthma, immune deficiencies, and bronchomalacia, and the absence of clear diagnostic criteria further complicates making an accurate diagnosis.<sup>61</sup> The prevalence of PCD, being 1:10,000 to 1:20,000, may therefore be and underestimate.<sup>61</sup> Mutations in 30 genes have been

associated with PCD and explain 60 to 70% of the cases.<sup>3,61</sup> The inheritance pattern associated with PCD is usually autosomal recessive; however, X-linked or autosomal dominant inheritance have also been reported in a few cases.<sup>61</sup> Roughly 60% of proteins that are encoded by PCD genes are found in protein complexes and axonemal structures that are typically found in motile cilia such as inner and outer dynein arms, radial spokes, the central microtubule pair, and nexin links (→Fig. 1), whereas the remaining proteins are cytoplasmic with a role in (pre)assembly and transportation of dynein components.<sup>3</sup>

### Why Is Genetic Screening Important?

The phenotypic spectrum of ciliopathies as a whole is very broad, but phenotypic overlap between certain ciliopathies can be marked, such as in JBTS and MKS or BBS and ALMS.<sup>20</sup> A correct diagnosis can thus not always be made solely based on the clinical phenotype but requires molecular testing. Molecular information facilitates making an accurate diagnosis and is therefore essential for proper genetic counseling, prognosis, recurrence risk estimation, and clinical management in the clinic. In the past years, gene panels and exomes have been analyzed by next-generation sequencing (NGS) technologies. This resulted in the detection of numerous pathogenic mutations and dozens of novel disease genes in a nick of time. NGS thus enormously facilitated our ability to make an early diagnosis and to recognize important genotype-phenotype correlations.<sup>62,63</sup> This knowledge contributes to the clinician's ability to correctly inform patients about expected disease progression and allows clinicians to form appropriate interdisciplinary teams leading to optimal clinical care and disease management. For example, early diagnosis is highly beneficial to PCD patients in whom progression to irreversible lung disease continues unless treated early on.<sup>64</sup> Gene and mutation identification also allows for development of personalized therapies such as targeted gene therapy. This is important as no cures are available for ciliopathies to date. Gene therapy successes have been booked in patients with a variety of hereditary disorders,<sup>65–68</sup> including retinal degeneration. The latter is a common feature in many ciliopathies and is probably treatable in the future.<sup>69</sup> In addition to the retina, researchers also started to investigate possibilities for gene therapy in ciliopathies affecting the respiratory system.<sup>70–72</sup> For example, ciliary beating was restored in ex vivo cell cultures from PCD patients harboring mutations in *DNAI1* by introducing a healthy copy of this gene via lentiviral transduction.<sup>72</sup>

### Ciliary Genes and Next-Generation Sequencing

#### Research

Since 1994,<sup>73</sup> 127 ciliopathy genes have been identified (→Table 1), initially with linkage studies and positional cloning (1980s–2005), later with single nucleotide polymorphism (SNP) microarrays and candidate gene analysis (2006–2010), and most recently with various NGS methods (2009–

2015).<sup>74–81</sup> Targeted gene panels have been sequenced with NGS methods in dozens or even hundreds of subsets of patients for efficient and cheap mutation detection in known genes in a variety of ciliopathies, including, but not limited to, JBTS, BBS, ALMS, NPHP, and PCD.<sup>62,63,82,83</sup> Some gene panels not only targeted known ciliary disease genes but also included candidate genes, for example genes with a predicted ciliary function. These panels have proven to be successful tools to identify novel disease genes by various groups.<sup>81,83–85</sup> Currently, we can genetically explain 45 to 90% of JBTS and MKS patients,<sup>63,86,87</sup> 55 to 70% of CED and SRTD patients,<sup>32,88</sup> 84% of EVC patients,<sup>89</sup> 80% of BBS and ALMS patients,<sup>44</sup> 21% of NPHP patients,<sup>90</sup> and 65% of PCD patients with panels.<sup>91</sup> The advantage of gene panel testing versus genomic methods such as whole-exome sequencing (WES), whereby all exons of the genome are sequenced, is that panels usually result in higher coverage data at a relatively low cost and with high-throughput.<sup>92,93</sup> In addition, the chance of identification of incidental findings, which are clinically relevant findings that are unrelated to the clinical problem for which screening was initially intended, is extremely low when only known ciliopathy-specific genes are being sequenced<sup>94</sup>; however, it is difficult to add new genes to existing gene panels.<sup>94</sup> The possibility of detection of incidental findings is an important concern when analyzing WES data, however, WES has proven to be highly successful for the identification of a mutation in a gene that has not previously been implicated in ciliary disease. Furthermore, retrospective analysis of the data is possible.<sup>94</sup> A recent research study reported that WES provides a molecular diagnosis in 44% of ciliopathy patients.<sup>95</sup>

### Diagnostics

In past and current genome diagnostics for ciliopathies, prescreening methods such as (SNP) microarrays for homozygosity mapping (and copy number detection, CNV) or targeted panels with only known mutations in ciliary disease genes have been used to reduce costs by limiting the list of genes to be tested by gene-specific methods such as Sanger sequencing. More recently, WES started to be implemented as a diagnostic test for ciliopathies and is considered to be cost-effective when a clinician needs to investigate more than two genes to identify the genetic cause of disease, a scenario that often applies when a ciliopathy patient presents in the clinic considering the enormous genetic heterogeneity in these disorders.<sup>95,96</sup> While incidental findings are a concern in WES, these can be easily circumvented in diagnostics by first (or only) analyzing known ciliopathy genes and leaving the remainder of WES data concealed. This approach has proven to be an effective tool for gene and mutation identification with reasonable throughput and acceptable costs.<sup>96,97</sup> Diagnostic WES has an estimated success rate of approximately 30% for ciliopathies in our institute (personal communication, D. Lugtenberg and [www.genome-diagnosticsnijmegen.nl](http://www.genome-diagnosticsnijmegen.nl)). Other groups reported general success rates for rare diseases that vary between 20 and 45% based on various studies that include thousands of patients.<sup>98–101</sup>

## Current Next-Generation Sequencing Obstacles

Although NGS sequencing has tremendously improved mutation detection for numerous disorders, ciliopathies included, there are still several hurdles to take with respect to accurate variant detection and prioritization.

### Technical Challenges

In WES the coverage, referring to the number of times that a genomic region is read, is not always complete for each ciliopathy gene, which prevents detection of causative variants. One gene that is not sufficiently covered in any standard WES procedure is *PKD1*. This is explained by the existence of six *PKD1* pseudogenes that share greater than 97% sequence identity with the first 33 exons of *PKD1*, which complicates standard alignment algorithms.<sup>102,103</sup> This problem can be resolved by combining NGS with long-range PCR<sup>104,105</sup> or by using custom-designed targeted enrichment libraries<sup>106</sup> and applying mapping algorithms that facilitate specific mapping of reads to the *PKD1* gene.<sup>103</sup> Pseudogenes are not the sole cause for the occurrence of low coverage; genomic regions with high GC contents and repetitive sequences are also difficult to read.<sup>107</sup> Another NGS challenge is represented by the occurrence of pathogenic mosaicisms and postzygotic mutations.<sup>108</sup> Such variation is difficult to detect because of the sporadic occurrence of these variants in sequence reads. Stringent filtering criteria may thus prevent us from identifying these mutations in WES data. Also, this type of variation may not be detectable at all when occurring in other tissues than the tested specimen. Other variants that cannot be detected by WES are noncoding mutations, translocations, and imprinting changes.<sup>95,97</sup> Copy number variations (CNVs) can be detected by different algorithms, but detection may be limited to three or more exons depending on the software used.<sup>109</sup>

### Variant Interpretation

Besides technical improvements, genome analysis would also highly benefit from improved variant understanding. Many WES-detected variants have not been associated with a clinical phenotype before and their clinical significance is therefore unclear. Such variants are referred to as variants of unknown clinical significance (VUS).<sup>110</sup> VUS may be found in genes that have previously been associated with ciliopathies and in genes in which clinical variation has not been described yet. One can distinguish between benign and pathogenic variants based on bioinformatic information, but also by assessing effects of VUS *in vitro* in ciliated cells derived from patient's shed deciduous teeth, skin biopsy, or urine samples.<sup>111,112</sup> Besides cellular phenotyping, simple organisms with ciliated cells such as *Danio rerio* (zebrafish), *Caenorhabditis elegans* (worm), and *Drosophila melanogaster* (fruit fly) can also be used to model mutational effects.<sup>113–115</sup> Although functional testing would allow for a better understanding of VUS, this type of testing is often not available in a clinical setting and is difficult to implement as each variant or gene requires a different assay. Another method that facilitates



variant interpretation in both research and clinic is data sharing and public registration of identified variants and associated phenotypes<sup>95</sup>; if patients with similar ciliopathy features are identified with identical variants in known disease genes or if deleterious variants in the same (previously unreported) gene are found, these replicative findings can render important clues about the nature of a variant. An initiative that promotes data sharing for rare disorders is the “Matchmaker Exchange” database (<http://www.matchmakerexchange.org/>). When genotypic and phenotypic data are entered on this Web site, it is registered in various connected databases that in turn allows bonding between scientists and clinical staff with matching interests,<sup>95</sup> which will benefit patients and their families in the long run.

## Future Perspectives

This review discussed mutation detection for ciliary disorders through gene panels and WES in both research and diagnostics. We did not highlight the power of whole-genome sequencing yet, while this is the technology of the future. The general advantage of WGS compared with WES is that it leads to improved mutation detection in protein-coding DNA.<sup>116</sup> In addition, WGS allows detection of variation in noncoding genomic regions. Although our understanding of variation in noncoding DNA is still poor, future research will allow for improved interpretation of variants in these regions, probably in particular in conserved regulatory elements such as promoters, enhancers, insulators, and other regulators.<sup>117</sup> Other WGS challenges concern data storage and throughput, which rely on the availability of excellent bioinformatic platforms; however, WGS platforms and procedures are improving and fastening as we speak. For example, it was recently shown that it is possible to effectively conduct WGS and data analysis within 26 to 50 hours periods in ill neonates.<sup>118,119</sup> This strategy is also of interest for newborns with a suspected ciliary disorder as clinical features of classic syndromes may be incomplete and difficult to recognize in infancy, while molecular knowledge allows accurate and early diagnosis and prognosis and directs decisions for optimal disease management in these children. Besides implementing NGS for ciliopathies in postnatal diagnostics, there also is a need to conduct such testing prenatally as clinical features of ciliopathies may present as early as the first trimester of pregnancy.<sup>120</sup> In addition, improved prenatal testing also allows for optimal management and counseling for parents who have a risk of giving birth to a child with a ciliopathy. The future implementation of NGS-based methods in prenatal diagnostics will rely on short turnaround times of clinical reports as limited time is available for counseling and decision making. Prenatal diagnostics will further benefit from the possibility to conduct genetic screening on fetal DNA derived from maternal blood as that avoids the need for invasive amniocentesis and chorionic villus sampling. Notably, NGS is already successfully being used for detection of aneuploidies and CNVs in fetal DNA extracts from maternal blood, whereas technological advances may allow for genome-wide single nucleotide variation (SNV) detection in fetal-maternal DNA in the future.<sup>121–123</sup>

In conclusion, NGS-based screening revolutionized the ciliopathy research and diagnostics in recent years, and this process is still ongoing. While technical and bioinformatic challenges remain, NGS methods are gradually being implemented in clinical laboratories with reasonable throughput, for acceptable costs, and with significant mutation identification success for ciliopathies. In the future, return of molecular reports will accelerate with turnaround times of only one or a few days, and screening of ciliopathy panels, WES, and WGS will probably evolve as part of noninvasive procedures in prenatal genome diagnostics. These developments will further contribute to improved health care for families affected by ciliary disorders.

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