Spectrum of Clinical Diseases Caused By Disorders of Primary Cilia

Stephanie M. Ware¹, Meral Gunay-Aygun², and Friedhelm Hildebrandt³

¹Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; ²National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland; and ³Howard Hughes Medical Institute and Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, Michigan

The ciliopathies are a category of diseases caused by disruption of the physiological functions of cilia. Ciliary dysfunction results in a broad range of phenotypes, including renal, hepatic, and pancreatic cyst formation; situs abnormalities; retinal degeneration; anosmia; cerebellar or other brain anomalies; postaxial polydactyly; bronchiectasis; and infertility. The specific clinical features are dictated by the subtype, structure, distribution, and function of the affected cilia. This review highlights the clinical variability caused by dysfunction of motile and nonmotile primary cilia and emphasizes the genetic heterogeneity and phenotypic overlap that are characteristics of these disorders. There is a need for additional research to understand the shared and unique functions of motile and nonmotile cilia and the pathophysiology resulting from mutations in cilia, basal bodies, or centrosomes. Increased understanding of ciliary biology will improve the diagnosis and management of primary ciliary dyskinesia, syndromic ciliopathies, and cilia-related cystic diseases.

Keywords: primary cilia; ciliopathy; heterotaxy; nephronophthisis; cyst

Ciliopathies are phenotypically and genetically heterogeneous disorders that share ciliary dysfunction as a common pathological mechanism. Motile cilia are primarily found on epithelial cells that line the respiratory tract, brain ventricles, and oviducts. These cilia have nine microtubule doublets surrounding a central pair, a "9+2" arrangement that is visible by electron microscopy of cross-sectional views. Absence or dysfunction of motile cilia causes primary ciliary dyskinesia (PCD). In contrast, primary cilia lack the central microtubule doublet and have a "9+0" arrangement of microtubules. Primary cilia extend from the surface of almost all cell types in the human body, including epithelial cells such as those lining the kidney tubules and bile ducts as well as nonepithelial cells such as chondrocytes and neurons (1). Primary cilia are present as monocilia and exist as a single extension per cell. Initially, cilia were described as motile or nonmotile, with the latter group encompassing primary cilia or sensory cilia (Table 1). However, the discovery of motile primary cilia at the embryonic node and the identification of sensory functions for motile cilia has rendered these historic designations imperfect. Although these broad classifications have utility, as the complexities of cilia structure and

function are better understood, further delineation of subtypes may occur. For example, the rod and cone cells of the retina possess a very unique type of primary cilium (connecting cilium) equipped with a specialized expanded tip that forms the outer segment of these photoreceptor cells (2). The node or organizer, a transient embryonic structure required for left-right patterning, also possesses a unique type of primary cilium that lacks a central pair of microtubules but is nevertheless motile. Dysfunction of cilia at the node can result in *situs inversus* or heterotaxy, a phenotype that is characteristic of PCD and primary, nonmotile ciliopathies. Understanding the biological function of motile and nonmotile cilia will provide insight into the underlying pathophysiology of ciliary disorders and the wide array of clinical phenotypes resulting from their disruption. The number of diseases known to be caused by ciliary pathology has increased dramatically in the last decade (summarized in Table 2). The diagnosis and management of patients with ciliary disorders will benefit from elucidating the shared and unique functions directed by motile and nonmotile cilia in a tissue- and developmental-stage-specific context.

FUNCTIONS OF PRIMARY CILIA

Primary cilia, both nonmotile cilia and motile cilia at the node, function as sensory organelles and coordinators of signal transduction pathways such as hedgehog (Hh), wntless (WNT), platelet-derived growth factor- α , and fibroblast growth factor pathways (3, 4). In addition, primary cilia are important for the regulation of intracellular calcium. Nonmotile cilia are sensory antennas of the cell, and intact nonmotile cilia function is required for maintenance of the balance between the canonical and noncanonical (planar cell polarity) parts of the WNT pathway. For example, disruption of planar cell polarity contributes to the pathogenesis of polycystic kidney disease (PKD) by resulting in abnormalities in multicellular activities that maintain tubular diameter, such as mitotic spindle orientation and convergence and extension movements (5). Nonmotile cilia also sense mechanical stimuli such as fluid flow in the lumen of tubular structures (kidney tubules and bile ducts) and convert these to alterations in calcium fluxes within the cell (1). Proteins defective in human PKD are required for this mechanosensory function of nonmotile cilia. Hence, during embryologic development, primary cilia regulate fundamental processes, such as determination of asymmetry of internal organs and neural tube and limb patterning. Nonmotile cilia are also required for the maintenance of differentiated tissues, such as retina and renal tubules. In addition, they play a role in the pathogenesis of certain types of obesity, probably through their role in appetite control and adipocyte differentiation (6).

HETEROTAXY SPECTRUM DISORDERS

Heterotaxy, or *situs ambiguus*, is an abnormal arrangement of thoracic and/or abdominal viscera. *Situs inversus* is another type of laterality disorder in which all internal organs are reversed. Typically, patients with *situs inversus* do not have the congenital anomalies

(Received in original form March 15, 2011; accepted in final form April 25, 2011)

The conference was supported by a grant from the National Institutes of Health (R13HL105073-01) and by the Primary Ciliary Dyskinesia Foundation. The authors' research is supported by the National Institutes of Health grants HL088639 (S.M.W.) and DK068305 and DK064614 (F.H.) and Burroughs-Wellcome Fund #1008496 (S.M.W.).

Correspondence and requests for reprints should be addressed to Stephanie M. Ware, M.D., Ph.D., Cincinnati Children's Hospital Medical Center, 240 Albert Sabin Way, MLC 7020, Cincinnati, OH 45244. E-mail: stephanie.ware@cchmc.org

Proc Am Thorac Soc Vol 8, pp 444-450, 2011

DOI: 10.1513/pats.201103-025SD

Internet address: www.atsjournals.org

TABLE 1. CLASSIFICATION OF CILIA

Category	Microtubule Arrangement	Cilia per Cell	Motility	Function	Cell Types
Motile cilia	9+2	Multiple	Planar motion	Mechanical; possible secondary chemosensory functions	Airways, spermatozoa, fallopian tubes, choroid plexus, and brain ventricles
Primary cilia	9+0	One	Rotary motion	Mechanosensory; chemosensory	Embryonic node
Primary or sensory cilia	9+0	One	Nonmotile	Mechanosensory; chemosensory	Renal tubules, pancreatic or bile ducts, bone and cartilage, retina, cochlea, developing heart, fibroblasts, etc.

that occur in heterotaxy syndrome. Embryologically, heterotaxy and situs inversus fall into the category of disorders of laterality because they have their root in the abnormal development of the left–right body axis. Primary cilia present on the embryonic node are required for the generation of the left–right axis during embryonic development (7, 8). The ciliated embryonic node is unusual in that it contains motile primary cilia that generate fluid flow and non-motile primary cilia that act as mechanosensors. Abnormal situs is a hallmark feature of a number of ciliopathies.

The nomenclature related to heterotaxy has traditionally been confusing, with both anatomic and clinical designations (e.g., right isomerism, asplenia syndrome) (9). Any internal organ that is asymmetrically positioned can be abnormal in individuals with heterotaxy. Improper symmetry, such as bilateral trilobed lungs, and failure to regress symmetric embryonic structures, such as persistent bilateral superior vena cava, are additional manifestations of heterotaxy. Midline defects occur in approximately 40% of patients (10). The wide phenotypic

TABLE 2. SUMMARY OF CILIOPATHIES

Ciliopathy	Features	Gene(s)	Pulmonary involvement
Primary ciliary dyskinesia	Bronchiectasis, sinusitis, otitis media, infertility, situs defects	DNAI1, DNAH5, DNAH11, DNAI2, KTU, TXNDC3, RPGR, RSPH4A, RSPH9, LRRC50, CCDC40, CCDC39	Prominent
Polycystic kidney disease	Renal cysts; hepatic fibrosis; autosomal dominant and recessive forms	PKHD1, PKD1, PKD2	Reported: bronchiectasis
Nephronophthisis	Renal cysts with or without extra-renal symptoms	NPHP1-4, IQCB1, CEP290, GLIS2, RPGRIP1L, NEK8, SDCCAG8, TMEM67, TTC21B	Reported in conjunction with allelic syndromes
Senior-Loken syndrome	Juvenile nephronophthisis, Leber amaurosis	NPHP1-6, SDCCAG8	Not reported
Leber congenital amaurosis	Visual impairment in first year of life; pigmentary retinopathy	GUCY2D, RPE65, LCA3-14 (including LCA10, CEP290)	Reported: abnormal respiratory cilia
Meckel-Gruber syndrome	Renal cysts, CNS anomalies (encephalocele), polydactyly, congenital heart defects	MKS1, TMEM216, TMEM67, CEP290, RPGRIP1L, CC2D2A	Not reported
Joubert and related syndromes	hypoplasia of the cerebellar vermis (molar tooth sign), dysregulated breathing pattern, retinal dystrophy, renal anomalies	JBT51, TMEM216, AHI1, NPHP1, CEP290, TMEM67, RPGRIP1L, ARL13B, CC2D2A	Not reported: episodic tachypnea ± apnea described as central in origin
Bardet-Biedl syndrome	Obesity, polydactyly, retinitis pigmentosa, anosmia, congenital heart defects	BBS1, 2, ARL6, BBS4,5, MKKS, BBS7, TTC8, BBS9, 10, TRIM32, BBS12, MKS1, CEP290, C2ORF86; modifiers MKS1, MKS3, CCDC28B	Not reported, but evidence for airway ciliary dysfunction in animal models
Oral-facial-digital syndrome type I	Oral cavity, face, and digit anomalies; CNS abnormalities; cystic kidney disease; X-linked with male lethality	OFD1	Reported: PCD phenotype
Alstrom syndrome	Dilated cardiomyopathy, obesity, sensorineural hearing loss, retinitis pigmentosa, endocrine abnormalities, renal and hepatic disease	ALMS1	Frequent: pneumonia, COPD, moderate to severe interstitial fibrosis
McKusick-Kaufman syndrome	Urogenital anomalies including hydrometrocolpos, postaxial polydactyly, congenital heart defects	MKKS (BBS6)	Not reported
Ellis van Creveld syndrome	Skeletal dysplasia; congenital heart defects; polydactyly; ectodermal dysplasia	EVC1, EVC2	Reported: respiratory distress due to narrow thorax
Jeunechondrodysplasia	Skeletal dysplasia; thoracic deformities; polydactyly; renal cysts; retinitis pigmentosa	IFT80	Frequent: pulmonary hypoplasia, respiratory distress, restrictive lung disease
Cranioectodermal dysplasia (Sensenbrenner syndrome)	Cranioectodermal dysplasia; narrow thorax, dental anomalies, hepatic and renal involvement	IFT122, WDR35	Not reported
Short rib polydactyly	Lethal skeletal dysplasia, polydactyly, multiple congenital anomalies	NEK1, DYNC2H1	Frequent: pulmonary hypoplasia, respiratory distress, restrictive lung disease
Heterotaxy (?)	Visceral situs anomalies, asplenia or polysplenia, congenital heart defects, biliary atresia, midline defects		Frequent: situs anomalies; reported: PCD

Definition of abbreviations: CNS = central nervous system; PCD = primary ciliary dyskinesia.

spectrum and range of congenital anomalies that can be seen in heterotaxy syndrome has hindered clinical care and research progress.

“Classic” heterotaxy, in which a characteristic cardiovascular malformation is identified in conjunction with visceral situs anomalies, comprises approximately 3% of congenital heart defect cases and has an estimated prevalence of 1 in 10,000 live births. However, this most likely underestimates the true incidence of heterotaxy spectrum disorders. Heterotaxy is a clinically and genetically heterogeneous condition, and patients with a combination of cardiovascular malformations and visceral situs anomalies represent the severe end of this spectrum. Isolated cardiovascular malformations may be caused by mutations in the same genes that cause heterotaxy, and therefore the true incidence of these defects of laterality is not known with certainty (11). Overall, male patients with heterotaxy outnumber female patients 2:1, although within the polysplenia subtype the gender distribution is equal. Known environmental risk factors for heterotaxy include twin gestation, maternal diabetes, and maternal cocaine use (12).

The clinical evaluation for patients with heterotaxy syndrome is focused on delineating the anatomy and managing the congenital anomalies present (13). Most frequently, patients with heterotaxy present in the newborn period with cyanotic congenital heart disease. Echocardiography is important for defining cardiac anatomy. In some cases, chest MRA is useful for better visualization of the vasculature. Abdominal ultrasound is necessary to determine the position of the liver and stomach, spleen position and number, and renal anatomy. Other imaging includes head ultrasound or brain MRI, upper gastrointestinal series to evaluate malrotation, chest radiograph and spine films, and consideration of hepatobiliary scintigraphy. A blood smear for Howell-Jolly bodies is an initial evaluation for splenic function. Chromosome microarray analysis is indicated due to the association with chromosome abnormalities. In addition, molecular testing for *ZIC3*, *NODAL*, *CFC1*, and *FOXH1*, four genes known to cause heterotaxy, is available on a clinical basis.

Consideration should be given to evaluation for PCD in patients with heterotaxy. The prevalence of PCD in this population is unknown, but recent clinical research and animal models suggest that the underlying etiology may be identical for a subset of patients in these groups. Neonatal respiratory distress (not related to cardiovascular malformations) is a frequent manifestation of PCD. Chronic wet, productive cough; recurrent pneumonia; and persistent rhinitis are important features that should trigger evaluation. Bronchiectasis, chronic otitis media with conductive hearing loss, hydrocephalus, or retinitis pigmentosa are features of motile ciliary dysfunction that should be recognized as symptoms unrelated to the cardiovascular malformations in heterotaxy and should prompt evaluation by the appropriate specialists, including pulmonologists. Further clinical research is necessary to better define the degree of overlap between PCD and heterotaxy and to provide better management of comorbidities.

Heterotaxy is highly heritable, indicating a strong genetic influence, although complex or multifactorial causation may predominate. X-linked heterotaxy is caused by mutations or deletions of the zinc-finger transcription factor *ZIC3*. We have investigated the mechanistic basis of X-linked heterotaxy in mouse and *Xenopus* models (14–16). The results indicate that *Zic3* acts early in development upstream of *Nodal* expression at the node. Further identification of the developmental pathways disrupted by *Zic3* loss of function will elucidate important candidate genes for human heterotaxy and will further delineate shared and divergent functions with genes causing PCD and other ciliopathies.

Mutations in genes important in the generation of left–right patterning, such as *NODAL* and *CFC1*, have been shown to

cause heterotaxy (17–19). Gene mutations identified in human laterality disorders have been reviewed recently (13). The majority of the mutations identified have reduced penetrance and variable expressivity. Although most cases of heterotaxy are single occurrences in a pedigree, approximately 10% of infants with heterotaxy have a family history of a close relative with a congenital heart defect. Epidemiologic data strongly indicate the important role of genetic factors in heterotaxy, but there is a fundamental lack of information about the genetic basis of disease in the vast majority of cases.

OVERVIEW OF SYNDROMIC CILIOPATHIES

Diseases of the nonmotile cilia are commonly associated with a spectrum of fibrocystic changes in the kidneys and the liver, including PKD, nephronophthisis (NPHP) and renal cystic dysplasia, congenital hepatic fibrosis, Caroli’s disease, and polycystic liver disease (20, 21). Other features include developmental anomalies of the cerebellum and midbrain, retinal degeneration, colobomas of the retina and iris, polydactyly, abnormal bone growth, obesity, anosmia and other sensory defects, situs defects, and pancreatic cysts or dysplasia (Table 2). Autosomal dominant (ADPKD) and recessive (ARPKD) polycystic kidney diseases and NPHP are the most common hepatorenal fibrocystic diseases. Pleiotropic syndromic ciliopathies with multisystem involvement include Joubert syndrome (JS) and related cerebellar disorders, Bardet-Biedl (BBS), Alstrom syndrome, Meckel-Gruber (MKS), Senior-Loken syndrome, and oral-facial-digital type 1 (OFD1) syndromes. Skeletal ciliopathies include Jeune chondrodysplasia, cranioectodermal dysplasia, short-rib polydactyly, and Ellis-Van Creveld (EVC) syndromes.

Liver disease in ciliopathies is not a primary disease of the hepatocytes but rather is a developmental defect of the portobiliary system, termed ductal plate malformation (DPM) (21, 22). DPM is defective remodeling of the biliary system characterized by retention of excessive numbers of embryonic bile duct remnants, abnormal portal veins, and periportal fibrosis. DPM of the peripheral biliary system causes congenital hepatic fibrosis, whereas DPM of the central biliary tree results in Caroli’s disease. The combination of the two is referred to as Caroli’s syndrome. The characteristics of liver disease in ARPKD and ADPKD are different. ARPKD is characterized by congenital hepatic fibrosis and Caroli’s disease and is often complicated by portal hypertension, whereas ADPKD patients develop polycystic liver disease and typically do not have portal hypertension (20, 21). In contrast to Caroli’s disease/Caroli’s syndrome, in which liver cysts are dilated parts of the bile tree itself, polycystic liver disease is characterized by isolated cysts that are not in continuity with the bile tree.

JS and related cerebellar disorders are a heterogeneous group of disorders in which the defining common pathology is the so-called “molar tooth sign” on axial brain MRI that is caused by a combination of mid- and hindbrain anomalies, including cerebellar vermis hypoplasia and abnormal superior cerebellar peduncles (23). Oculomotor apraxia and speech apraxia are typical. Variable features in JS include retinal degeneration, colobomas, renal disease (commonly in the form of NPHP), congenital hepatic fibrosis/Caroli’s syndrome, and polydactyly. BBS is characterized by retinal degeneration, cognitive impairment, obesity, and hypothalamic hypogonadism in male patients; variable features include renal disease, congenital hepatic fibrosis, polydactyly, cardiac defects, situs inversus, and abnormalities of internal genital organs in female patients (24). Alstrom syndrome is also characterized by retinal dystrophy, obesity, cardiomyopathy, progressive sensorineural hearing impairment, and insulin resistance. MKS represents the most severe end of the

nonmotile ciliopathy spectrum, with the typical triad of occipital encephalocele, cystic dysplastic kidneys, and postaxial polydactyly (25). Congenital hepatic fibrosis is an invariable feature in MKS. OFD1 is an X-linked dominant, male-lethal, nonmotile ciliopathy characterized by prominent external features, including oral clefts, hamartomas, or cysts of the tongue, and digital anomalies and visceral involvement, including PKD and biliary and pancreatic cystic disease (26). Jeune chondrodysplasia, which is also referred to as asphyxiating thoracic dysplasia, is characterized by small thorax due to short ribs, short stature that is mainly caused by short limbs, and polydactyly (27). Jeune patients who survive the small thorax-related respiratory distress develop NPHP, retinopathy, and fibrocystic disease of the pancreas. EVC is characterized by short stature associated with short limbs, congenital cardiac defects, teeth abnormalities, congenital hepatic fibrosis, and situs anomalies in some cases (28). Cranioectodermal dysplasia is characterized by frontal predominance and widely spaced eyes; teeth, hair, and nail abnormalities; congenital hepatic fibrosis; and a NPHP-like renal pathology (29).

Genetic heterogeneity (multiple genes causing similar clinical phenotype) and genotypic and phenotypic overlap are two characteristics of syndromic ciliopathies (30, 31). There are multiple genes identified for JS and related cerebellar disorders, BBS, and MKS, still not accounting for all patients. These disorders were originally defined based on their most prominent clinical features. As we understand these disorders better, we realize that the borders between them are blurred at the phenotypic and genotypic levels.

CYSTIC KIDNEY DISEASES AND NEPHRONOPHTHISIS-RELATED CILIOPATHIES

Nephronophthisis (NPHP), an autosomal recessive cystic kidney disease, represents the most common genetic cause of terminal renal failure occurring in the first three decades of life. It may be associated with retinitis pigmentosa (RP) in the renal-retinal disorder Senior-Loken syndrome or with cerebellar vermis aplasia and mental retardation in JS. Ten different recessive genes have been identified as mutated in NPHP-related ciliopathies (NPHP-RC) with and without extrarenal manifestations.

NPHP1 is mutated in NPHP type 1 (32). Its gene product, nephrocystin-1, localizes to transition fibers of cilia and acts in focal adhesion signaling. Mutations in the *NPHP2/inversin* gene cause infantile NPHP (type 2) with the rare association of situs inversus and RP. Demonstration of expression of *NPHP1* and *NPHP2* in primary cilia of renal epithelial cells (33) supported the “ciliopathy theory” for the pathogenesis of cystic kidney diseases (34). This theory states that the products (“cystoproteins”) of all genes mutated in cystic kidney disease in humans, mice, or zebrafish are expressed in primary cilia, basal bodies, or centrosomes of renal epithelial cells (35). Identification of *NPHP3* as mutated in NPHP type 3 supported this theory and revealed an *Nphp3* mutation as causing the renal cystic disease in *pcy* mice (36). For this animal model, efficient treatment was demonstrated (37).

Some of the general features of renal cystic diseases are exemplified by studies on the *NPHP* gene products nephrocystins. Positional cloning of *NPHP4* as mutated in Senior-Loken syndrome (38) led to the demonstration that its gene product, nephrocystin-4, is conserved in *Caenorhabditis elegans* and expressed together with nephrocystin-1 in ciliated head and tail neurons of the nematode (39). The role of primary cilia function for retinal-renal syndromes was confirmed by identification of the novel *NPHP5* gene (40), demonstrating interaction of nephrocystin-5 with calmodulin and the retinitis pigmentosa

GTPase regulator (RPGR). Recently, several signaling pathways have been implicated in the downstream signaling pathways that connect ciliary and basal body function to the renal cystic phenotype. These include the Wnt signaling and planar cell polarity pathways (41–43) and the hedgehog signaling pathway (44).

Recessive mutation of *NPHP6/CEP290* was found to cause JS (45). *NPHP6* encodes a centrosomal protein, nephrocystin-6, which modulates the activity of ATF4/CREB2, a transcription factor implicated in cAMP-dependent renal cyst formation. We demonstrated that abrogation of *nphp6* function in zebrafish causes planar cell polarity (convergent extension) defects and recapitulates the human phenotype of JS. Hypomorphic mutations in *NPHP6/CEP290* were shown to cause retinal degeneration (*rds16*) in mice (46) and represent a major cause of Leber’s congenital amaurosis in humans (47). Identification of the *NPHP6* gene, therefore, established a link between centrosome function and tissue architecture in the pathogenesis of cystic kidney disease and in central nervous system development. Mutation of *GLIS2/NPHP7* causes NPHP type 7 in humans and mice (48). Differential gene expression studies on *Glis2* mutant kidneys demonstrated that genes promoting epithelial-to-mesenchymal transition and fibrosis are up-regulated in the absence of *Glis2*, demonstrating that *Glis2* is essential for the maintenance of renal tissue architecture through prevention of apoptosis and fibrosis. Recently, mutations of *RPGRIP1L/NPHP8* were shown to cause MKS and JS (49), and it has been demonstrated that *NEK8* mutations may rarely cause NPHP (50).

GENETIC AND PHENOTYPIC OVERLAP OF CILIOPATHIES

Ciliary dysfunction leads to a wide range of phenotypes, many of which overlap. In recessive ciliopathies, the nature of the two recessive mutations determines the severity and extent of organ involvement, leading to seemingly different disorders. In this context, loss-of-function mutations cause severe, early-onset, dysplastic, multiorgan disease, whereas reduced-function mutations cause mild, late-onset, degenerative disease with limited organ involvement. For example, recent data indicate that mutations in *TTC21B*, which encodes the intraflagellar transport protein IFT139, cause isolated NPHP or Jeune asphyxiating thoracic dystrophy depending on mutation type (51). Causal genes can also act as modifier alleles in clinically distinct disorders. In the case of *TTC21B*, sequence-based interrogation of a large ciliopathy cohort identified pathogenic rare variants in approximately 5% of cases. These alleles are proposed to act in trans with other disease causing genes to influence the range of ciliopathy phenotypes.

In human ciliary disorders, motor and sensory functions have been considered distinct properties of motile and nonmotile cilia, respectively. However, there are recent data that indicate that motile cilia of the airway are important for sensory reception (52, 53). It is theoretically possible that defects in certain ciliary proteins whose functions are important for the motile and nonmotile cilia can result in dysfunction of both types of cilia, thereby causing diverse phenotypes. For example, recently seven patients with Leber’s congenital amaurosis due to *CEP290* mutations were shown to have defective respiratory cilia (54). In addition, patients with autosomal dominant polycystic kidney disease have been shown to have increased prevalence of radiographic bronchiectasis (55). Additional examples of subtle or subclinical phenotypic overlap may become apparent with the growing clinical appreciation of the spectrum of ciliopathy features.

Heterotaxy, PCD, and other ciliopathies that occasionally have abnormal laterality as features share a similar underlying pathogenesis centering on the role of cilia. Heterotaxy may result from defects in any one of a series of signaling or morphogenetic processes that are required for the establishment of the left-right axis. In contrast, *situs inversus* caused by PCD usually results from specific defects in the structure and function of cilia at the node. Recent data indicate that not less than 6% of patients with PCD have heterotaxy, highlighting the overlap of these conditions (56). Situs defects are also observed in nonmotile ciliopathies, including RHPD, EVC, JC, and rare cases of BBS, JS, and OFD1. There is a lack of clarity regarding the mechanisms underlying the development of situs inversus versus heterotaxy as phenotypes. An improved mechanistic understanding of the steps leading to the development of abnormal laterality is critical to delineate the interrelatedness of these disorders and to direct proper management and therapy.

RESEARCH AND FUTURE DIRECTIONS

Within the last 5 years, the improved technology available for genetic analyses has had a dramatic impact on the investigation of ciliopathies. This has facilitated a more rapid identification of causative genes, investigation of modifier alleles, expansion and refinement of genotype-phenotype correlations, and improvement of clinical diagnostics.

The use of genome-wide copy number analysis coupled with *in vitro* and *in vivo* analyses of gene function is a powerful technique for the identification of novel genetic causes of ciliopathies. Imbalances in chromosomal copy number resulting in deletion or duplication of a small number of genes are a known cause of congenital heart defects in a small number of patients with genetic syndromes. Until recently, it has not been possible to evaluate the entire genome for these genetic alterations. Recently, whole-genome, single-nucleotide polymorphism genotyping was used to evaluate chromosomal copy number imbalances in a well characterized cohort of patients with complex cardiovascular malformations and heterotaxy syndrome. The results indicate that 15 to 30% of patients have pathogenic copy number variation. Efforts are ongoing to identify dosage-sensitive genes within these regions of chromosomal imbalance that will allow for identification of novel genes required for cardiac development and patterning (57). Identification of specific genes underlying heterotaxy spectrum congenital heart disease will lead to the development of novel diagnostics and therapeutics and improved management.

Sequence-based analysis is another strategy that has been enhanced by the development of novel genomic technology and subsequently has proven tremendously valuable for the study of ciliopathies. Massively parallel resequencing improves the ability to interrogate large numbers of disease-causing genes in well phenotyped cohorts, and as a result there is a wider appreciation of the genetic architecture and phenotypic spectrum of the ciliopathies (58, 59). These methods are also translating into improved availability of testing on a clinical basis. However, challenges remain in the interpretation of rare variants in a clinical setting. Exome capture technology allows unbiased interrogation of all coding region of the genome, allowing identification of novel disease-causing genes. This approach is particularly useful for autosomal recessive conditions. Recently, exome capture identified 12 different truncating mutations of *SDCCAG8* (serologically defined colon cancer antigen 8) in 20 individuals with Senior-Loken syndrome of 10 different families (60). *SDCCAG8* is localized at both centrioles and interacts directly with OFD1. Depletion of *sdccag8* causes kidney cysts and a body axis defect in zebrafish and induces cell polarity

defects in three-dimensional renal cell cultures. Further gene identification in NPHP will result in the definition of functional networks of primary cilia, centrosomes, and planar cell polarity as they pertain to the pathogenic mechanisms of NPHP-associated syndromes and other cystic kidney diseases.

CONCLUSIONS

Primary cilia are ubiquitous organelles that serve a sensory function. Disruption of the function of primary cilia results in clinically heterogeneous disorders that may affect a single organ or result in a syndromic phenotype. There is substantial clinical and genetic heterogeneity. There is some evidence suggesting that certain ciliary defects might affect primary and motile cilia associated with PCD. Additional research is needed to better understand the biological functions of these diverse classes of cilia, the genetic basis of disease, and the management of clinical symptoms caused by functional disruption.

Author Disclosure: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgments: The authors gratefully acknowledge the sponsorship and meeting organization provided by the Primary Ciliary Dyskinesia Foundation.

References

1. Fliegauf M, Benzing T, Omran H. When cilia go bad: cilia defects and ciliopathies. *Nat Rev Mol Cell Biol* 2007;8:880–893.
2. Adams NA, Awadein A, Toma HS. The retinal ciliopathies. *Ophthalmic Genet* 2007;28:113–125.
3. Christensen ST, Pedersen LB, Schneider L, Satir P. Sensory cilia and integration of signal transduction in human health and disease. *Traffic* 2007;8:97–109.
4. Cardenas-Rodriguez M, Badano JL. Ciliary biology: understanding the cellular and genetic basis of human ciliopathies. *Am J Med Genet C Semin Med Genet* 2009;151C:263–280.
5. Singla V, Reiter JF. The primary cilium as the cell's antenna: signaling at a sensory organelle. *Science* 2006;313:629–633.
6. Mok CA, Heon E, Zhen M. Ciliary dysfunction and obesity. *Clin Genet* 2010;77:18–27.
7. Hamada H. Breakthroughs and future challenges in left-right patterning. *Dev Growth Differ* 2008;50:S71–S78.
8. Levin M. Left-right asymmetry in embryonic development: a comprehensive review. *Mech Dev* 2005;122:3–25.
9. Zhu L, Belmont JW, Ware SM. Genetics of human heterotaxias. *Eur J Hum Genet* 2006;14:17–25.
10. Ticho BS, Goldstein AM, Van Praagh R. Extracardiac anomalies in the heterotaxy syndromes with focus on anomalies of midline-associated structures. *Am J Cardiol* 2000;85:729–734.
11. Ware SM, Peng J, Zhu L, Fernbach S, Colicos S, Casey B, Towbin J, Belmont JW. Identification and functional analysis of *zic3* mutations in heterotaxy and related congenital heart defects. *Am J Hum Genet* 2004;74:93–105.
12. Kuehl KS, Loffredo C. Risk factors for heart disease associated with abnormal sidedness. *Teratology* 2002;66:242–248.
13. Sutherland MJ, Ware SM. Disorders of left-right asymmetry: heterotaxy and situs inversus. *Am J Med Genet C Semin Med Genet* 2009;151C:307–317.
14. Purandare SM, Ware SM, Kwan KM, Gebbia M, Bassi MT, Deng JM, Vogel H, Behringer RR, Belmont JW, Casey B. A complex syndrome of left-right axis, central nervous system and axial skeleton defects in *zic3* mutant mice. *Development* 2002;129:2293–2302.
15. Ware SM, Harutyunyan KG, Belmont JW. Heart defects in x-linked heterotaxy: evidence for a genetic interaction of *zic3* with the nodal signaling pathway. *Dev Dyn* 2006;235:1631–1637.
16. Ware SM, Harutyunyan KG, Belmont JW. *Zic3* is critical for early embryonic patterning during gastrulation. *Dev Dyn* 2006;235:776–785.
17. Mohapatra B, Casey B, Li H, Ho-Dawson T, Smith L, Fernbach SD, Molinari L, Niesch SR, Jefferies JL, Craigen WJ, et al. Identification and functional characterization of nodal rare variants in heterotaxy

- and isolated cardiovascular malformations. *Hum Mol Genet* 2009;18: 861–871.
18. Goldmuntz E, Bamford R, Karkera JD, dela Cruz J, Roessler E, Muenke M. Cfc1 mutations in patients with transposition of the great arteries and double-outlet right ventricle. *Am J Hum Genet* 2002;70: 776–780.
 19. Roessler E, Ouspenskaia MV, Karkera JD, Velez JI, Kantipong A, Lachawan F, Bowers P, Belmont JW, Towbin JA, Goldmuntz E, et al. Reduced nodal signaling strength via mutation of several pathway members including foxh1 is linked to human heart defects and holoprosencephaly. *Am J Hum Genet* 2008;83:18–29.
 20. Gunay-Aygun M. Liver and kidney disease in ciliopathies. *Am J Med Genet C Semin Med Genet* 2009;151C:296–306.
 21. Gunay-Aygun M, Heller T, Gahl WA. Congenital hepatic fibrosis overview. In: GeneReviews at GeneTests: Medical genetics information resource (database online) [accessed March 10, 2011]. Available from: <http://www.genetests.org>. University of Washington, Seattle, WA; 2008.
 22. Desmet VJ. Congenital diseases of intrahepatic bile ducts: variations on the theme “ductal plate malformation”. *Hepatology* 1992;16:1069–1083.
 23. Parisi MA, Doherty D, Chance PF, Glass IA. Joubert syndrome (and related disorders) (omim 213300). *Eur J Hum Genet* 2007;15:511–521.
 24. Beales RA. Bardet-biedl syndrome [accessed March 10, 2011]. GeneReviews; 2007. Available from: www.genereviews.org
 25. Alexiev BA, Lin X, Sun CC, Brenner DS. Meckel-gruber syndrome: pathologic manifestations, minimal diagnostic criteria, and differential diagnosis. *Arch Pathol Lab Med* 2006;130:1236–1238.
 26. Chetty-John S, Piwnicka-Worms K, Bryant J, Bernardini I, Fischer RE, Heller T, Gahl WA, Gunay-Aygun M. Fibrocystic disease of liver and pancreas; under-recognized features of the x-linked ciliopathy oral-facial-digital syndrome type 1 (ofd i). *Am J Med Genet A* 2010;152A: 2640–2645.
 27. Tuysuz B, Baris S, Aksoy F, Madazli R, Ungur S, Sever L. Clinical variability of asphyxiating thoracic dystrophy (jeune) syndrome: evaluation and classification of 13 patients. *Am J Med Genet A* 2009; 149A:1727–1733.
 28. Ruiz-Perez VL, Goodship JA. Ellis-van creveld syndrome and weyers acrodermal dysostosis are caused by cilia-mediated diminished response to hedgehog ligands. *Am J Med Genet C Semin Med Genet* 2009;151C:341–351.
 29. Zaffanello M, Diomedei-Camassei F, Melzi ML, Torre G, Callea F, Emma F. Sensenbrenner syndrome: a new member of the hepatorenal fibrocystic family. *Am J Med Genet A* 2006;140:2336–2340.
 30. Tobin JL, Beales PL. The nonmotile ciliopathies. *Genet Med* 2009;11: 386–402.
 31. Gunay-Aygun M, Parisi MA, Doherty D, Tuchman M, Tsilou E, Kleiner DE, Huizing M, Turkbey B, Choyke P, Guay-Woodford L, et al. Mks3-related ciliopathy with features of autosomal recessive polycystic kidney disease, nephronophthisis, and joubert syndrome. *J Pediatr* 2009;155:386–392, e381.
 32. Hildebrandt F, Otto E, Rensing C, Nothwang HG, Vollmer M, Adolphs J, Hanusch H, Brandis M. A novel gene encoding an sh3 domain protein is mutated in nephronophthisis type 1. *Nat Genet* 1997;17:149–153.
 33. Otto EA, Schermer B, Obara T, O'Toole JF, Hiller KS, Mueller AM, Ruf RG, Hoefele J, Beckmann F, Landau D, et al. Mutations in invs encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. *Nat Genet* 2003;34:413–420.
 34. Watnick T, Germino G. From cilia to cyst. *Nat Genet* 2003;34:355–356.
 35. Hildebrandt F, Otto E. Cilia and centrosomes: a unifying pathogenic concept for cystic kidney disease? *Nat Rev Genet* 2005;6:928–940.
 36. Olbrich H, Fliegauf M, Hoefele J, Kispert A, Otto E, Volz A, Wolf MT, Sasmaz G, Trauer U, Reinhardt R, et al. Mutations in a novel gene, nphp3, cause adolescent nephronophthisis, tapeto-retinal degeneration and hepatic fibrosis. *Nat Genet* 2003;34:455–459.
 37. Gattone VH II, Wang X, Harris PC, Torres VE. Inhibition of renal cystic disease development and progression by a vasopressin v2 receptor antagonist. *Nat Med* 2003;9:1323–1326.
 38. Otto E, Hoefele J, Ruf R, Mueller AM, Hiller KS, Wolf MT, Schuermann MJ, Becker A, Birkenhager R, Sudbrak R, et al. A gene mutated in nephronophthisis and retinitis pigmentosa encodes a novel protein, nephroretinin, conserved in evolution. *Am J Hum Genet* 2002;71:1161–1167.
 39. Wolf MT, Lee J, Panther F, Otto EA, Guan KL, Hildebrandt F. Expression and phenotype analysis of the nephrocystin-1 and nephrocystin-4 homologs in caenorhabditis elegans. *J Am Soc Nephrol* 2005;16: 676–687.
 40. Otto EA, Loeys B, Khanna H, Hellemans J, Sudbrak R, Fan S, Muerb U, O'Toole JF, Helou J, Attanasio M, et al. Nephrocystin-5, a ciliary iq domain protein, is mutated in senior-loken syndrome and interacts with rpgr and calmodulin. *Nat Genet* 2005;37:282–288.
 41. Ross AJ, May-Simera H, Eichers ER, Kai M, Hill J, Jagger DJ, Leitch CC, Chapple JP, Munro PM, Fisher S, et al. Disruption of bardet-biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. *Nat Genet* 2005;37:1135–1140.
 42. Simons M, Gloy J, Ganner A, Bullerkotte A, Bashkurov M, Kronig C, Schermer B, Benzing T, Cabello OA, Jenny A, et al. Inversin, the gene product mutated in nephronophthisis type ii, functions as a molecular switch between wnt signaling pathways. *Nat Genet* 2005;37: 537–543.
 43. Fischer E, Legue E, Doyen A, Nato F, Nicolas JF, Torres V, Yaniv M, Pontoglio M. Defective planar cell polarity in polycystic kidney disease. *Nat Genet* 2006;38:21–23.
 44. Huangfu D, Liu A, Rakeman AS, Murcia NS, Niswander L, Anderson KV. Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature* 2003;426:83–87.
 45. Sayer JA, Otto EA, O'Toole JF, Nurnberg G, Kennedy MA, Becker C, Hennies HC, Helou J, Attanasio M, Fausett BV, et al. The centrosomal protein nephrocystin-6 is mutated in joubert syndrome and activates transcription factor atf4. *Nat Genet* 2006;38:674–681.
 46. Chang B, Khanna H, Hawes N, Jimeno D, He S, Lillo C, Parapuram SK, Cheng H, Scott A, Hurd RE, et al. In-frame deletion in a novel centrosomal/ciliary protein cep290/nphp6 perturbs its interaction with rpgr and results in early-onset retinal degeneration in the rd16 mouse. *Hum Mol Genet* 2006;15:1847–1857.
 47. den Hollander AI, Koenekoop RK, Yzer S, Lopez I, Arends ML, Voeseke KE, Zonneveld MN, Strom TM, Meitinger T, Brunner HG, et al. Mutations in the cep290 (nphp6) gene are a frequent cause of leber congenital amaurosis. *Am J Hum Genet* 2006;79:556–561.
 48. Attanasio M, Uhlenhaut NH, Sousa VH, O'Toole JF, Otto E, Anlag K, Klugmann C, Treier AC, Helou J, Sayer JA, et al. Loss of glis2 causes nephronophthisis in humans and mice by increased apoptosis and fibrosis. *Nat Genet* 2007;39:1018–1024.
 49. Delous M, Baala L, Salomon R, Laclef C, Vierkotten J, Tory K, Golzio C, Lacoste T, Besse L, Ozilou C, et al. The ciliary gene rpgr11 is mutated in cerebello-oculo-renal syndrome (joubert syndrome type b) and meckel syndrome. *Nat Genet* 2007;39:875–881.
 50. Otto EA, Trapp ML, Schultheiss UT, Helou J, Quarmby LM, Hildebrandt F. Nek8 mutations affect ciliary and centrosomal localization and may cause nephronophthisis. *J Am Soc Nephrol* 2008;19: 587–592.
 51. Davis EE, Zhang Q, Liu Q, Diplas BH, Davey LM, Hartley J, Stoetzel C, Szymanska K, Ramaswami G, Logan CV, et al. Ttc21b contributes both causal and modifying alleles across the ciliopathy spectrum. *Nat Genet* 2011;43:189–196.
 52. Shah AS, Ben-Shahar Y, Moninger TO, Kline JN, Welsh MJ. Motile cilia of human airway epithelia are chemosensory. *Science* 2009;325: 1131–1134.
 53. Bloodgood RA. Sensory reception is an attribute of both primary cilia and motile cilia. *J Cell Sci* 2010;123:505–509.
 54. Papon JF, Perrault I, Coste A, Louis B, Gerard X, Hanein S, Fares-Taie L, Gerber S, Defoort-Dhellemmes S, Vojtek AM, et al. Abnormal respiratory cilia in non-syndromic leber congenital amaurosis with cep290 mutations. *J Med Genet* 2010;47:829–834.
 55. Driscoll JA, Bhalla S, Liapis H, Ibricevic A, Brody SL. Autosomal dominant polycystic kidney disease is associated with an increased prevalence of radiographic bronchiectasis. *Chest* 2008;133:1181–1188.
 56. Kennedy MP, Omran H, Leigh MW, Dell S, Morgan L, Molina PL, Robinson BV, Minnick SL, Olbrich H, Severin T, et al. Congenital heart disease and other heterotaxic defects in a large cohort of patients with primary ciliary dyskinesia. *Circulation* 2007;115:2814–2821.

57. Fakhro KA, Choi M, Ware SM, Belmont JW, Towbin JA, Lifton RP, Khokha MK, Brueckner M. Rare copy number variations in congenital heart disease patients identify unique genes in left-right patterning. *Proc Natl Acad Sci USA* 2011;108:2915–2920.
58. Janssen S, Ramaswami G, Davis EE, Hurd T, Airik R, Kasanuki JM, Van Der Kraak L, Allen SJ, Beales PL, Katsanis N, *et al.* Mutation analysis in bardet-biedl syndrome by DNA pooling and massively parallel resequencing in 105 individuals. *Hum Genet* 2011;129:79–90.
59. Otto EA, Ramaswami G, Janssen S, Chaki M, Allen SJ, Zhou W, Airik R, Hurd TW, Ghosh AK, Wolf MT, *et al.* Mutation analysis of 18 nephronophthisis associated ciliopathy disease genes using a DNA pooling and next generation sequencing strategy. *J Med Genet* 2011; 48:105–116.
60. Otto EA, Hurd TW, Airik R, Chaki M, Zhou W, Stoetzel C, Patil SB, Levy S, Ghosh AK, Murga-Zamalloa CA, *et al.* Candidate exome capture identifies mutation of *sdccag8* as the cause of a retinal-renal ciliopathy. *Nat Genet* 2010;42:840–850.