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Genetics and Biology of Primary Ciliary Dyskinesia

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Summary

Ciliopathies are a growing class of disorders caused by abnormal ciliary axonemal structure and function. Our understanding of the complex genetic and functional phenotypes of these conditions has rapidly progressed. Primary ciliary dyskinesia (PCD) remains the sole genetic disorder of motile cilia dysfunction. However, unlike many Mendelian genetic disorders, PCD is not caused by mutations in a single gene or locus, but rather, autosomal recessive mutation in one of many genes that lead to a similar phenotype. The first reported PCD mutations, more than a decade ago, identified genes encoding known structural components of the ciliary axoneme. In recent years, mutations in genes encoding novel cytoplasmic and regulatory proteins have been discovered. These findings have provided new insights into the functions of the motile cilia, and a better understanding of motile cilia disease. Advances in genetic tools will soon allow more precise genetic testing, mandating that clinicians must understand the genetic basis of PCD. Here, we review genetic mutations, their biological impact on cilia structure and function, and the implication of emerging genetic diagnostic tools.

Keywords

cilia; primary ciliary dyskinesia; ciliogenesis; genetic sequencing; genetic testing; rare lung disease

Primary ciliary dyskinesia (PCD) is a rare disease of childhood, the prototype for motile ciliary dysfunction. PCD results from abnormal ciliary function, leading to neonatal respiratory distress, chronic sinopulmonary disease causing sinusitis, bronchiectasis, recurrent ear infections, and infertility. The cilia are also important for establishing left-right asymmetry during embryogenesis, thus cilia dysfunction can lead to *situs inversus* and a spectrum of *situs ambiguous* with associated congenital heart defects. The first report of bronchiectasis and situs abnormalities was in 1904 by Zivert [1]. This same association was later coined Kartagener's syndrome, based on the description of a series of patients, presenting with recurrent sinusitis, lung infections, and dextrocardia [2, 3]. Inclusion of

siblings and other family members suggested a genetic cause. Almost 40 years passed before Afzelius provided biological credence to the pathogenesis of PCD in a description of ultrastructural changes in the ciliary axoneme of individuals with immotile cilia and Kartagener's syndrome [4-6]. During the past decade, advances in DNA sequencing, genomics, and proteomics have driven the identification of mutations in almost 30 genes that are causative of motile cilia defects in PCD. At the same time, the discovery of each mutant gene has expanded our understanding of cilia biology and ciliogenesis.

The goal of this review is to provide an update regarding the genetics of PCD so that the clinician can be familiar with the importance of biologic defects in cilia functions, recognize the potential genotype-phenotype relationships, as well as understand the biologic and genetic bases of PCD. This is particularly important when evaluating the patient with recurrent respiratory tract infections. It must be recognized that the genetic causes of PCD are in contrast to cystic fibrosis, another well-known genetic cause of bronchiectasis. While cystic fibrosis is a result of mutations in a single gene causing dysfunction of a single protein (CFTR), PCD is the result of recessive mutations in one of many genes, owing to the complexity of the cilia structure and the process of ciliogenesis. Moreover, most mutations are private, unique to families and reflect the personalized nature of the genetics of PCD.

Secondly, genetic testing for PCD continues to evolve and is currently available on a limited basis for analysis of mutations in a few genes. Academic and commercial development of platforms is anticipated to make testing available for many more mutations in the coming years. Thus, it is essential that clinicians are familiar with the genes that when mutated can cause PCD. Moreover, the genetic basis of an estimated 30 to 40% of patients with PCD and overlapping syndromes are unknown. An appreciation of the structure of the cilia and fundamental stages in ciliogenesis will aid the clinician in understanding the role of new genes as they are discovered. In addition, as mutations in novel genes are identified using genome sequencing, it is important to understand how the protein product is validated as cilia-related. Finally, though still conceptual, the reader will gain insight into the challenge of developing therapies for PCD.

Cilia types and ciliopathies

Cilia are segregated into two classes, motile and primary. Syndromes associated with defects in cilia of either class are termed ciliopathies. Primary cilia have sensory and signaling roles, and are present on most non-dividing cells, with prominent functions in osteoblasts, neurons and renal tubule cells during development and for homeostasis [7, 8]. Primary cilia have evolved unique functions in the cells of the retina and inner ear, as well as in the epithelia of the renal tubule, where cilia sense flow [9-11]. Like PCD, primary cilia syndromes are the result of mutations in one of many genes with specific functions in primary cilia. Unlike PCD, features of these syndromes consist of combinations of sensory (blindness, deafness), skeletal, neural tube, developmental, and cognitive defects, as well as renal cysts. Genes mutated in primary cilia syndromes are also expressed in cells with motile cilia, but rarely is ciliary motility affected, with the exception of some overlap syndromes [12].

Motile cilia are found on the multiciliated cells that line the respiratory tract, ependymal cells of the brain ventricles, and fallopian tubes. The flagellum that propels spermatozoa has a similar ultrastructure. A unique type of solitary, motile cilia are transiently present during early development in a midline structure called the embryonic node. The current paradigm is that nodal cilia move with a directional, spinning motion, to signal the primary cilia that surround the node to govern left-right asymmetry during embryogenesis. Failure of ciliary function in their various tissues is responsible for the constellation of findings in PCD.

Programs of ciliogenesis

Identifying the specific function of proteins that are mutated in PCD is a major challenge, owing to the complexity of the defined and predicted pathways for generation of ciliated cells and the assembly of motile cilia. A mutation in any one of the proteins required to build or regulate the cilium could cause PCD. The assembly of cilia is regulated by several transcription factors, each with their own transcriptome. The identification of regulatory programs has been complemented by the identification of human and experimental mutations in transcription factors that cause PCD syndromes. Notch signaling pathway is among the earliest regulators involved, with reduced activity levels driving progenitor cells to the motile ciliated phenotype, while higher levels favor secretory and mucous cell differentiation (Figure 1) [13]. Notch activation is followed by activity of MCIDAS and MYB, that regulate early steps in multilineage commitment and differentiation of airway epithelial cells [14]. Other transcription factors, RFX2, RFX3 and FOXJ1 are required for the activation of genes necessary for anchoring basal bodies at the apical surface of cells, and regulating the transcription of structural proteins during ciliogenesis [15-17]. FOXJ1 has been coined the “master” ciliogenesis gene owing to severe disruption of cilia formation in animal models lacking FOXJ1. The later is due to failure of the basal bodies to properly dock to the cell membrane. These animals also manifest the classic symptoms of motile cilia dysfunction, which includes *situs inversus*, hydrocephalus, sinusitis, and lung disease [16, 17]. Roles in ciliogenesis of Regulatory factor X (Rfx) proteins were first identified in the roundworm (*Caenorhabditis elegans*), indicating the importance of less complex organisms in sorting out ciliogenesis [18]. The RFX transcription factors act at early stages in the ciliogenesis program, and their dysfunction affects both motile and sensory primary cilia [19, 20]. Moreover, RFX2 and RFX3 share program with FOXJ1, and are potential candidates for mutations that causes PCD [21-24].

Animal models for discovery of candidate PCD genes and validation

As noted, a challenge in the field is the identification of candidate genes that may be mutant in PCD, and to determine if a mutation is related to cilia function. The ciliary axoneme is phylogenetically conserved, which has been exploited in PCD gene discovery.

Chlamydomonas reinhardtii, a single cell, biflagellated alga that is motile has been widely used [25, 26]. The ability to isolate large quantities of biochemically pure algal flagella has permitted proteomic analysis. These analyses have identified over 600 polypeptides in a *Chlamydomonas* flagellum, with many orthologues conserved in human cilia [27]. Approximately 50 genetic loci have been identified and the causative genes have been described for most of them. As in humans, gene mutations that affect the outer dynein arms

and inner dynein arms, radial spokes, nexin links, and central pair microtubules, were reported. Indeed, 25 of the 29 genes linked to PCD have *Chlamydomonas* orthologues (Table 1), which provides an adaptable and reproducible model to study the effects of specific mutations on the ciliary structure and function. Moreover, screening algal cells with abnormal motility or flagellar structure is routinely used to study the function of orthologous ciliary proteins, many of which have been linked to human disease [28].

Organisms bearing cilia or flagella that can be cultivated in large numbers such as *Chlamydomonas*, *Tetrahymena* and sea urchin allow the use of protein extraction methods that can be coupled with electron microscopy to provided insights into various substructures in cilia and flagella. For instance, cryoelectron tomography of biochemically pure ciliary and flagellar preparations from these model organisms, have allowed the resolution and localization of individual proteins [29-31]. In addition, the waveform analysis of *Chlamydomonas* flagella is simplified compared to human cilia. The beat is nearly planar, which allows the entire beat cycle to be filmed [32, 33] and permits detailed analysis of the effect of mutations on flagellar motion and beat frequency. Other experimental models include frog (*Xenopus*) larvae, which have cilia on the skin during development (in tadpoles), flies (*Drosophila melanogaster*), and zebrafish (*Danio rerio*). These models rely on the conservative nature of many cilia proteins [25]. Together with discoveries in mammalian cilia proteins, these findings have facilitated the collection of ciliogenesis-related transcriptomes and cilia proteomes that provide lists of cilia genes that could be linked to newly found disease mutations [34].

PCD genetics

PCD is usually inherited as an autosomal recessive trait. However, rare cases of autosomal dominant and X-linked transmission, causing PCD-like phenotype, have been reported [35, 36]. Consanguinity commonly contributes to autosomal recessive inheritance. PCD is often found in closed communities, among geographically or culturally isolated groups and thus, the same mutation may be observed in extended families. Therefore, it is essential that the clinician obtain a careful family history, not overlooking family structures and symptoms in other family members, including second and third degree relatives. Symptoms such as recurrent pneumonia or infertility in distant family members may be clue to that a gene is harbored within a large family. There is no evidence that carrying a single mutant allele imparts any signs of symptoms of PCD, other than possibly a slightly decreased level of nasal nitric oxide that is of unknown importance [37].

Mutations in PCD genes were initially determined on a candidate basis but now are generally found by genome sequencing. The identification of PCD-associated genes has traditionally relied on sequencing of candidate genes encoding proteins that compose the ciliary structure. The role of other cilia-related genes has similarly been elucidated based on gene discovery using *in vitro* experimental models and proteomic analysis [38-41]. More recently, massive parallel sequencing and whole exome or genome sequencing in individuals with PCD has allowed more rapid identification of multiple, new mutations in families of PCD subjects [42-44]. Typically, the genome of parents and siblings are sequenced and compared to the affected individual to determine the significance of polymorphisms.

Regardless of the gene affected, most reported mutations are nonsense or deletion mutations that result in a truncated protein and loss of function [45-49]. Rare variants in sequence such as missense mutations that change a single amino acid are more difficult to attribute to disease. Mutations of PCD-related genes identified in patient or a population require confirmation, which is usually performed by using *in vitro* cell culture of airway epithelial cells [43] or by examining the ortholog gene in a model organism (e.g., *Chlamydomonas*, *Xenopus*, *Danio*). It is worth noting however, that many of these systems rely on complete genetic disruption, otherwise referred to as gene knock-out (when the change is at the genomic level) or gene-knock down (when the change is at the mRNA level). The effect of some gene variants on cilia function requires more precise and targeted gene manipulation, which has been a limitation in PCD genetic research. It is thus possible that some gene variants result in mildly diminished cilia function and subtle patient symptoms without all of the classic features of PCD.

Genes associated with primary ciliary dyskinesia

The mutant genes associated with PCD encode proteins that are involved either in axonemal structural and functional components, regulatory complexes, and ciliary assembly or preassembly complexes. A significant gap in research prevents accurate classification of many ciliary (and PCD-related) proteins into one of these classes. In other cases, such as DNAH5, a protein that we recognize as a portion of the ultrastructure of the outer dynein arm, is in fact, a functional, force-generating protein with ATPase activity, required for the ciliary power stroke. Therefore, the current organization of PCD mutations is often based on ultrastructure as detected by electron microscopy (Table 1). It is predicted that the reported mutations explain roughly 70% of PCD cases.

The earliest reported gene mutations associated with PCD were genes that encoded proteins most easily identified in the ultrastructure in the cilia axoneme. The first two PCD-associated genes identified were *DNAI1* [47] and *DNAH5* [46], which encode components of the outer dynein arm, and are estimated to account for more than 30% of all cases [50]. *DNAI1* was initially identified using a candidate gene approach, and mutations were reported in a large cohort of PCD subjects, estimating a prevalence of 9% of all identified PCD subjects [51]. *DNAH5* was discovered using homozygosity mapping in large affected consanguineous families [46, 52].

In the following decade, at least 25 additional genes have been implicated as causative of PCD. Mutations in different genes result in varied cilia motility and ultrastructure. Mutations in genes encoding outer dynein arm proteins, such as DNAH5 and DNAI1, typically render the cilia immotile or markedly hypokinetic. Other genes encoding structural and functional components of the ciliary axoneme have been associated with disease. These include genes that encode outer dynein arm proteins: *DNAL1* [53], *DNAI2* [54], *TXNDC3* [55], and *DNAH11* [56]; the outer arm docking complex: *CCDC151* [57] and *CCDC114* [44]; the dynein-regulatory complex (nexin link): *CCDC39* [49] and *CCDC40* [48]; and the central apparatus and radial spokes: *HYDIN* [58], *RSPH4A*, and *RSPH9* [59]. Mutations in the latter two genes are associated with absence of the central pair and motility defects [59, 60]. Functionally, cilia lacking the central apparatus have a unique phenotype, manifest as a

rotational waveform. Subjects with this class of mutations are also less likely to have *situs* abnormalities, since the nodal cilia also lack the central pair and normally have a whirling motion [58].

Although most of the mutations associated with PCD result in an identifiable structural defect, a handful of gene mutations produce abnormal motility without a clear structural defect by transmission EM and instead have been diagnosed by genetic testing (usually sequencing). One such example is mutations in *DNAH11* that encodes an outer dynein arm protein [45, 52]. Interestingly, ciliated cells examined using light microscopy have a normal or “hyperkinetic” beat frequency. Other gene mutations that do not produce a clear ultrastructural defect include those in *CCDC65* [61]. Based on the *Chlamydomonas* orthologue, it is localized to the dynein regulatory complex, and is estimated to be a linear protein that is 30nm in length. Standard resolution of electron microscope technology is limited to sections 60nm in size. Structural changes within the region of CCDC65 are obscured by the presence of unaffected inner dynein arms. The structural change produced by mutations in CCDC65 for instance, are better appreciated by using more sophisticated imaging modalities such as cryoelectron tomography.

Mutations in other genes, such as the coiled-coil domain containing proteins CCDC39 and CCDC40, produce inconsistent ultrastructural abnormalities, characterized by disordered microtubules in some but not all cilia, which underscores the observation that current diagnostic testing by electron microscopy will miss some PCD cases. Of note, *CCDC39* and *CCDC40* mutations are almost exclusively null alleles, which indicate that complete absence of protein is required to lead to disease [48]. CCDC39 and CCDC40 have recently been observed to function as a cilia “ruler” to promote the accurate spacing of the radial spokes along the axoneme [31]. These proteins are necessary for construction of the intricate scaffolding mechanism that allows structures of the cilia axoneme to align in a 96-nm repeat pattern along the microtubule doublets.

Advances in genetic sequencing, especially whole exome or genome analysis, has led to the discovery of proteins that were not found in the proteome of isolated *Chlamydomonas* flagella or human cilia. However, many of these are found by comparative genomics [25]. Several of these genes encode cytoplasmic proteins, rather than structural elements of the cilia, and have been linked to PCD mutations. These include the so-called “preassembly” proteins, which are presumed to have roles in cilia assembly or protein transport, and mutations lead to ultrastructural abnormalities (typically absent or truncated inner and outer dynein arms). This set of PCD-related proteins include *HEATR2* [43], *DNAAF1* [38], *DNAAF2* [40], *DNAAF3* [41], *CCDC103* [62], *LRRC6* [63], *DYX1C1* [64], *ZMYND10* [65], and *ARMC4* [66] (Table 1 and Figure 2).

The first PCD-associated preassembly protein discovered was DNAAF2 [40], which was initially identified in a mutant Japanese rice fish (*Oryzias latipes*) and in an outer and inner arm defective strain in *Chlamydomonas*. Only later was a DNAAF2 mutation found in PCD subjects who lacked outer and inner dynein arms. Localized within the apical region of the cell cytoplasm, DNAAF2 belongs to the PIH (proteins interacting with Hsp90) family. The protein interacts with DNAI2 and the chaperone heat shock protein HSP70 to presumably

facilitate preassembly or transport of dynein complexes into the cilia [40, 67]. While mutations in the human orthologue have not yet been identified, MOT48 is a PIH protein localized to the cell body that was identified in a *Chlamydomonas* mutant (*ida10*) that display altered waveforms. MOT84 is associated with preassembly of a subset of inner dynein arms, and is thought to be required for the stability of dynein heavy chain components. Mutations in other preassembly proteins DNAAF1 (LRRC50) [38, 55] and DNAAF3 [41] are also associated with outer dynein arm defects in subjects with PCD. Evidence for assembly roles of these proteins is substantiated in PCD-mutant cells by the presence of components of the inner dynein arm in the cytoplasm that fail to move into the cilium. The exact mechanism by which preassembly occurs in the cytoplasm is poorly understood. It is postulated that preassembly proteins interact in a stepwise fashion to build portions of the outer and inner dynein arms.

Another important assembly component is HEATR2, which was implicated in outer and inner dynein arm assembly [43]. Similar to other preassembly factors, HEATR2 is expressed in the cytoplasm of ciliated cells. However, HEATR2 is diffusely expressed in the cytoplasm rather than in the apical region, which suggests it functions at different stages of dynein assembly. Other proteins with HEAT-containing repeats have been implicated in ciliary and nuclear import [68], suggesting that HEATR2 can potentially have similar functions.

Genes associated with reduced cilia numbers

Multiciliated respiratory epithelia average 200 cilia per cell. Reports of subjects with poorly ciliated airway cells have attributed these findings to infection, errors in preparation of pathologic samples, or otherwise to a poorly understood rare disease, termed ciliary aplasia [69-71]. Some of these subjects presented with clinical symptoms similar to classical PCD, including repeated sinopulmonary infections and infertility. Some rare instances of associated congenital hydrocephalus are also reported [71].

Genome sequencing of patients with symptoms consistent with PCD and marked reduction in the number of motile cilia in the airway cells has recently uncovered mutations in one of two factors, Cyclin O (CCNO) and a gene called Multiciliate differentiation and DNA synthesis associated cell cycle protein (MCIDAS) [72, 73]. CCNO is required for centriole production. Mutations in CCNO result in reduction in the number of basal bodies and mislocalization of basal bodies in the cell cytoplasm, consistent with a defect in basal body replication and migration to the cell surface. A role of MCIDAS in the proper formation of ciliated cells was initially described in the skin of *Xenopus* tadpoles and has been found to control centriole replication required for normal ciliated cell formation [74, 75]. Both CCNO and MCIDAS reside in a highly conserved region on chromosome 5 next to CDC20B, which has also been linked to ciliated cell differentiation [76, 77]. Both CCNO and MCIDAS appear to function upstream to FOXJ1 and may involved in cross-talk with other transcription factors such as Myb [14, 72, 74]. Pinning the genetic basis for ciliary aplasia on transcription factors that are important for cilia formation emphasizes the wide spectrum of both genotypes and phenotypes of the motile cilia disease, and the limitations of electron microscopy that focus only on cross sections of the ciliary axoneme as means of diagnosing PCD.

Mutation tolerance and compound heterozygosity

High throughput sequencing has provided us with novel gene mutations and many variants, raising new questions related to the meaning of sequence variants and null mutations in one or two different alleles that are linked to cilia function. Like many inheritable diseases, the genetic load increases with consanguinity, explaining the higher frequency of identified cases in closed populations [78]. However, it is curious that mutations leading to PCD persist in these families despite their association with male and possibly female infertility. Moreover, consanguinity has the potential to purge harmful mutations due to the increased incidence of homozygosity [79]. A potential explanation of the observed persistence of these mutations in these families is increased mutation tolerance in some genes or pathways that lead to common effects (i.e., decreased mucociliary clearance) [80]. One could postulate that these individuals can tolerate mutations in multiple PCD related genes that affect the same ciliary structure at the same time, thus propagating these mutations and preventing negative selection. A second issue is related to the possibility that mutations in a single allele of two different known PCD genes could result in symptomatic disease. To date, this phenomena has not been identified, however as more is known about the relationship of specific cilia proteins in a single pathway, it is possible that a mild PCD-like syndrome could occur due to complex, compound heterozygosity.

Genotype-phenotype relationships

While genetic defects have been linked with specific ultrastructural abnormalities of the ciliary axoneme, there has not been clear relationship between ultrastructure, genotypes, and respiratory phenotypes. Historically, clinical and genetic heterogeneity of PCD has complicated efforts to provide a clear genotype-phenotype relationship. Moreover, the onset and progression of respiratory disease has not been systematically evaluated or linked to mutated genes. Multi-national study groups composed of specialized PCD clinics have only recently assembled large prospective clinical and genetic studies for longitudinal evaluation of subjects with specific genotypes.

To date, multicenter analyses have uncovered limited information on genotype-phenotype relationships. One unexpected finding is related to *CCDC39* and *CCDC40* mutations, which result in absent inner dynein arm, misplaced radial spokes, and microtubular disorganization in only a subpopulation of cilia. Motility defects are variable, ranging from stiffened cilia and reduced ciliary beat frequency to complete immotility. Longitudinal studies of subjects with microtubular disorganization defects (primarily biallelic mutations in *CCDC39* or *CCDC40*) have revealed an association with more severe lung disease as measured by lung function [81]. Since mutations in other genes also lead to ciliary dysmotility or immotility, the basis of severe pulmonary phenotype in subjects with *CCDC39* and *CCDC40* mutations is unexplained.

Diagnostic challenges and the role of genetic testing

Routine genetic testing must be put in context of other methods, as the diagnosis of PCD can be challenging using current tools. Historically, transmission electron microscopy has been

the gold standard, and identification of consistent defects in axonemal ultrastructure with typical phenotypic features was considered sufficient to make the diagnosis. As discussed above, ultrastructural examination of cilia as a diagnostic test for PCD has significant limitations and drawbacks. As alternatives, nasal nitric oxide (NO) concentrations, qualitative tests to assess ciliary motion, and immunofluorescence imaging have been used to screen or test for PCD [82–84]. Such techniques have largely been used in research tools. Each has technical limitations and is not widely available for routine clinical use. The use of nasal nitric oxide measurement as a screening tool for PCD is gaining momentum and is being employed by several PCD centers in Europe and North America. Low levels of nitric oxide are also observed in individuals with CF, thus screening for CF as part of the diagnostic work for PCD is important [82, 85].

Formal genetic testing for PCD is in its infancy. Currently, there are several companies that offer whole exome or selective sequencing for specific diseases, including PCD. Sequencing of PCD genes is now limited to the most common alleles including DNAH5, DNAI1, and DNAH11. However, on the horizon are broader panels, including those with almost 30 known PCD-related genes. In addition, PCD consortiums and other academic research groups have developed genetic screening tools for diagnosis. Clinicians who see patients with suspected PCD must be cognizant of the limitations of sequencing tools. The clinical importance of poorly described gene variants is unknown and the likelihood that new mutations will be uncovered is high given the private nature of most PCD mutations. Thus, there is substantial value in referral to specialized center for genetic testing, well-organized studies of families on a research basis, and the engagement of specialized genetic counselors, prior to the wide use of genetic screening for PCD.

Conclusions

PCD is a heterogenic disease, with mutations found in a relatively large number of genes that code for proteins involved in different points of cilia assembly, structure, and function. The number of genes associated with PCD has rapidly grown, and genetic testing as a diagnostic tool is becoming a reality. Biallelic, disease-causing mutations are currently linked to approximately 70% of known cases, though commercial laboratories test are available for only some of these mutations. Nevertheless, it is reasonable to expect that genetic testing will become the preferred diagnostic approach for PCD.

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Educational Aims

- 1) Appreciate the heterogeneity and complexity of primary ciliary dyskinesia genetics.
- 2) Understand how mutations in affected genes impact cilia structure and regulation, leading to primary ciliary dyskinesia.
- 3) Recognize issues related to the use of genetic testing as a diagnostic tool for primary ciliary dyskinesia.

Future Directions

- Design and use of DNA microchips for the rapid diagnosis of PCD.
- Genotype-phenotype mapping to understand the heterogeneity of PCD.
- Elucidation of the mechanism of cilia assembly and function to allow for gene specific therapies.

Figure 1.

Multiciliated cell differentiation. An airway epithelial progenitor cell, possessing a primary cilium, is directed toward the multiciliated cell type in a low Notch signaling condition. Under the influence of multiple transcription factors, hundreds of centrioles are generated, dock as basal bodies, and nucleate motile cilia in a step-wise process.

Figure 2.

Ultrastructural defects associated with mutations in PCD-causative genes. Shown are cross sectional diagrams of a normal motile ciliary axoneme and those with specific ultrastructural defects that are observed in PCD.

Table 1

Chlamydomonas orthologues of PCD associated genes.

Gene name	Locus	OMIM	<i>Chlamydomonas</i> orthologue
Outer dynein arm truncation			
DNAH5	Chromosome 5	603335	ODA2/Cre11.g476050
TXNDC3	Chromosome 7	607421	FAP67/Cre12.g558700
DNAI1	Chromosome 9	604366	ODA9/DIC1/cre12.g536550
DNAI2	Chromosome 17	605483	DIC2/Cre12.g506000
DNAL1	Chromosome 14	610062	DIC1/Cre12.g536550
CCDC114	Chromosome 19	615038	DCC2/Cre16.g666150
ARMC4	Chromosome 10	615408	--
CCDC151	Chromosome 19	615956	ODA10/Cre08.g361200
Outer dynein arm defect			
CCDC103	Chromosome 17	614677	CCDC103/PR46/Cre06.g253404
Outer and inner dynein arm truncation			
LRRC6	Chromosome 8	614677	MOT48/Cre17.g739850
HEATR2	Chromosome 7	614864	HTR2/Cre09.g395500
DYX1C1	Chromosome 15	608706	Dyx1C1/Cre11.g467560
DNAAF1	Chromosome 16	613190	ODA7/DNAAF1/Cre01.g029150
DNAAF3	Chromosome 19	614566	PF22/DNAAF3/Cre01.g001657
DNAAF2	Chromosome 14	612517	PF13/DNAAF2/Cre09.g411400
SPAG1	Chromosome 8	603395	--
C21orf59	Chromosome 21	615494	FBB18/Cre16.g688450
ZMYND10	Chromosome 3	607070	ZMYND10/cre08.g358750
Central apparatus defect			
HYDIN	Chromosome 16	610812	Hydin/Cre01.g025400
RSPH4A	Chromosome 6	612647	RSP4/PF1/Cre05.g242500
Radial spoke defects			
RSPH9	Chromosome 6	612648	RSP9/PF17/Cre07.g330200
RSPH1	Chromosome 21	609314	RSP1/Cre03.g201900
Nexin-dynein regulatory complex defect			
CCDC164	Chromosome 2	615288	DRC1/Cre13.g607750
Variable axonemal disorganization			
CCDC39	Chromosome 3	613798	PF8/Cre17.g701250
CCDC40	Chromosome 17	613799	PF7/Cre17.g698353

Gene name	Locus	OMIM	<i>Chlamydomonas</i> orthologue
Rare cilia			
CCNO	Chromosome 5	607752	--
MCIDAS	Chromosome 5	614086	--
Normal axonemal ultrastructure			
DNAH11	Chromosome 7	603339	ODA4/Cre09.g403800
CCDC65	Chromosome 12	611088	DRC2/Cre13.g607750

Chlamydomonas genes are found at: http://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Creinhardtii