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REVIEW

Male Fertility

Genetic factors contributing to human primary ciliary dyskinesia and male infertility

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Primary ciliary dyskinesia (PCD) is an autosomal-recessive disorder resulting from the loss of normal ciliary function. Symptoms include neonatal respiratory distress, chronic sinusitis, bronchiectasis, situs inversus, and infertility. However, only 15 PCD-associated genes have been identified to cause male infertility to date. Owing to the genetic heterogeneity of PCD, comprehensive molecular genetic testing is not considered the standard of care. Here, we provide an update of the progress on the identification of genetic factors related to PCD associated with male infertility, summarizing the underlying molecular mechanisms, and discuss the clinical implications of these findings. Further research in this field will impact the diagnostic strategy for male infertility, enabling clinicians to provide patients with informed genetic counseling, and help to adopt the best course of treatment for developing directly targeted personalized medicine.

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INTRODUCTION

Primary ciliary dyskinesia (PCD) (Mendelian Inheritance in Man, MIM 242650) is a rare genetic disorder affecting approximately one in 20 000 individuals worldwide, which is multisystemic and caused by motility defects in the cilia and flagella.[1,2] Specifically, ineffective cilia movement results in limited mucociliary clearance in the upper and lower respiratory tract, leading to rhinitis, sinusitis, rhinorrhea, chronic cough, recurrent respiratory infections, infertility, and ultimately, scarring of the lungs in the form of bronchiectasis.[3] PCD is associated with situs inversus in 50% of the patients, which is termed Kartagener syndrome (KS; MIM 244400).[4]

In humans, motile cilia have a common microtubule-based ultrastructure (axoneme), comprising nine peripheral microtubule doublets surrounding a central microtubule pair,[5] which are linked to a variety of microtubule-associated proteins. These proteins include the inner dynein arm (IDA) and outer dynein arm (ODA) motor complexes, which project from the peripheral microtubule doublets; the radial spokes, which provide a radial scaffold between the central pair and peripheral microtubules to facilitate signal transduction from the center out to the dynein arms to govern ciliary beats and waveforms;[6] and nexin–dynein regulatory complexes, which attach between adjacent peripheral doublets to facilitate IDA attachment and regulate dynein activity.[7]

Male infertility has often been described as part of the clinical symptoms of PCD, but this particular aspect of the PCD physiopathology has not been systematically explored and is generally poorly described in scientific reports. The sperm of infertile male PCD patients is usually immotile and presents various ultrastructural defects of the flagella, such as missing dynein arms, microtubular translocations, and lack of radial spokes. There has been recent research progress on PCD, contributing to the identification and characterization of the numerous proteins required for adequate axonemal molecular structure and assembly. In this review, we focus on the progress made in identifying the genes related to PCD that are directly or potentially associated with male infertility, provide an update of the genetic etiology, summarizing the proposed underlying molecular mechanisms, and discuss the clinical implications arising from these findings.

COILED-COIL DOMAIN-CONTAINING 39 (*CCCD39*)

FAP59, the *Chlamydomonas* ortholog of human *CCDC39* (MIM 613798), which is located on chromosomal region 3q26.33 and consists of twenty exons, was predicted to be essential for motile ciliary function, as no orthologs have been found in nonciliated organisms ("CiliaCut") or in *Caenorhabditis elegans* ("MotileCut").[8]

Merveille *et al.*[9] found that a substantial proportion of human PCD cases with axonemal disorganization and abnormal ciliary beating is associated with loss-of-function mutations in *CCDC39*. Furthermore, functional analyses indicated that CCDC39 localizes to the ciliary axonemes and is essential for the assembly of IDA and the dynein regulatory complex.

Antony *et al.*[10] sequenced the *CCDC39* and *CCDC40* genes in 54 "radial spoke defect" families, as these are the two genes identified so far to cause this defect. These findings highlighted a key role of both genes in the development of PCD with axonemal disorganization and IDA loss. IDA defects account for about 16% of all PCD cases, and were found in 14% up to 29% of all PCD cases in other cohorts.

DYNEIN, AXONEMAL, ASSEMBLY FACTOR 1–3 (*DNAAF1–3*)

The most frequent defects in PCD involve ODAs and IDAs, the large multiprotein complexes responsible for cilia-beat generation

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and regulation, respectively. Duquesnoy *et al.*[11] investigated whether PCD might result from mutations in the human gene *LRRC50* (also named *DNAAF1*; MIM 611088), which is located at chromosome 16q24.1 and encodes a member of the superfamily of leucine-rich repeat (LRR)-containing proteins. Functional analyses performed in *Chlamydomonas reinhardtii* and in another flagellated protist, *Trypanosoma brucei*, support a key role for *DNAAF1* in the cytoplasmic preassembly of the dynein arms. Furthermore, Loges *et al.*[12] demonstrated that DNAAF1 deficiency disrupts the assembly of distal and proximal dynein, axonemal, heavy chain 5 (*DNAH5*)- and dynein, axonemal, intermediate chain 1 (*DNAI2*)-containing ODA complexes, as well as dynein, axonemal, light chain 1 (*DNALI1*)-containing IDA complexes, resulting in immotile cilia.

Kintoun (*KTU*, previously termed C14 or f104, also named *DNAFF2*; MIM 612517) is located on chromosome 14q21.3 and consists of three exons, encoding cDNAs of 2511 bp or 2367 bp (an in-frame splice variant lacking exon 2). This gene was first identified in a medaka mutant, and was found to be mutated in PCD patients from two affected families.[13] In the absence of *DNAFF2*, both ODAs and IDAs are missing or defective in the axoneme, leading to general loss of motility. Biochemical and immunohistochemical studies have shown that *KTU*/*PF13* is one of the long-sought after proteins involved in the preassembly of dynein arm complexes in the cytoplasm before being loaded for intraflagellar transport to the ciliary compartment.[14]

The gene *DNAAF3* (MIM 614566) on human chromosome 19q13 (previously designated C19 or f51) encodes a 588-amino acid protein (GenBank NP_849159).[15] Lunt *et al.*[16] showed that PF22/DNAAF3 is essential for the preassembly of dyneins into complexes prior to their transport into cilia. Loss-of-function mutations were identified in the human *DNAAF3* gene in patients from families with situs inversus, causing defects in the assembly of the inner and outer dynein arms. Moreover, *DNAAF3* loss prevents correct assembly of the inner and outer dynein arms, abolishing the motility of respiratory cilia, and giving rise to classical PCD associated with defective left–right organ asymmetry and male infertility.[17]

DNAH5

DNAH5 (MIM 603335) was identified based on homozygosity mapping and a candidate gene approach.[18] This 79-exon gene (with one alternative first exon) encodes a heavy-chain protein that localizes to the outer dynein arm and is the homolog of the dynein c-heavy chain of *Chlamydomonas reinhardtii*.

A PCD-related locus was identified at chromosome 5p15.2, which contains *DNAH5*, the human ortholog of the *Chlamydomonas* ODA γ-heavy chain gene.[19] Recessive mutations of *DNAH5* result in nonfunctional DNAH5 proteins,[20] and affected patients have dysmotile respiratory cilia with ODA defects. Mutations in *DNAI1*, encoding an ODA intermediate chain orthologous to *Chlamydomonas IC78*, also cause ODA defects in patients with PCD.[21]

In the normal ciliated airway epithelium, *DNAH5* and *DNAH9* show a specific regional distribution along the ciliary axoneme, indicating the existence of at least two distinct ODA types. Cilia with complete axonemal DNAH5 deficiency were immotile, whereas cilia with distal DNAH5 deficiency showed residual motility. In addition, the observation of the absence of DNAH5 within the respiratory ciliary compartment but a normal DNAH5 distribution within the sperm flagellum in a patient with *DNAH5* mutations raised the possibility that the two organelle types are assembled via distinct mechanisms.[22]

DNAI1

The first gene in which mutations were found to be associated with PCD was *DNAI1* (MIM 604366).[23] *DNAI1* is an axonemal dynein intermediate chain gene that was isolated from *Chlamydomonas reinhardtii*, a unicellular alga with two flagella showing an axonemal structure similar to that of human respiratory cilia and sperm tails.[24] *DNAI1* is localized on chromosome 9p13-p21 and is composed of twenty exons encoding a 699-amino acid protein.

Guichard *et al.*[25] demonstrated a link between ciliary function and situs determination, given that heterozygosity for a compound mutation in *DNAI1* results in PCD with situs solitus or situs inversus (KS). Zariwala *et al.*[26] found that mutations in *DNAI1* cause PCD with ODA defects, and are likely the genetic origin of clinical disease in some PCD patients with ultrastructural defects in the ODA.

DNAI2

DNAI2 (MIM 605483), the human ortholog of *Chlamydomonas* intermediate ODA chain *IC69/IC2*, which is located on chromosome 17q25, comprises 14 exons extending over a 39-kb genomic distance.[27–29]

Applying a positional and functional candidate gene approach, Loges *et al.*[30] identified homozygous loss-of-function *DNAI2* mutations (IVS11þ1G>A) in four individuals from a family with PCD and ODA defects. *DNAI2* and *DNAH5* mutations affect the assembly of proximal and distal ODA complexes, whereas *DNAI1* mutations mainly disrupt the assembly of proximal ODA complexes. Mutations in an ODA intermediate dynein chain are reported in 2%–13% of all PCD patients with defined ODA defects.

DYSLEXIA SUSCEPTIBILITY 1 CANDIDATE 1 (*DYX1C1*)

DYX1C1 (MIM 608706) was initially identified as a candidate dyslexia gene due to a single-balanced translocation t(2;15)(q11;q21) that coincidentally segregates with dyslexia in a family, and was confirmed in subsequent single nucleotide polymorphism association studies.[31] *DYX1C1* is located on chromosome 15q21.3 and comprises ten exons (translation starts at exon 2) encompassing 77.93 kb of genomic DNA. Follow-up gene association studies have provided both positive[32–34] and negative[35–37] support for the association with dyslexia. Molecular and cellular analyses of *DYX1C1* have indicated potential functional roles with chaperonins,[38,39] estrogen receptor trafficking,[40] and neuronal migration.[41,42]

Tarkar *et al.*[43] found that deletion of *Dyx1c1* exons 2–4 in mice caused a phenotype resembling PCD. Ultrastructural and immunofluorescence analyses of *DYX1C1*-mutant motile cilia in mice and humans revealed disruptions of the ODA and IDA. DYX1C1 localizes to the cytoplasm of respiratory epithelial cells, its interactome is enriched for molecular chaperones, and it interacts with the cytoplasmic ODA/IDA assembly factor DNAAF2/KTU.

HEAT REPEAT-CONTAINING 2 (*HEATR2*)

HEATR2 (MIM 614864), located on chromosome 7p22.3, encodes a member of the family of ten other uncharacterized HEAT repeat-containing proteins in humans. Preliminary analyses have shown that *HEATR2* gene and protein sequences are highly conserved, and that HEATR2 is enriched in organisms with motile cilia and flagella.[44]

Horani *et al.*[45] demonstrated that airway epithelial cells isolated from PCD-affected individuals showed markedly reduced HEATR2 levels, absence of dynein arms, and loss of ciliary beating. Moreover,

immunohistochemistry studies in human airway epithelial cells showed that HEATR2 was localized to the cytoplasm and not to the cilia, which suggests a role for this protein in either dynein arm transport or assembly.

HYDIN, AXONEMAL CENTRAL PAIR APPARATUS PROTEIN (*HYDIN*)

Homozygous-recessive *Hydin* mutations are lethal in the 1st week of life in hy3 mice, due to hydrocephalus from abnormal ependymal ciliary motility.[46–49]

With homozygosity mapping, Olbrich *et al*.[50] identified a PCD-associated locus at the chromosomal region 16q21-q23, which contains *HYDIN* (MIM 610812). However, a nearly identical 360-kb paralogous segment (*HYDIN2*) in the chromosomal region 1q21.1 complicated the mutational analysis. Electron microscopy tomography of *HYDIN* mutant respiratory cilia showed results consistent with the effects of loss-of-function mutations, in that the C2b projection of the central pair apparatus was lacking; similar findings were reported in *Hydin*-deficient *Chlamydomonas* and mice. High-speed video microscopy demonstrated markedly reduced beating amplitudes of the respiratory cilia and stiff sperm flagella.

LRR-CONTAINING 6 (*LRRC6*)

LRRC6 (MIM 614930) is expressed in the flagella of *Chlamydomonas reinhardtii* and in human cilia.[51] In mice, *Lrrc6* transcripts were found to be expressed in tissues that have flagella or motile cilia.[52] In addition, a putative PCD-related locus containing *LRRC6* was identified in a genome-wide linkage study performed in familial cases of PCD.[53] Most importantly, the phenotypic features of several animal models with mutations in *LRRC6* orthologs are consistent with ciliary defects. In zebrafish, seahorse mutants display a curved body with pronephric cysts,[54] which was associated with left–right abnormalities in half of the cases.[55] In *Drosophila*, a null allele of the *LRRC6* ortholog called *tilB* was found to be responsible for the ciliary dysfunction of sensory neurons of the auditory organ and male sterility.[56] In humans, *LRRC6*, which is located on chromosome 8q24.22, consists of 12 exons, and the only predicted transcript (RefSeq accession number NM_012472.3) encodes a 466-amino acid residue protein with five N-terminal LRR motifs. The LRRs of LRRC6 contain the consensus sequence LxxLxLxxNxIxxIxxLxzx Lxx ("z" indicates frequent deletions), which defines the SDS22-like subfamily of LRR-containing proteins.[57] Each LRR is a beta-strand-turn/alpha-helix structure, and together, these motifs are known to form a solenoid (repeated structural units that form a continuous superhelix).[58] LRRC6 also contains an LRR-cap that shields the solenoid, a coiled-coil (CC) domain, a polylysine motif, and a C-terminal alpha-crystallin p23-like domain.

Kott *et al*.[59] demonstrated that in spite of the structural and functional similarities between LRRC6 and DNAAF1, another LRR-containing protein was associated with the same PCD phenotype, the two proteins are not redundant. Therefore, the evolutionarily conserved LRRC6 emerges as an additional player in IDA assembly, a process that is essential for proper axoneme building and that appears to be much more complex than previously thought.

Horani *et al*.[60] revealed a single novel mutation in *LRRC6* in PCD patients, which fits the model of autosomal-recessive genetic transmission, leading to a change of a highly conserved aminoacid from aspartic acid to histidine (Asp146His). These findings suggest that LRRC6 plays a role in dynein arm assembly or trafficking, and its mutation leads to PCD with laterality defects.

RADIAL SPOKE HEAD HOMOLOGS

In humans, *RSPH1* (MIM609314; *RSPH*: radial spoke head homologs) is located on chromosomal region 21q22.3 and consists of nine exons; the only predicted transcript (RefSeq accession number NM_080860.2) encodes a 309-aminoacid residue protein with five N-terminal membrane occupation and recognition of nexus (MORN) repeats,[61] followed by a linker and a sixth MORN repeat. *RSPH1* is the homolog of *Chlamydomonas RSP1*.

Kott *et al*.[62] combined homozygosity mapping and whole-exome sequencing in a consanguineous individual with central complex defects, and identified a nonsense mutation in *RSPH1*. *RSPH1* mutations appear to play an important role in the etiology of this PCD phenotype, which includes radial spoke defects, thereby unveiling the importance of RSPH1 in the proper building of the central complex and radial spokes in humans.

Daniels *et al*.[63] described a novel splice-site mutation (c.921+3_6delAAGT) in *RSPH4A* (MIM 612647), which leads to a premature translation termination signal, in nine subjects with PCD (seven families). Loss-of-function of this mutation was confirmed with quantitative ciliary ultrastructural analysis, measurement of ciliary beat frequency and waveform, and transcript analysis. All nine individuals carrying the c.921+3_6delAAGT splice-site mutation in *RSPH4A* were Hispanic with ancestry tracing to Puerto Rico. This mutation is considered to be a founder mutation and a common cause of PCD without situs abnormalities in patients of Puerto Rican descent.

Castleman *et al*.[64] identified mutations in two positional candidate genes, *RSPH9* on chromosome 6p21.1 and *RSPH4A* on chromosome 6q22.1. Haplotype analysis identified a common ancestral founder effect of an *RSPH4A* mutation present in the United Kingdom-Pakistani pedigrees. Both *RSPH9* (MIM 612648) and *RSPH4A* encode protein components of the axonemal radial spoke head. *In situ* hybridization of murine *Rsph9* showed that gene expression was restricted to regions containing motile cilia. Investigation of the effect of knockdown or mutations of *RSPH9* orthologs in zebrafish and *Chlamydomonas* indicates that radial spoke head proteins are important in maintaining normal movement in motile, "9 + 2"-structure cilia and flagella. This effect could be rescued by the reintroduction of gene expression for restoration of a normal beat pattern in zebrafish. Disturbance in the function of these genes was not associated with defects in left–right axis determination in humans or zebrafish.

Onoufriadis *et al*.[65] found that mutations in *RSPH1*, *RSPH4A*, and *RSPH9*, which all encode homologs of components of the "head" structure of ciliary radial spoke complexes identified in *Chlamydomonas*, cause clinical phenotypes that appear to be indistinguishable except at the gene level. Using high-resolution immunofluorescence, they identified loss of RSPH4A and RSPH9 associated with *RSPH1*-mutated cilia, suggesting that *RSPH1* mutations may result in loss of the entire spoke head structure.

ZINC FINGER, MYND-TYPE-CONTAINING 10 (*ZMYND10*)

ZMYND10 (also known as *BLU*; MIM 607070), which is located on chromosome 3p21.3, encodes a protein containing a C-terminal myeloid, nervy, and DEAF-1 (MYND) domain. ZMYND10 is highly enriched in ciliated cells compared to nonciliated cells,[66] but little is known about its function. ZMYND10 is known to act as a tumor suppressor, inhibiting the clonogenic growth of nasopharyngeal carcinoma cells, arresting the cell cycle at the G1 phase, downregulating c-Jun N-terminal kinase and cyclin D1 promoter activities, and inhibiting the phosphorylation of c-Jun.[67]

In mice, *Zmynd10* mRNA is restricted to regions containing motile cilia. In a *Drosophila* model of PCD, ZMYND10 is exclusively expressed in cells with motile cilia, chordotonal sensory neurons, and sperm. In these cells, P-element-mediated gene silencing caused IDA and ODA defects, proprioception deficits, and sterility due to immotile sperm. Human ZMYND10 interacts with LRRC6, another cytoplasmically localized protein that is altered in PCD.[68] Moore *et al*.[68] concluded that ZMYND10 is a cytoplasmic protein required for IDA and ODA assembly, and that its variants cause ciliary dysmotility and PCD with laterality defects. Using whole-exome and candidate-gene Sanger resequencing in PCD-affected families afflicted with combined IDA and ODA defects, they found that 6/38 (16%) of the subjects carried biallelic mutations in *ZMYND10*.

Zariwala *et al*.[69] identified mutations in *ZMYND10* that result in the absence of the axonemal protein components DNAH5 and DNALI1 from respiratory cilia. Animal models also support the association between ZMYND10 and human PCD, given that *Zmynd10* knockdown in zebrafish caused ciliary paralysis, leading to cystic kidneys and otolith defects, and that knockdown in *Xenopus* interfered with ciliogenesis. Thus, a cytoplasmic protein complex containing ZMYND10 and LRRC6 is necessary for motile ciliary function.

Summary and perspectives

A male infertility phenotype has often been described as one of the clinical symptoms of PCD, but this particular aspect of the physiopathology has not yet been systematically explored and is often only scarcely described in scientific reports (**Table 1**). PCD/KS severely affects the quality of life of patients, and male infertility resulting from the disease should receive more attention. The gaining popularity and progress in assisted reproductive technology will certainly benefit infertile couples affected by this disease. However, full understanding of the underlying molecular mechanisms of PCD/KS will require further studies. This is a very exciting time in the field of the genetics of infertility. We have so far witnessed only

Table 1: Primary ciliary dyskinesia gene mutations and their consequences for axonemal ultrastructure and sperm phenotype

Gene name	*Full name*	*MIM number*	*Axonemal localization*	*Axonemal defects*	*Infertility phenotype*	*Cytogenetic location*
CCDC39	Coiled-coil domain containing 39	6132798	Assembly of IDA, N-DRC, and radial spokes	Absence of inner dynein arms and nexin links Axonemal disorganization with mislocalized peripheral doublet Displacement or absence of the central pair	Oligoasthenozoospermia midpiece is narrowed flagellum is shortened	3q26.33
DNAAF1 (LRRC50)	Dynein, axonemal, assembly factor 1	613190	Assembly of dynein arm complexes in the cytoplasm	Absence of both outer and inner dynein arms	Male infertility reported but no details were provided	16q24.1
DNAAF2 (KTU)	Dynein, axonemal, assembly factor 2	612517	Assembly of dynein arm complexes in the cytoplasm	Absence of both outer and inner dynein arms	Asthenozoospermia	14q21.3
DNAAF3	Dynein, axonemal, assembly factor 3	614566	Assembly of dynein arm complexes in the cytoplasm	Absence of both outer and inner dynein arms	Male infertility reported but no details were provided	19q13
DNAH5	Dynein, axonemal, heavy chain 5	603335	Outer dynein arm heavy chain	Outer dynein arm defect	Asthenozoospermia	5p15.2
DNAI1	Dynein, axonemal, intermediate chain 1	604366	Outer dynein arm intermediate chain	The outer dynein arms are shortened or missing	Asthenozoospermia	9p13-p21
DNAI2	Dynein, axonemal, intermediate chain 2	605483	Outer dynein arm intermediate chain	Outer dynein arm defect	Male infertility reported but no details were provided	17q25
DYX1C1 (DNAAF4)	Dyslexia susceptibility 1 candidate 1	608706	Assembly of dynein arm complexes in the cytoplasm	Absence of both outer and inner dynein arms	Asthenozoospermia	15q21.3
HEATR2	HEAT repeat containing 2	614864	Assembly or stability of axonemal dynein arms	Absence of outer dynein arms and partial lack of inner dynein arms	Male infertility reported but no details were provided	7p22.3
HYDIN	HYDIN, axonemal central pair apparatus protein	610812	C2b projection	Lack the C2b projection of the central pair	Asthenozoospermia	16q21-q23
LRRC6	Leucine-rich repeat containing 6	614930	Assembly of dynein arm complexes in the cytoplasm	Absence of both outer and inner dynein arms	Asthenozoospermia	8q24.22
RSPH1	Radial spoke head 1 homolog	609314	Radial spoke component	Central pair microtubule complex and radial spoke defects	Male infertility reported but no details were provided	21q22.3
RSPH4A	Radial spoke head 4A homolog	612647	Radial spoke component	Central pair microtubule complex and radial spoke defects	Male infertility reported but no details were provided	6q22.1
RSPH9	Radial spoke head 9 homolog	612648	Radial spoke component	Central pair microtubule complex and radial spoke defects	Male infertility reported but no details were provided	6p21.1
ZMYND10	Zinc finger, MYND-type containing 10	607070	Assembly of dynein arm complexes in the cytoplasm	Lack of outer and inner dynein arms	Male infertility reported but no details were provided	3p21.3

MIM: mendelian Inheritance in Man; IDA: inner dynein arm; N-DRC: nexin-dynein regulatory complex

the tip of the iceberg, but we are confident that the rest will come to light in the foreseeable future.

AUTHOR CONTRIBUTIONS

LD, YWS, and ZYJ drafted the manuscript, and PL revised it critically for important intellectual content. All authors have read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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