OPEN ACCESS

Recent advances in primary ciliary dyskinesia genetics

Małgorzata Kurkowiak,[1,2] Ewa Ziętkiewicz,[1] Michał Witt[1,2]

[1]Department of Molecular and Clinical Genetics, Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland
[2]International Institute of Molecular and Cell Biology, Warsaw, Poland

Correspondence to
Dr Małgorzata Kurkowiak, Department of Molecular and Clinical Genetics, Institute of Human Genetics, Polish Academy of Sciences, 32 Strzeszyńska Street, Poznań 60-479, Poland; mszczepaniak86@gmail.com

Received 31 August 2014
Accepted 8 October 2014
Published Online First 28 October 2014

ABSTRACT

Primary ciliary dyskinesia (PCD) is a rare genetically heterogeneous disorder caused by the abnormal structure and/or function of motile cilia. The PCD diagnosis is challenging and requires a well-described clinical phenotype combined with the identification of abnormalities in ciliary ultrastructure and/or beating pattern as well as the recognition of genetic cause of the disease. Regarding the pace of identification of PCD-related genes, a rapid acceleration during the last 2–3 years is notable. This is the result of new technologies, such as whole-exome sequencing, that have been recently applied in genetic research. To date, PCD-causative mutations in 29 genes are known and the number of causative genes is bound to rise. Even though the genetic causes of approximately one-third of PCD cases still remain to be found, the current knowledge can already be used to create new, accurate genetic tests for PCD that can accelerate the correct diagnosis and reduce the proportion of unexplained cases. This review aims to present the latest data on the relations between ciliary structure aberrations and their genetic basis.

CILIARY STRUCTURE AND PRIMARY CILIARY DYSKINESIA

Cilia and flagella are highly evolutionary conserved organelles present on the variety of eukaryotic cells. Axoneme, the core of the cytoskeletal structure of cilia and flagella, emanates from the basal body, which is necessary for anchoring the cilium to the apical side of the cell.[1] Cilia and flagella are continuously assembled and disassembled at their distal tips. Intraflagellar transport is essential for the transport of flagellar/ciliary components from the basal body to their assembly region and for the removal of turnover products from the flagellar/ciliary tip. Anterograde (base-to-tip) transport with the heterotrimeric kinesin-2 and retrograde (tip-to-base) transport with the cytoplasmic dynein occur at a constant rate without pauses.[2–4]

The axoneme consists of microtubule (MT) doublets (figure 1) associated with the large number of multiprotein components. In humans, axonemes with either 9+2 or 9+0 microtubular pattern identified on cross sections are present. In the 9+2 cilia, nine peripheral MT doublets surround a central pair (CP) of MTs, while in the 9+0 cilia no CP is present. Cilia can be divided into two major groups: motile and non-motile (primary). Subgroups with different ultrastructural patterns can be distinguished depending on the structure and function they perform:

- motile cilia with the 9+2 pattern (nine peripheral microtubular doublets, CP and associated structures like outer dynein arms (ODAs), inner dynein arms (IDAs), nexin-dynein regulatory complexes (N-DRCs), radial spokes (RSs)); found on the apical surface of epithelial cells in the airways (respiratory cilia), brain (ependymal cilia), female reproductive system (cilia in fallopian tube) or in male reproductive system (sperm flagella);[5–8]
- motile cilia with the 9+0 pattern (they lack CP but still contain ODAs and IDAs); found in the embryo (nodal cilia);[9 10]
- non-motile cilia with the 9+2 pattern; found in the inner ear (kinocilia);[11]
- non-motile cilia with the 9+0 pattern (no CP and associated structures), acting mainly as mechanosensor and chemosensors; they are present in kidney (renal cilia),[12 13] bile duct (cholangiocyte cilia),[14] pancreas (cilia in pancreatic duct),[15] bone or cartilage (cilia in osteocyte or chondrocyte)[16 17] or in the eye (photoreceptor connecting cilia).[18]

The ultrastructural elements of cilia are composed of many proteins; their definition in human cilia is largely based on the information from *Chlamydomonas reinhardtii*, a unicellular biflagellate green alga.[19] A broad knowledge on the function of flagellar proteins could be used in the studies of ciliary proteins thanks to the high evolutionary conservation of these organelles.

Each peripheral doublet consists of MT A and MT B, built of 13 and 11 protofilaments, respectively; protofilaments are composed of α and β tubulin heterodimers. The MTs A and B of the neighbouring peripheral doublets interconnect with each other by the N-DRC. From each MT A, ODAs and IDAs extend; both are large force-producing multiprotein complexes (molecular motors).[20] ODA complexes face towards the boundary of the cilium while IDA complexes are directed towards the CP. The peripheral doublets are connected to the CP via RSs. The CP complex, RSs, IDA isoforms f (figure 1) and N-DRC are important for the coordination of the dynein motor activity.[21–23] All these components, except ODA, are distributed with the 96 nm periodicity along the axoneme. ODAs are repeated every 24 nm; in addition, two forms of ODA can be distinguished: proximal (characterised by the presence of two heavy dynein chains, DNAH5 and DNAH9) and distal, which do not contain DNAH9.[24]

CLINICAL FEATURES AND DIAGNOSIS OF PCD

A wide range of different functions played by cilia present on nearly all types of vertebrate cells underlies their importance in the functioning of the organism. Symptoms triggered by dysfunctional

Open Access
Scan to access more free content

To cite: Kurkowiak M, Ziętkiewicz E, Witt M. *J Med Genet* 2015;**52**:1–9.

J Med Genet: first published as 10.1136/jmedgenet-2014-102755 on 28 October 2014. Downloaded from http://jmg.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

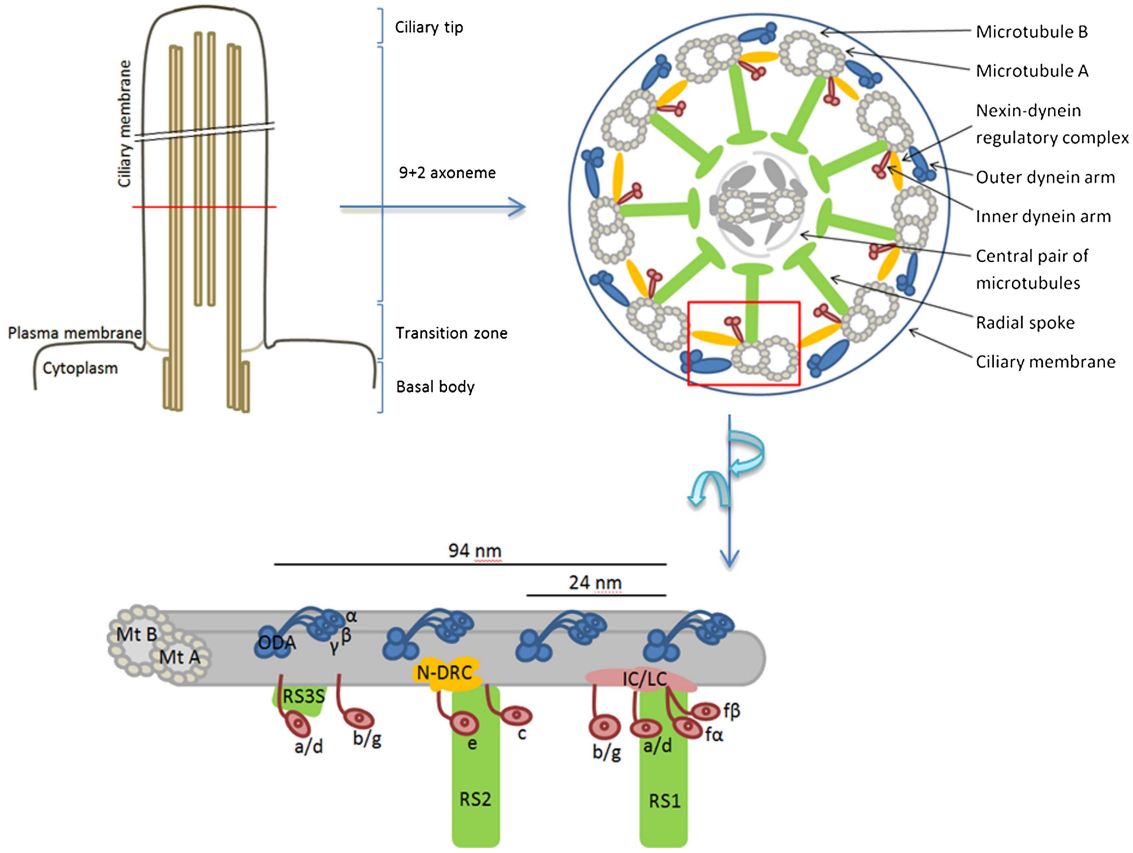

Figure 1 Schematic diagram of the ciliary axoneme in *Chlamydomonas*. Upper left: longitudinal section of a cilium presenting the location of axonemal microtubules in different parts of the cilium. Red line indicates the site of a cross section presented on the right site. The cross section shows nine microtubule doublets surrounding the central pair of microtubules. The microtubules are interconnected via radial spokes, N-DRCs and dynein arms; no dynein arms are present in the transition region. All these structures except ODA are distributed with the 96 nm periodicity along the axoneme; ODA are present every 24 nm. The placement of all these structures in relation to each other within the 96 nm repeat is depicted at the bottom. a/d, b/g, c and e, single-headed IDA isoforms; fα and fβ, double-headed IDA isoform f; IC/LC, intermediate chain-light chain complex of inner dynein arms (IDA); Mt A, microtubule A; Mt B, microtubule B; N-DRC, nexin-dynein regulatory complex; ODA, outer dynein arms with marked α, β and γ heavy chains; RS1, radial spoke 1, RS2; radial spoke 2; RS3S, radial spoke 3 stump. Scheme based on the original data published previously.[98]

cilia form a broad and phenotypically heterogeneous category of overlapping disorders, commonly named ciliopathies.

Primary ciliary dyskinesia (PCD, OMIM id: 242650), a rare, genetically heterogeneous autosomal-recessive disease, is the major ciliopathy related to the dysfunction of motile cilia. The incidence of PCD is estimated at 1:10 000–1:20 000 live births.[25 26] Extrapolating these data, approximately 70 new cases can be born each year in the white population worldwide.[27]

The history of PCD dates back to 1904 when Dr A. K. Siewert reported the coexistence of bronchiectasis and *situs inversus*.[28] A few decades later, in 1933, Manes Kartagener described multiple cases with bronchiectasis, *situs inversus* and sinusitis and named this disorder the Kartagener syndrome.[29] Then in 1976, Björn Afzelius, using transmission electron microscopy (TEM), identified an absence of dynein arms in the axoneme of respiratory cilia and sperm flagella in patients with Kartagener syndrome. He concluded that the observed lack of dynein arms must be the cause of ciliary immotility leading to all symptoms of Kartagener syndrome including male infertility due to azoospermia.[30]

Since then, much more has been learned about the pathophysiology of PCD. This complex disease appears early in life and, if misdiagnosed, may lead to severe symptoms like bronchiectasis or chronic lung disease.[31 32] PCD symptoms involve organs where motility of cilia has an impact on their normal functioning. The most prominent PCD features relate to the upper and lower respiratory tract and usually appear early after birth. Those symptoms include neonatal respiratory distress syndrome, which comprises chest congestion, coughing, tachypnoea (rapid breathing) and hypoxia.[31 32] In cases where dextrocardia, *situs ambiguous* or *situs inversus* are present in the infant, PCD should be considered as a highly possible diagnosis.[33 34] Later, chronic sinusitis and secretory otitis media might occur, which, together with chronic middle ear effusion, frequently lead to hearing loss.[35–37] There are also lower respiratory tract symptoms like wheezing, chronic wet cough frequently with sputum production, chronic bronchitis and recurrent pneumonia; after several years these symptoms may lead to bronchiectasis in the middle and lower lobes.[27] Upper respiratory tract abnormalities include persistent rhinitis, mucosal congestion, nasal passages oedema and infrequently (more prevalent in adults) nasal polyps.[38] In older children and adults, chronic and recurrent sinusitis as well as chronic mucopurulent sputum production are common features. Bacteria commonly identified in sputum samples after microbiological testing in those patients are *Haemophilus influenzae*, *Staphylococcus aureus* and *Streptococcus pneumoniae*.[39] Older

J Med Genet: first published as 10.1136/jmedgenet-2014-102755 on 28 October 2014. Downloaded from http://jmg.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

Table 1 A comprehensive list of primary ciliary dyskinesia (PCD)-related genes

Gene	Protein localisation/function	Ultrastructural defect in PCD	Typical functional defect in PCD*	Reference
DNAI1	ODA	ODA defect	Immotile cilia	53, 62–65
DNAH5	ODA	ODA defect	Immotile cilia	54, 42 43
DNAI2	ODA	ODA defect	Immotile cilia	24
TXNDC3 (NME8)	ODA	ODA defect	Immotile cilia	66
DNAL1	ODA	ODA defect	Impaired motility	67
CCDC114	ODA docking complex	ODA defect	Immotile cilia	68, 69
ARMC4	ODA docking complex	ODA defect	Immotile cilia	70 71
CCDC151	ODA targeting and docking	ODA defect	Immotile cilia	72, 73
CCDC103	Cytoplasmic, ODA assembling	ODA defect	Immotile cilia	74
KTU (DNAAF2)	Cytoplasmic, DA assembling	ODA+IDA defect	Immotile cilia	75
LRRC50 (DNAAF1)	Cytoplasmic, DA assembling	ODA+IDA defect	Immotile cilia	55, 56
DNAAF3	Cytoplasmic, DA assembling	ODA+IDA defect	Immotile cilia	76
DYX1C1	Cytoplasmic, DA assembling	ODA+IDA defect	Immotile cilia	77
HEATR2	Cytoplasmic, DA assembling	ODA+IDA defect	Immotile cilia	78
LRRC6	Cytoplasmic, DA assembling	ODA+IDA defect	Immotile cilia	79, 80
ZMYND10	Cytoplasmic, DA assembling	ODA+IDA defect	Immotile cilia	7, 81
SPAG1	Cytoplasmic, DA assembling	ODA+IDA defect	Immotile cilia	82
C21orf59	Cytoplasmic, DA assembling	ODA+IDA defect		83
CCDC39	N-DRC	MT disorganisation and IDA defect	Hyperkinetic, stiff cilia	57, 84
CCDC40	N-DRC	MT disorganisation and IDA defect	Hyperkinetic, stiff cilia	84, 85
CCDC164	N-DRC	N-DRC defect; not visible	Reduced amplitude	86
CCDC65	N-DRC	N-DRC defect	Impaired motility	83
RSPH4A	RS	MT disorganisation (CP-RS defect); not visible	Circular beat or stiff cilia	87–89
RSPH9	RS	MT disorganisation (CP-RS defect)	Circular beat	87–89
RSPH1	RS	MT disorganisation (CP-RS defect)	Different beating patterns	89–91
HYDIN	CP	CP projection defect; not visible	Reduced amplitude, discoordination	92
DNAH11	ODA	Normal ultrastructure	Hyperkinetic cilia, reduced amplitude	40, 41
RPGR	Cytoplasmic	Syndromic PCD with retinitis pigmentosa		58, 59
OFD1	Cytoplasmic	Syndromic PCD with orofacialdigital syndrome		60
CCNO	Apical cytoplasm	Reduction of multiple motile cilia		44

*For more details on the ciliary movement impairment, see ref. 61
BF, cilia beat frequency; CP, central pair; DA, dynein arms; IDA, inner dynein arm; MT, microtubule; N-DRC, nexin-dynein regulatory complex; ODA, outer dynein arm; RS, radial spoke.

individuals with advanced lung disease exhibit greater frequency of *Pseudomonas aeruginosa* and non-tuberculous mycobacteria occurrence.[31 38] With time, lung functions may deteriorate to the stage of a severe respiratory failure, when lobectomy and lung transplantation are highly recommended.[26] Moreover, male infertility due to the sperm tail dysmotility, as well as decreased fertility in women, is observed.[31]

Recognition of PCD and the correct diagnosis are often delayed due to the clinical symptoms overlapping with other chronic airway disorders. The diagnosis is easier when the patient exhibits abnormal placement of the internal organs observed on the chest X-ray.[25 26] As the disease is progressive, late recognition may result in a worse prognosis for patients due to an inadequate previous treatment. Thus, PCD diagnosis requires a well-described clinical phenotype combined with the identification of abnormalities in the ciliary ultrastructure and/or beating pattern.

Patients with PCD exhibit distinct structural and functional defects of cilia, ranging from almost normal ultrastructure but abnormal beat pattern, through the lack of dynein arms resulting in cilia immotility, to a complete absence of cilia.[24 40–44] Up to now, a combination of several diagnostic methods/techniques is used to properly identify the disease, including nasal nitric oxide measurement,[45] TEM analysis of the ciliary ultrastructure,[46–48] high-resolution immunofluorescence (IF) microscopy[49 50] and high-speed video microscopy (HSVM) analysis of ciliary waveform and the beat frequency.[32] In some cases, to differentiate PCD from clinically similar secondary ciliary dyskinesia, ciliogenesis de novo is required, and cultures of the respiratory epithelium cells (submerged[51] or air–liquid interface[38 52]) are performed.

Correct recognition of PCD can be aided by performing genetic tests, which would detect underlying genetic causes of the ciliary dysfunction. However, because of the complexity of the ciliary structure and function, PCD is genetically highly heterogeneous. Recently, the availability of high-throughput sequencing techniques contributed to the swift identification of new PCD-causative genes (further referred to as PCD genes), but with almost 30 such genes identified so far, genetic causes of approximately one-third of PCD cases still need to be explained.

GENETICS OF PCD

The identification of PCD genes has been based on linkage studies, candidate gene approaches and proteomic analyses, combined with sequencing of potentially causative genes.[53 54] Linkage analysis and homozygosity mapping have been used to indicate the regions of interest in the genome of PCD families and cohorts.[54 55] Selection of the candidate genes was based on data from model organisms; in particular, *Chlamydomonas* mutants with the defective structure and/or function of flagella delivered many candidate genes for PCD studies in

J Med Genet: first published as 10.1136/jmedgenet-2014-102755 on 28 October 2014. Downloaded from http://jmg.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

human.[53 56 57] For many years, search for mutation in the candidate genes was based on Sanger sequencing. Recently, the next-generation whole-exome and whole-genome sequencing technology became available, revolutionising identification of new mutations and new genes related to PCD.

Table 1 provides a comprehensive list of PCD-related genes. To date, mutations in 27 genes have been identified in patients with classical PCD symptoms. Two more genes, *RPGR* and *OFD1*, are mutated in rare syndromic PCD cases (combined with retinitis pigmentosa and orofacialdigital syndrome, respectively).[58–60] Mutations in another gene, *CCNO*, found in 16 patients, are involved in the disease characterised by the paucity of motile cilia, with the PCD-like clinical consequences. *CCNO* gene encodes cyclin O, and its mutations result in the defective generation and placement of the mother centriole.[44]

The PCD genes can be divided according to their protein product location and function, or to the ultrastructural/functional defect in patients with PCD. These aspects are summarised in table 1 and figure 2. Figure 2 provides a schematic representation of the probable localisation of PCD-relevant proteins within the human ciliary structure components.

Genes encoding ODA components

Mutations in these genes result in a typical defect of the axonemal ultrastructure, characterised by the absence or severe shortening of ODA. This can be observed in TEM cross sections (lack of ODA) or in IF analysis using antibodies directed against the ODA marker proteins (no reaction with anti-DNAH5 and anti-DNAH9).[50] The defects cause complete or almost complete immotility of cilia.[61]

The first PCD gene, *DNAI1*, identified in 1999 using a candidate gene approach,[53] is the human ortholog of *Chlamydomonas IC78* gene and encodes intermediate dynein chain 1 of ODA. The majority of *DNAI1* mutations, clustering in three exons (13, 16 and 17) and in intron 1 (IVC1 +2_3insT), explain up to 14% of PCD cases.[62–65]

The second identified gene, *DNAH5*, discovered using homozygosity mapping and linkage analysis performed in one consanguineous family,[54] is the human ortholog of *Chlamydomonas* gene encoding γ-axonemal heavy dynein chain. *DNAH5* encodes heavy dynein chain 5, the major molecular motor of ODAs. Mutations in *DNAH5* identified in further studies[42 43] account together for more than 25% of all PCD cases.[43] The most frequently found mutations cluster in exons 34, 50, 63, 76 and 77.[49]

Other genes encoding structural components of ODA, *DNAI2, DNAL1* and *TXNDC3*, are less frequently involved in PCD. Mutations in *DNAI2*, encoding intermediate dynein chain 2, were identified using candidate gene approach[24]; mutations have been found in five PCD families so far.[7 24]

TXNDC3 (also known as *NME8*) was identified as the human ortholog of the sea urchin ODA component *IC1*[66]; two compound mutations have been found in a single patient. *DNAL1* was considered a good candidate for PCD since DNAL1 protein was shown to specifically bind DNAH5. But for a long time the search for mutations was ineffective.[65] Finally, a homozygous point mutation in *DNAL1* was found in two Bedouin patients with absent or shortened ODAs.[67]

IF analysis of the respiratory cilia from PCD individuals with *DNAI2, DNAH5* and *DNAI1* mutations revealed that mutations in *DNAI1* affect assembly of proximal ODA complexes (containing DNAH5 and DNAH9), while mutations in *DNAI2* and *DNAH5* disturb the assembly of both ODA complexes, proximal and distal (which do not contain DNAH9).[24]

Genes related to assembling of dynein arms

Multisubunit dynein arm complexes, produced and preassembled in the cytoplasm, are transported to the cilia and anchored into the axonemal MTs via the ODA docking complex. Recently, mutations in a number of genes involved in the assembly, targeting and docking processes have been identified in individuals with ODA or combined ODA/IDA defects.

ODA defects have been shown to result from mutations in *CCDC114*, encoding ODA-docking complex component required for attachment of ODA to the MT A,[68] and in *CCDC151*, encoding protein required for the axonemal assembly of ODA-docking complexes.[69] Mutations in these two genes have been found in singular PCD families. More frequent mutations have been found in *ARMC4*, encoding the protein, which may be important in targeting or anchoring of ODA to the MT A.[70 71]

Furthermore, cytoplasmic proteins were identified, which most probably play a role in preassembly or transport of both ODA and IDA components. *KTU (DNAAF2)*, encoding protein localising to the apical part of the cell cytoplasm, was the first preassembly factor described to cause PCD.[75] It was identified in medaka fish mutant with dynein arms defect; the loss of both dynein arms and immotility of cilia have been observed in two patients with mutations in *KTU*.[75] Other genes encoding factors involved in the cytoplasmic preassembly pathway in PCD pathogenesis are: rarely involved *LRRC50* (*DNAAF1*),[55 56] *DNAAF3*,[76] *HEATR2*[78] and *C21orf59*;[83] frequently or very frequently involved *DYX1C1* (protein interacts with KTU),[77] *LRRC6*,[7 79 80] *ZMYND10* (protein interacts with LRRC6)[7 81] and *SPAG1*.[82] Mutations in all these genes lead to the combined ODA and IDA deficiency.

IF analysis in individuals (mostly Pakistani) with mutations in *CCDC103* demonstrated that respiratory cilia partially lack ODA complexes (with the ODA marker protein DNAH5 accumulating in the apical part of cytoplasm) but exhibit normal localisation of IDA marker protein DNALI1.[74] CCDC103 has been proposed to act during ODA assembly in the cytoplasm as well as in the proximal part of cilia.[74]

Genes encoding radial spokes or CP component

Abnormalities in MTs arrangement involving absence of the CP, with the 9+0 pattern or 8+1 pattern (one of peripheral doublets shifted towards the axoneme centre), are the most common feature observed in TEM cross sections in patients with mutations in some genes.[31 93] These defects result in a range of altered beat patterns.[61 87]

RSPH4A and RSPH9 are radial spoke head proteins and most probably play a role in signal transduction between CP and dynein arms as they interact with CP on their head side and with IDA on the other end (figures 1 and 2). Mutations in *RSPH9* and *RSPH4A* have been first detected using linkage analysis and homozygosity mapping in several consanguineous Bedouin families that shared PCD phenotype with CP–RS abnormalities.[87] Other mutations in *RSPH4A* are relatively frequent in other populations.[88 89 94] In the East European PCD population study, no *RSPH9* mutations were found, suggesting they could be exclusively related to Bedouin families[88]; recently, other *RSPH9* mutations have been reported in four families of unspecified origin.[89] Recently, mutations in another gene encoding radial spoke head protein, *RSPH1*, have been detected in a number of patients with PCD with the CP–RS defect[89–91]; *RSPH1* appears to be the most common gene mutated in individuals with this particular defect.[89]

J Med Genet: first published as 10.1136/jmedgenet-2014-102755 on 28 October 2014. Downloaded from http://jmg.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

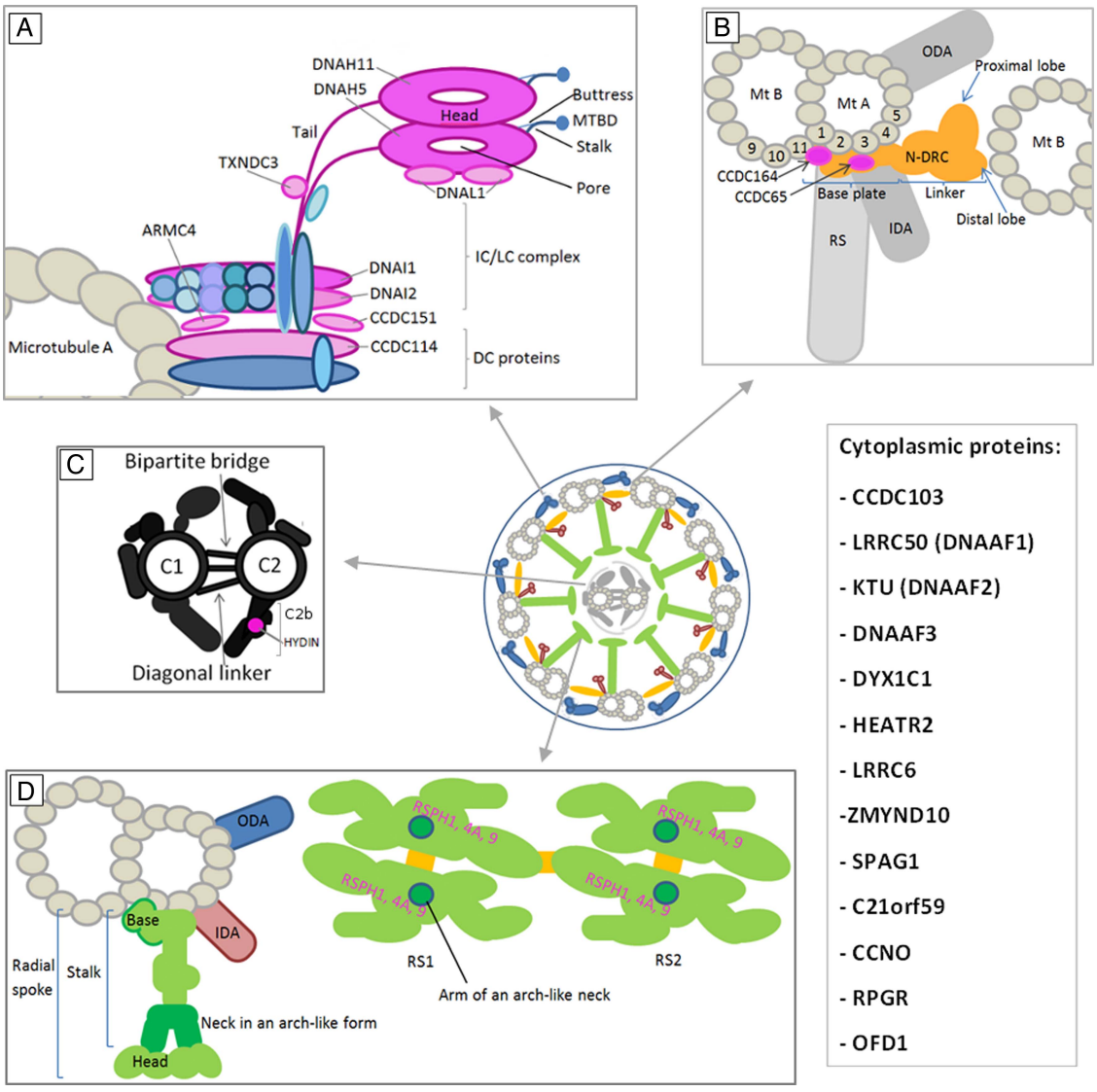

Figure 2 The probable localisation of identified primary ciliary dyskinesia (PCD) genes products within the human ciliary structure components. The schemes of human axonemal complexes are adapted from *Chlamydomonas* data. The proteins related to PCD are indicated in purple and their names are provided. Cytoplasmic proteins involved in PCD phenotype are listed in the box on the right. (A) ODA consists of heavy dynein chains β and γ (comprising a tail, the AAA-ring-like structure, which is a motor unit, and the stalk with MTBD and coiled-coil protrusion called strut or buttress, which strengthens the base of the stalk), LCs, ICs and DC proteins. (B) N-DRC consists of the base plate and linker that further comprises proximal and distal lobes. (C) CP scheme involves microtubules C1 and C2 with their projections (including C2b) interconnected by a bipartite bridge and diagonal linker. (D) RS structure is depicted in a cross-sectional (left) and a bottom (right) view. RSPH1, 4A and 9 are components of RS head (pink). CP, central pair; DC, docking complex; IC, intermediate chain; IDA, inner dynein arm; LC, light chain; Mt A and B, microtubule A and B; MTBD, microtubule binding domain; N-DRC, nexin-dynein regulatory complex; ODA, outer dynein arm; RS, radial spoke; β and γ, dynein heavy chains β and γ. Schemes based on the original data published previously.[20 21 87 99–101]

Mutations in *HYDIN*, found so far in five families, cause much more subtle defects.[92] Mutant cilia lack the C2b projection in the CP complex (figure 2), but most TEM cross sections appear normal, with no MT disorganisation typical for mutations in the radial spoke proteins. Patients demonstrate only subtle abnormalities in the ciliary waveform, which can be correctly recognised only when aided by the high-speed videomicroscopy analysis.[92] These features impede correct diagnosis of PCD in patients with *HYDIN* mutations.

Genes encoding proteins of the N-DRC

The studies on *Chlamydomonas* mutants provided an insight into the complexity of the N-DRC structure, and a number of protein components of N-DRC have been identified, including DRC4, DRC1 and FAP250.[22 83 86] IF analysis of human cilia performed using GAS11 (aka GAS8), the human ortholog of DRC4, has been used as a protein marker of N-DRC in IF studies in human PCD.[50] So far, no mutation has been identified in *GAS11* but mutations in other N-DRC proteins have been found.

The first two mutant genes associated with defects in N-DRC and IDA complexes in PCD were *CCDC39* and *CCDC40*.[57 84 85] Broad analysis of the axonemal structure and function in the individuals with mutations revealed the same defects. The cilia lacked N-DRC, IDA and RS, which resulted in MT disorganisation, and the hyperkinetic and stiff ciliary beat pattern.[57 84 85] Such a wide range of ultrastructural defects indicated that both proteins could be components of another

J Med Genet: first published as 10.1136/jmedgenet-2014-102755 on 28 October 2014. Downloaded from http://jmg.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

axonemal complex related or attached to N-DRC.[86 87] Mutations in these genes have been found in a large number of patients with PCD.

Two other genes related to N-DRC defects were found in a few families. Patients with PCD with mutations in *CCDC164*, a human ortholog of *Chlamydomonas DRC1*, exhibited the absence of N-DRC but no IDA or MT defects were found.[86] Moreover, it has been shown that CCDC164 mutations do not disturb proper localisation of CCDC39. The impairment of the ciliary beat pattern and beat frequency observed in *CCDC164* mutant cilia were less severe than in *CCDC39* mutants.[86]

Patients with mutations in *CCDC65*, the human ortholog of *Chlamydomonas* FAP250, displayed reduction of IDA and N-DRC, and stiff ciliary beat pattern.[83] Moreover, some TEM cross sections showed MT disorganisation advocating the role of N-DRC in stabilisation of axonemal structures organisation.[83]

Genes responsible for abnormalities in ciliary movement with normal ultrastructure

Some patients with PCD presenting clinical manifestations consistent with PCD phenotype have no detectable ultrastructural defects of cilia. In these cases, however, abnormalities in the ciliary beat pattern can be found. Cilia are stiff (nonflexible) and hyperkinetic[40] or static,[95] resulting in the impaired mucociliary clearance and clinical PCD phenotype. So far, *DNAH11*, encoding heavy dynein chain 11 of ODA, has been the only identified gene related to such a phenotype.[40] Mutations in this gene are relatively common in PCD individuals with normal axonemal ultrastructure and explain about 20% of this particular phenotype.[41]

GENETIC TESTING IN PCD

For a long time, genetic testing available was based on the identification of the most common mutations in two genes responsible for the ODA defect: IVS1+2_3insT and mutations in exons 13, 16 and 17 for *DNAI1*, and exons 34, 50, 63, 76 and 77 for *DNAH5*.[49] Mutations in these two genes explain about 50%–60% of PCD cases with the ODA defect. With ODA defects observed in ~60% of PCD cases, mutations in *DNAH5* and *DNAI1* can be expected in only 30%–35% of the whole PCD population.[49] This proportion well illustrates the fact that genetic testing comprising only selected genes in a heterogeneous disease like PCD is not sufficiently effective. Moreover, genotyping only the most frequent mutations in genes characterised by high allelic heterogeneity often results in the identification of only a single mutated allele, which is not sufficient for the diagnosis; in such cases, the whole gene should be screened to search for the second, trans-allelic mutation.[49] The scope of mutations has been expanded including other PCD genes in addition to the first two ones.[96]

The one-by-one screening of a number of genes in genetically heterogeneous disorders like PCD is, therefore, neither time-effective nor cost-effective. The whole-exome sequencing (WES) enables rapid and relatively reasonably priced identification of mutations in genetically heterogeneous disorders like PCD. The WES approach has been recently used for the identification of new genes associated with PCD,[7 68 70] but this technology is still reserved for the research field and not applied in the diagnostics. However, with dropping sequencing costs, there is a chance for WES to become a routine test in the future. Indeed, this is currently happening: a broad diagnostic gene panel service has just been initiated within the North Thames Regional Genetics service provided at Great Ormond Street Hospital.[97]

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

A rapid acceleration in the pace of identification of PCD-causing genes during the last 2–3 years is notable. This is the result of new technologies that have been launched recently and applied in genetic research, like whole-genome SNP-based homozygosity mapping or WES. Nevertheless, genetic causes of approximately one-third of PCD cases remain to be explained.

The genes already identified can be used in genetic screening, enabling earlier recognition of PCD and implementation of an appropriate treatment from the earliest age. This would have a huge impact on the patients, vastly improving the quality of life, professional and private. In this context, it is important that many of the PCD genes identified so far are related to particular PCD phenotypes, which facilitates and simplifies genetic screening if preceded by TEM, IF or HSVM analysis.

It should be emphasised that while early studies focused on the identification of genes encoding proteins that build easily recognised ultrastructural components of cilia, like ODA, IDA or RS, the recent advances in understanding of the ciliary structure and assembly have shifted the attention to the genes encoding proteins involved in the processes of assembling, targeting and docking of these ciliary components.

The recent findings indicate the existence of sequential stages of dynein arms assembly. Functional characterisation of the genes encoding cytoplasmic proteins, which are not a part of the axoneme structure, can help to explain the processes during the axoneme assembly pathway. Moreover, cytoplasmic proteins involved in ciliogenesis might be more sensitive/responsive to potential drugs and new treatment modalities due to their better physical accessibility than the structural axonemal proteins.

Acknowledgements MK is supported by the project 'Studies of nucleic acids and proteins—from basic to applied research', sponsored by the International PhD Projects Programme of Foundation for Polish Science. The project is co-financed by the European Union Regional Development Fund. MK is also supported by the project 'Scholarship support for PhD. students specialising in majors strategic for Wielkopolska's development', Sub-measure 8.2.2 Human Capital Operational Programme, co-financed by European Union under the European Social Fund. EZ is supported by the National Science Centre (grant no. 2011/01/B/NZ4/04840). This work has been supported from the European Union Seventh Framework Programme (FP7/2007-2013) funds under grant agreement no. (305404) (BESTCILIA) (MW).

Contributors MK performed the literature research, prepared figures and table, and wrote the manuscript. EZ and MW reviewed the paper.

Competing interests None.

Provenance and peer review Commissioned; externally peer reviewed.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

REFERENCES

1 Wirschell M, Yamamoto R, Alford L, Gokhale A, Gaillard A, Sale W. Regulation of ciliary motility: conserved protein kinases and phosphatases are targeted and anchored in the ciliary axoneme. *Arch Biochem Biophys* 2011;510:93–100.
2 Rompolas P, Pedersen L, Patel-King R, King S. Chlamydomonas FAP133 is a dynein intermediate chain associated with the retrograde intraflagellar transport motor. *J Cell Sci* 2007;120(Pt 20):3653–65.
3 Schneider M, Ulland M, Sloboda R. A protein methylation pathway in Chlamydomonas flagella is active during flagellar resorption. *Mol Biol Cell* 2008;19:4319–27.
4 Lin H, Nauman N, Albee A, Hsu S, Dutcher S. New mutations in flagellar motors identified by whole genome sequencing in Chlamydomonas. *Cilia* 2013;2:14.

J Med Genet: first published as 10.1136/jmedgenet-2014-102755 on 28 October 2014. Downloaded from http://jmg.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

5 Tissir F, Qu Y, Montcouquiol M, Zhou L, Komatsu K, Shi D, Fujimori T, Labeau J, Tyteca D, Courtoy P, Poumay Y, Uemura T, Goffinet AM. Lack of cadherins Celsr2 and Celsr3 impairs ependymal ciliogenesis, leading to fatal hydrocephalus. *Nat Neurosci* 2010;13:700–7.
6 Bylander A, Lind K, Goksör M, Billig H, Larsson DG. The classical progesterone receptor mediates the rapid reduction of fallopian tube ciliary beat frequency by progesterone. *Reprod Biol Endocrinol* 2013;11:33.
7 Zariwala MA, Gee HY, Kurkowiak M, Al-Mutairi DA, Leigh MW, Hurd TW, Hjeij R, Dell SD, Chaki M, Dougherty GW, Adan M, Spear PC, Esteve-Rudd J, Loges NT, Rosenfeld M, Diaz KA, Olbrich H, Wolf WE, Sheridan E, Batten TF, Halbritter J, Porath JD, Kohl S, Lovric S, Hwang DY, Pittman JE, Burns KA, Ferkol TW, Sagel SD, Olivier KN, Morgan LC, Werner C, Raidt J, Pennekamp P, Sun Z, Zhou W, Airik R, Natarajan S, Allen SJ, Amirav I, Wieczorek D, Landwehr K, Nielsen K, Schwerk N, Sertic J, Köhler G, Washburn J, Levy S, Fan S, Koerner-Rettberg C, Amselem S, Williams DS, Mitchell BJ, Drummond IA, Otto EA, Omran H, Knowles MR, Hildebrandt F. ZMYND10 is mutated in primary ciliary dyskinesia and interacts with LRRC6. *Am J Hum Genet* 2013;93:336–45.
8 Ben Khelifa M, Coutton C, Zouari R, Karaouzène T, Rendu J, Bidart M, Yassine S, Pierre V, Delaroche J, Hennebicq S, Grunwald D, Escalier D, Pernet-Gallay K, Jouk PS, Thierry-Mieg N, Touré A, Arnoult C, Ray PF. Mutations in DNAH1, which encodes an inner arm heavy chain dynein, lead to male infertility from multiple morphological abnormalities of the sperm flagella. *Am J Hum Genet* 2014;94:95–104.
9 Yoshiba S, Shiratori H, Kuo IY, Kawasumi A, Shinohara K, Nonaka S, Asai Y, Sasaki G, Belo JA, Sasaki H, Nakai J, Dworniczak B, Ehrlich BE, Pennekamp P, Hamada H. Cilia at the node of mouse embryos sense fluid flow for left-right determination via Pkd2. *Science* 2012;338:226–31.
10 Wang G, Yost HJ, Amack JD. Analysis of gene function and visualization of cilia-generated fluid flow in Kupffer's vesicle. *J Vis Exp* 2013;10:e50038.
11 Kindt KS, Finch G, Nicolson T. Kinocilia mediate mechanosensitivity in developing zebrafish hair cells. *Dev Cell* 2012;23:329–41.
12 Bergmann C. ARPKD and early manifestations of ADPKD: the original polycystic kidney disease and phenocopies. *Pediatr Nephrol* 2014. Published Online First: 1 March 2014. doi 10.1007/s00467-013-2706-2
13 Ko JY, Park JH. Mouse models of polycystic kidney disease induced by defects of ciliary proteins. *BMB Rep* 2013;46:73–9.
14 Larusso NF, Masyuk TV. The role of cilia in the regulation of bile flow. *Dig Dis* 2011;29:6–12.
15 Gallagher AR, Esquivel EL, Briere TS, Tian X, Mitobe M, Menezes LF, Markowitz GS, Jain D, Onuchic LF, Somlo S. Biliary and pancreatic dysgenesis in mice harboring a mutation in Pkhd1. *Am J Pathol* 2008;172:417–29.
16 Muhammad H, Rais Y, Miosge N, Ornan EM. The primary cilium as a dual sensor of mechanochemical signals in chondrocytes. *Cell Mol Life Sci* 2012; 69:2101–7.
17 Ho L, Ali SA, Al-Jazrawe M, Kandel R, Wunder JS, Alman BA. Primary cilia attenuate hedgehog signalling in neoplastic chondrocytes. *Oncogene* 2013;32:5388–96.
18 Di Gioia SA, Letteboer SJ, Kostic C, Bandah-Rozenfeld D, Hetterschijt L, Sharon D, Arsenijevic Y, Roepman R, Rivolta C. FAM161A, associated with retinitis pigmentosa, is a component of the cilia-basal body complex and interacts with proteins involved in ciliopathies. *Hum Mol Genet* 2012;21:5174–84.
19 Pazour GJ, Agrin N, Leszyk J, Witman GB. Proteomic analysis of a eukaryotic cilium. *J Cell Biol* 2005;170:103–13.
20 Schmidt H, Gleave E, Carter A. Insights into dynein motor domain function from a 3.3-Å crystal structure. *Nat Struct Mol Biol* 2012;19:492–7.
21 Heuser T, Raytchev M, Krell J, Porter M, Nicastro D. The dynein regulatory complex is the nexin link and a major regulatory node in cilia and flagella. *J Cell Biol* 2009;187:921–33.
22 Dymek E, Heuser T, Nicastro D, Smith E. The CSC is required for complete radial spoke assembly and wild-type ciliary motility. *Mol Biol Cell* 2011;22:2520–31.
23 Heuser T, Dymek E, Lin J, Smith E, Nicastro D. The CSC connects three major axonemal complexes involved in dynein regulation. *Mol Biol Cell* 2012;23:3143–55.
24 Loges NT, Olbrich H, Fenske L, Mussaffi H, Horvath J, Fliegauf M, Kuhl H, Baktai G, Peterffy E, Chodhari R, Chung EM, Rutman A, O'Callaghan C, Blau H, Tiszlavicz L, Voelkel K, Witt M, Zietkiewicz E, Neesen J, Reinhardt R, Mitchison HM, Omran H. DNAI2 mutations cause primary ciliary dyskinesia with defects in the outer dynein arm. *Am J Hum Genet* 2008;83:547–58.
25 Kuehni CE, Frischer T, Strippoli MP, Maurer E, Bush A, Nielsen KG, Escribano A, Lucas JS, Yiallouros P, Omran H, Eber E, O'Callaghan C, Snijders D, Barbato A; ERS Task Force on Primary Ciliary Dyskinesia in Children. Factors influencing age at diagnosis of primary ciliary dyskinesia in European children. *Eur Respir J* 2010;36:1248–58.
26 Barbato A, Frischer T, Kuehni C, Snijders D, Azevedo I, Baktai G, Bartoloni L, Eber E, Escribano A, Haarman E, Hesselmar B, Hogg C, Jorissen M, Lucas J, Nielsen KG, O'Callaghan C, Omran H, Pohunek P, Strippoli MP, Bush A. Primary ciliary dyskinesia: a consensus statement on diagnostic and treatment approaches in children. *Eur Respir J* 2009;34:1264–76.
27 Pifferi M, Di Cicco M, Piras M, Cangiotti A, Saggese G. Up to date on primary ciliary dyskinesia in children. *Early Hum Dev* 2013;89(Suppl 3):S45–8.
28 Sievert A. Über einen Fall von Bronchiectasie bei einem Patienten mit situs inversus viscerum. *Berliner klinische Wochenschrift* 1904;41:139–41.
29 Kartagener M. Zur Pathogenese der Bronchiektasien: Bronchiektasien bei Situs viscerum inversus. *Beiträge zur Klinik der Tuberkulose* 1933;83:489–501.
30 Afzelius B. A human syndrome caused by immotile cilia. *Science* 1976;193:317–19.
31 Noone P, Leigh M, Sannuti A, Minnix S, Carson J, Hazucha M, Zariwala MA, Knowles MR. Primary ciliary dyskinesia: diagnostic and phenotypic features. *Am J Respir Crit Care Med* 2004;169:459–67.
32 Stannard W, Chilvers M, Rutman A, Williams C, O'Callaghan C. Diagnostic testing of patients suspected of primary ciliary dyskinesia. *Am J Respir Crit Care Med* 2010;181:307–14.
33 Kennedy MP, Omran H, Leigh MW, Dell S, Morgan L, Molina PL, Robinson BV, Minnix SL, Olbrich H, Severin T, Ahrens P, Lange L, Morillas HN, Noone PG, Zariwala MA, Knowles MR. Congenital heart disease and other heterotaxic defects in a large cohort of patients with primary ciliary dyskinesia. *Circulation* 2007;115:2814–21.
34 Shapiro AJ, Davis SD, Ferkol T, Dell SD, Rosenfeld M, Olivier KN, Sagel SD, Milla C, Zariwala MA, Wolf W, Carson JL, Hazucha MJ, Burns K, Robinson B, Knowles MR, Leigh MW; for the Genetic Disorders of Mucociliary Clearance Consortium. Laterality defects other than situs inversus totalis in primary ciliary dyskinesia: insights into situs ambiguus and heterotaxy. *Chest* 2014. Published Online First: 27 February 2014. doi:10.1378/chest.13-1704
35 Sommer JU, Schäfer K, Omran H, Olbrich H, Wallmeier J, Blum A, Hörmann K, Stuck BA. ENT manifestations in patients with primary ciliary dyskinesia: prevalence and significance of otorhinolaryngologic co-morbidities. *Eur Arch Otorhinolaryngol* 2011;268:383–8.
36 Coren ME, Meeks M, Morrison I, Buchdahl RM, Bush A. Primary ciliary dyskinesia: age at diagnosis and symptom history. *Acta Paediatr* 2002;91:667–9.
37 Stannard W, O'Callaghan C. Ciliary function and the role of cilia in clearance. *J Aerosol Med* 2006;19:110–15.
38 Boon M, Smits A, Cuppens H, Jaspers M, Proesmans M, Dupont L, Vermeulen FL, Van Daele S, Malfroot A, Godding V, Jorissen M, De Boeck K. Primary ciliary dyskinesia: critical evaluation of clinical symptoms and diagnosis in patients with normal and abnormal ultrastructure. *Orphanet J Rare Dis* 2014;9:1–10.
39 Rogers GB, Carroll MP, Zain NM, Bruce KD, Lock K, Walker W, Jones G, Daniels TW, Lucas JS. Complexity, temporal stability, and clinical correlates of airway bacterial community composition in primary ciliary dyskinesia. *J Clin Microbiol* 2013;51:4029–35.
40 Pifferi M, Michelucci A, Conidi M, Cangiotti A, Simi P, Macchia P, Boner A. New DNAH11 mutations in primary ciliary dyskinesia with normal axonemal ultrastructure. *Eur Respir J* 2010;35:1413–16.
41 Knowles M, Leigh M, Carson J, Davis S, Dell S, Ferkol T, Olivier KN, Sagel SD, Rosenfeld M, Burns KA, Minnix SL, Armstrong MC, Lori A, Hazucha MJ, Loges NT, Olbrich H, Becker-Heck A, Schmidts M, Werner C, Omran H, Zariwala MA; Genetic Disorders of Mucociliary Clearance Consortium. Mutations of DNAH11 in patients with primary ciliary dyskinesia with normal ciliary ultrastructure. *Thorax* 2012;67:433–41.
42 Olbrich H, Häffner K, Kispert A, Völkel A, Volz A, Sasmaz G, Reinhardt R, Hennig S, Lehrach H, Konietzko N, Zariwala M, Noone PG, Knowles M, Mitchison HM, Meeks M, Chung EM, Hildebrandt F, Sudbrak R, Omran H. Mutations in DNAH5 cause primary ciliary dyskinesia and randomization of left-right asymmetry. *Nat Genet* 2002;30:143–4.
43 Hornef N, Olbrich H, Horvath J, Zariwala M, Fliegauf M, Loges NT, Wildhaber J, Noone PG, Kennedy M, Antonarakis SE, Blouin JL, Bartoloni L, Nüsslein T, Ahrens P, Griese M, Kuhl H, Sudbrak R, Knowles MR, Reinhardt R, Omran H. DNAH5 mutations are a common cause of primary ciliary dyskinesia with outer dynein arm defects. *Am J Respir Crit Care Med* 2006;174:120–6.
44 Wallmeier J, Al-Mutairi DA, Chen CT, Loges NT, Pennekamp P, Menchen T, Ma L, Shamseldin HE, Olbrich H, Dougherty GW, Werner C, Alsabah BH, Köhler G, Jaspers M Boon M, Griese M, Schmitt-Grohé S, Zimmermann T, Koerner-Rettberg C, Horak E, Kintner C, Alkuraya FS, Omran H. Mutations in CCNO result in congenital mucociliary clearance disorder with reduced generation of multiple motile cilia. *Nat Genet* 2014;46:646–51.
45 Leigh MW, Hazucha MJ, Chawla KK, Baker BR, Shapiro AJ, Brown DE, Lavange LM, Horton BJ, Qaqish B, Carson JL, Davis SD, Dell SD, Ferkol TW, Atkinson JJ, Olivier KN, Sagel SD, Rosenfeld M, Milla C, Lee HS, Krischer J, Zariwala MA, Knowles MR. Standardizing nasal nitric oxide measurement as a test for primary ciliary dyskinesia. *Ann Am Thorac Soc* 2013;10:574–81.
46 Papon J, Coste A, Roudot-Thoraval F, Boucherat M, Roger G, Tamalet A, Vojtek AM, Amselem S, Escudier E. A 20-year experience of electron microscopy in the diagnosis of primary ciliary dyskinesia. *Eur Respir J* 2010;35:1057–63.
47 Escudier E, Duquesnoy P, Papon J, Amselem S. Ciliary defects and genetics of primary ciliary dyskinesia. *Paediatr Respir Rev* 2009;10:51–4.
48 Funkhouser WK III, Niethammer M, Carson JL, Burns KA, Knowles MR, Leigh MW, Zariwala MA, Funkhouser WK Jr. A new tool improves diagnostic test performance

J Med Genet: first published as 10.1136/jmedgenet-2014-102755 on 28 October 2014. Downloaded from http://jmg.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

for transmission EM evaluation of axonemal dynein arms. *Ultrasrtuct Pathol* 2013;38:248–55.
49 Zariwala M, Knowles M, Omran H. Genetic defects in ciliary structure and function. *Annu Rev Physiol* 2007;69:423–50.
50 Omran H, Loges NT. Immunofluorescence staining of ciliated respiratory epithelial cells. *Methods Cell Biol* 2009;91:123–33.
51 Jorissen M, Van der Schueren B, Van den Berghe H, Cassiman JJ. Ciliogenesis in cultured human nasal epithelium. *ORL J Otorhinolaryngol Relat Spec* 1990;52:368–74.
52 Hirst RA, Jackson CL, Coles JL, Williams G, Rutman A, Goggin PM, Adam EC, Page A, Evans HJ, Lackie PM, O'Callaghan C, Lucas JS. Culture of primary ciliary dyskinesia epithelial cells at air-liquid interface can alter ciliary phenotype but remains a robust and informative diagnostic aid. *PLoS ONE* 2014;9:e89675.
53 Pennarun G, Escudier E, Chapelin C, Bridoux A, Cacheux V, Roger G, Clément A, Goossens M, Amselem S, Duriez B. Loss-of-function mutations in a human gene related to Chlamydomonas reinhardtii dynein IC78 result in primary ciliary dyskinesia. *Am J Hum Genet* 1999;65:1508–19.
54 Omran H, Häffner K, Völkel A, Kuehr J, Ketelsen UP, Ross UH, Konietzko N, Wienker T, Brandis M, Hildebrandt F. Homozygosity mapping of a gene locus for primary ciliary dyskinesia on chromosome 5p and identification of the heavy dynein chain DNAH5 as a candidate gene. *Am J Respir Cell Mol Biol* 2000;23:696–702.
55 Loges NT, Olbrich H, Becker-Heck A, Häffner K, Heer A, Reinhard C, Schmidts M, Kispert A, Zariwala MA, Leigh MW, Knowles MR, Zentgraf H, Seithe H, Nürnberg G, Nürnberg P, Reinhardt R, Omran H. Deletions and point mutations of LRRC50 cause primary ciliary dyskinesia due to dynein arm defects. *Am J Hum Genet* 2009;85:883–9.
56 Duquesnoy P, Escudier E, Vincensini L, Freshour J, Bridoux AM, Coste A, Deschildre A, de Blic J, Legendre M, Montantin G, Tenreiro H, Vojtek AM, Loussert C, Clément A, Escalier D, Bastin P, Mitchell DR, Amselem S. Loss-of-function mutations in the human ortholog of Chlamydomonas reinhardtii ODA7 disrupt dynein arm assembly and cause primary ciliary dyskinesia. *Am J Hum Genet* 2009;85:890–6.
57 Merveille AC, Davis EE, Becker-Heck A, Legendre M, Amirav I, Bataille G, Belmont J, Beydon N, Billen F, Clément A, Clercx C, Coste A, Crosbie R, de Blic J, Deleuze S, Duquesnoy P, Escalier D, Escudier E, Fliegauf M, Horvath J, Hill K, Jorissen M, Just J, Kispert A, Lathrop M, Loges NT, Marthin JK, Momozawa Y, Montantin G, Nielsen KG, Olbrich H, Papon JF, Rayet I, Roger G, Schmidts M, Tenreiro H, Towbin JA, Zelenika D, Zentgraf H, Georges M, Lequarré AS, Katsanis N, Omran H, Amselem S. CCDC39 is required for assembly of inner dynein arms and the dynein regulatory complex and for normal ciliary motility in humans and dogs. *Nat Genet* 2011;43:72–8.
58 Moore A, Escudier E, Roger G, Tamalet A, Pelosse B, Marlin S, Clément A, Geremek M, Delaisi B, Bridoux AM, Coste A, Witt M, Duriez B, Amselem S. RPGR is mutated in patients with a complex X linked phenotype combining primary ciliary dyskinesia and retinitis pigmentosa. *J Med Genet* 2006;43:326–33.
59 Bukowy-Bieryłło Z, Ziętkiewicz E, Loges NT, Wittmer M, Geremek M, Olbrich H, Fliegauf M, Voelkel K, Rutkiewicz E, Rutland J, Morgan L, Pogorzelski A, Martin J, Haan E, Berger W, Omran H, Witt M. RPGR mutations might cause reduced orientation of respiratory cilia. *Pediatr Pulmonol* 2013;48:352–63.
60 Budny B, Chen W, Omran H, Fliegauf M, Tzschach A, Wisniewska M, Jensen LR, Raynaud M, Shoichet SA, Badura M, Lenzner S, Latos-Bielenska A, Ropers HH. A novel X-linked recessive mental retardation syndrome comprising macrocephaly and ciliary dysfunction is allelic to oral-facial-digital type I syndrome. *Hum Genet* 2006;120:171–8.
61 Raidt J, Wallmeier J, Hjeij R, Onnebrink JG, Pennekamp P, Loges NT, Olbrich H, Häffner K, Dougherty GW, Omran H, Werner C. Ciliary beat pattern and frequency in genetic variants of primary ciliary dyskinesia. *Eur Respir J* 2014:pii:erj00520–2014. Published online first: 3 September 2014. doi 10.1183/09031936.00052014
62 Zariwala MA, Leigh MW, Ceppa F, Kennedy MP, Noone PG, Carson JL, Hazucha MJ, Lori A, Horvath J, Olbrich H, Loges NT, Bridoux AM, Pennarun G, Duriez B, Escudier E, Mitchison HM, Chodhari R, Chung EM, Morgan LC, de Iongh RU, Rutland J, Pradal U, Omran H, Amselem S, Knowles MR. Mutations of DNAI1 in primary ciliary dyskinesia: evidence of founder effect in a common mutation. *Am J Respir Crit Care Med* 2006;174:858–66.
63 Ziętkiewicz E, Nitka B, Voelkel K, Skrzypczak U, Bukowy Z, Rutkiewicz E, Humińska K, Przystałowska H, Pogorzelski A, Witt M. Population specificity of the DNAI1 gene mutation spectrum in primary ciliary dyskinesia (PCD). *Respir Res* 2010;11:1–11.
64 Failly M, Saitta A, Muñoz A, Falconnet E, Rossier C, Santamaria F, de Santi MM, Lazor R, DeLozier-Blanchet CD, Bartoloni L, Blouin JL. DNAI1 mutations explain only 2% of primary ciliary dykinesia. *Respiration* 2008;76:198–204.
65 Horváth J, Fliegauf M, Olbrich H, Kispert A, King S, Mitchison H, Zariwala MA, Knowles MR, Sudbrak R, Fekete G, Neesen J, Reinhardt R, Omran H. Identification and analysis of axonemal dynein light chain 1 in primary ciliary dyskinesia patients. *Am J Respir Cell Mol Biol* 2005;33:41–7.
66 Duriez B, Duquesnoy P, Escudier E, Bridoux A, Escalier D, Rayet I, Marcos E, Vojtek AM, Bercher JF, Amselem S. A common variant in combination with a nonsense mutation in a member of the thioredoxin family causes primary ciliary dyskinesia. *Proc Natl Acad Sci USA* 2007;104:3336–41.
67 Mazor M, Alkrinawi S, Chalifa-Caspi V, Manor E, Sheffield V, Aviram M, Parvari R. Primary ciliary dyskinesia caused by homozygous mutation in DNAL1, encoding dynein light chain 1. *Am J Hum Genet* 2011;88:599–607.
68 Knowles MR, Leigh MW, Ostrowski LE, Huang L, Carson JL, Hazucha MJ, Yin W, Berg JS, Davis SD, Dell SD, Ferkol TW, Rosenfeld M, Sagel SD, Milla CE, Olivier KN, Turner EH, Lewis AP, Bamshad MJ, Nickerson DA, Shendure J, Zariwala MA; Genetic Disorders of Mucociliary Clearance Consortium. Exome sequencing identifies mutations in CCDC114 as a cause of primary ciliary dyskinesia. *Am J Hum Genet* 2013;92:99–106.
69 Onoufriadis A, Paff T, Antony D, Shoemark A, Micha D, Kuyt B, Schmidts M, Petridi S, Dankert-Roelse JE, Haarman EG, Daniels JM, Emes RD, Wilson R, Hogg C, Scambler PJ, Chung EM; UK10K, Pals G, Mitchison HM. Splice-site mutations in the axonemal outer dynein arm docking complex gene CCDC114 cause primary ciliary dyskinesia. *Am J Hum Genet* 2013;92:88–98.
70 Onoufriadis A, Shoemark A, Munye MM, James CT, Schmidts M, Patel M, Rosser EM, Bacchelli C, Beales PL, Scambler PJ, Hart SL, Danke-Roelse JE, Sloper JJ, Hull S, Hogg C, Emes RD, Pals G, Moore AT, Chung EM; UK10K, Mitchison HM. Combined exome and whole-genome sequencing identifies mutations in ARMC4 as a cause of primary ciliary dyskinesia with defects in the outer dynein arm. *J Med Genet* 2014;51:61–7.
71 Hjeij R, Lindstrand A, Francis R, Zariwala MA, Liu X, Li Y, Damerla R, Dougherty GW, Abouhamed M, Olbrich H, Loges NT, Pennekamp P, Davis EE, Carvalho CM, Pehlivan D, Werner C, Raidt J, Köhler G, Häffner K, Reyes-Mugica M, Lupski JR, Leigh MW, Rosenfeld M, Morgan LC, Knowles MR, Lo CW, Katsanis N, Omran H. ARMC4 mutations cause primary ciliary dyskinesia with randomization of left/right body asymmetry. *Am J Hum Genet* 2013;93:357–67.
72 Hjeij R, Onoufriadis A, Watson CM, Slagle CE, Klena NT, Dougherty GW, Kurkowiak M, Loges NT, Diggle CP, Morante NF, Gabriel GC, Lemke KL, Li Y, Pennekamp P, Menchen T, Konert F, Marthin JK, Mans DA, Letteboer SJ, Werner C, Burgoyne T, Westermann C, Rutman A, Carr IM, O'Callaghan C, Moya E, Chung EM; UK10K Consortium, Sheridan E, Nielsen KG, Roepman R, Bartscherer K, Burdine RD, Lo CW, Omran H, Mitchison HM. CCDC151 mutations cause primary ciliary dyskinesia by disruption of the outer dynein arm docking complex formation. *Am J Hum Genet* 2014;95:257–74.
73 Alsaadi MM, Erzurumluoglu AM, Rodriguez S, Guthrie PA, Gaunt TR, Omar HZ, Mubarak M, Alharbi KK, Al-Rikabi AC, Day IN. Nonsense mutation in coiled coil domain containing 151 gene (CCDC151) causes Primary ciliary dyskinesia. *Hum Mutat* 2014. Published Online First: 16 September 2014. doi:10.1002/humu.22698
74 Panizzi J, Becker-Heck A, Castleman VH, Al-Mutairi D, Liu Y, Loges NT, Pathak N, Austin-Tse C, Sheridan E, Schmidts M, Olbrich H, Werner C, Häffner K, Hellman N, Chodhari R, Gupta A, Kramer-Zucker A, Olale F, Burdine RD, Schier AF, O'Callaghan C, Chung EM, Reinhardt R, Mitchison HM, King SM, Omran H, Drummond IA. CCDC103 mutations cause primary ciliary dyskinesia by disrupting assembly of ciliary dynein arms. *Nat Genet* 2013;44:714–19.
75 Omran H, Kobayashi D, Olbrich H, Tsukahara T, Loges NT, Hagiwara H, Zhang Q, Leblond G, O'Toole E, Hara C, Mizuno H, Kawano H, Fliegauf M, Yagi T, Koshida S, Miyawaki A, Zentgraf H, Seithe H, Reinhardt R, Watanabe Y, Kamiya R, Mitchell DR, Takeda H. Ktu/PF13 is required for cytoplasmic pre-assembly of axonemal dyneins. *Nature* 2008;456:611–16.
76 Mitchison HM, Schmidts M, Loges NT, Freshour J, Dritsoula A, Hirst RA, O'Callaghan C, Blau H, Al Dabbagh M, Olbrich H, Beales PL, Yagi T, Mussaffi H, Chung EM, Omran H, Mitchell DR. Mutations in axonemal dynein assembly factor DNAAF3 cause primary ciliary dyskinesia. *Nat Genet* 2012;44:381–9.
77 Tarkar A, Loges NT, Slagle CE, Francis R, Dougherty GW, Tamayo JV, Shook B, Cantino M, Schwartz D, Jahnke C, Olbrich H, Werner C, Raidt J, Pennekamp P, Abouhamed M, Hjeij R, Köhler G, Griese M, Li Y, Lemke K, Klena N, Liu X, Gabriel G, Tobita K, Jaspers M, Morgan LC, Shapiro AJ, Letteboer SJ, Mans DA, Carson JL, Leigh MW, Wolf WE, Chen S, Lucas JS, Onoufriadis A, Plagnol V, Schmidts M, Boldt K; UK10K, Roepman R, Zariwala MA, Lo CW, Mitchison HM, Knowles MR, Burdine RD, Loturco JJ, Omran H. DYX1C1 is required for axonemal dynein assembly and ciliary motility. *Nat Genet* 2013;45:995–1003.
78 Horani A, Druley TE, Zariwala MA, Patel AC, Levinson BT, Van Arendonk LG, Thornton KC, Giacalone JC, Albee AJ, Wilson KS, Turner EH, Nickerson DA, Shendure J, Bayly PV, Leigh MW, Knowles MR, Brody SL, Dutcher SK, Ferkol TW. Whole-exome capture and sequencing identifies HEATR2 mutation as a cause of primary ciliary dyskinesia. *Am J Hum Genet* 2012;91:685–93.
79 Kott E, Duquesnoy P, Copin B, Legendre M, Dastot-Le Moal F, Montantin G, Jeanson L, Tamalet A, Papon JF, Siffroi JP, Rives N, Mitchell V, de Blic J, Coste A, Clement A, Escalier D, Touré A, Escudier E, Amselem S. Loss-of-function mutations in LRRC6, a gene essential for proper axonemal assembly of inner and outer dynein arms, cause primary ciliary dyskinesia. *Am J Hum Genet* 2012;91:958–64.
80 Horani A, Ferkol TW, Shoseyov D, Wasserman MG, Oren YS, Kerem B, Amirav I, Cohen-Cymberknoh M, Dutcher SK, Brody SL, Elpeleg O, Kerem E. LRRC6 mutation causes primary ciliary dyskinesia with dynein arm defects. *PLoS ONE* 2013;8:e59436.

J Med Genet: first published as 10.1136/jmedgenet-2014-102755 on 28 October 2014. Downloaded from http://jmg.bmj.com/ on April 24, 2024 by guest. Protected by copyright.

81 Moore DJ, Onoufriadis A, Shoemark A, Simpson MA, zur Lage PI, de Castro SC, Bartoloni L, Gallone G, Petridi S, Woollard WJ, Antony D, Schmidts M, Didonna T, Makrythanasis P, Bevillard J, Mongan NP, Djakow J, Pals G, Lucas JS, Marthin JK, Nielsen KG, Santoni F, Guipponi M, Hogg C, Antonarakis SE, Emes RD, Chung EM, Greene ND, Blouin JL, Jarman AP, Mitchison HM. Mutations in ZMYND10, a gene essential for proper axonemal assembly of inner and outer dynein arms in humans and flies, cause primary ciliary dyskinesia. *Am J Hum Genet* 2013;93:346–56.

82 Knowles MR, Ostrowski LE, Loges NT, Hurd T, Leigh MW, Huang L, Wolf WE, Carson JL, Hazucha MJ, Yin W, Davis SD, Dell SD, Ferkol TW, Sagel SD, Olivier KN, Jahnke C, Olbrich H, Werner C, Raidt J, Wallmeier J, Pennekamp P, Dougherty GW, Hjeij R, Gee HY, Otto EA, Halbritter J, Chaki M, Diaz KA, Braun DA, Porath JD, Schueler M, Baktai G, Griese M, Turner EH, Lewis AP, Bamshad MJ, Nickerson DA, Hildebrandt F, Shendure J, Omran H, Zariwala MA. Mutations in SPAG1 cause primary ciliary dyskinesia associated with defective outer and inner dynein arms. *Am J Hum Genet* 2013;93:711–20.

83 Austin-Tse C, Halbritter J, Zariwala MA, Gilberti RM, Gee HY, Hellman N, Pathak N, Liu Y, Panizzi JR, Patel-King RS, Tritschler D, Bower R, O'Toole E, Porath JD, Hurd TW, Chaki M, Diaz KA, Kohl S, Lovric S, Hwang DY, Braun DA, Schueler M, Airik R, Otto EA, Leigh MW, Noone PG, Carson JL, Davis SD, Pittman JE, Ferkol TW, Atkinson JJ, Olivier KN, Sagel SD, Dell SD, Rosenfeld M, Milla CE, Loges NT, Omran H, Porter ME, King SM, Knowles MR, Drummond IA, Hildebrandt F. Zebrafish ciliopathy screen plus human mutational analysis identifies C21orf59 and CCDC65 defects as causing primary ciliary dyskinesia. *Am J Hum Genet* 2013;93:672–86.

84 Blanchon S, Legendre M, Copin B, Duquesnoy P, Montantin G, Kott E, Dastot F, Jeanson L, Cachanado M, Rousseau A, Papon JF, Beydon N, Brouard J, Crestani B, Deschildre A, Désir J, Dollfus H, Leheup B, Tamalet A, Thumerelle C, Vojtek AM, Escalier D, Coste A, de Blic J, Clément A, Escudier E, Amselem S. Delineation of CCDC39/CCDC40 mutation spectrum and associated phenotypes in primary ciliary dyskinesia. *J Med Genet* 2011;49:410–16.

85 Becker-Heck A, Zohn IE, Okabe N, Pollock A, Lenhart KB, Sullivan-Brown J, McSheene J, Loges NT, Olbrich H, Haeffner K, Fliegauf M, Horvath J, Reinhardt R, Nielsen KG, Marthin JK, Baktai G, Anderson KV, Geisler R, Niswander L, Omran H, Burdine RD. The coiled-coil domain containing protein CCDC40 is essential for motile cilia function and left-right axis formation. *Nat Genet* 2011;43:79–84.

86 Wirschell M, Olbrich H, Werner C, Tritschler D, Bower R, Sale WS, Loges NT, Pennekamp P, Lindberg S, Stenram U, Carlén B, Horak E, Köhler G, Nürnberg P, Nürnberg G, Porter ME, Omran H. The nexin-dynein regulatory complex subunit DRC1 is essential for motile cilia function in algae and humans. *Nat Genet* 2013;45:262–8.

87 Castleman VH, Romio L, Chodhari R, Hirst RA, de Castro SC, Parker KA, Ybot-Gonzalez P, Emes RD, Wilson SW, Wallis C, Johnson CA, Herrera RJ, Rutman A, Dixon M, Shoemark A, Bush A, Hogg C, Gardiner RM, Reish O, Greene ND, O'Callaghan C, Purton S, Chung EM, Mitchison HM. Mutations in radial spoke head protein genes RSPH9 and RSPH4A cause PCD with central-microtubular-pair abnormalities. *AJHG* 2009;84:197–209.

88 Ziętkiewicz E, Bukowy-Bieryłło Z, Voelkel K, Klimek B, Dmeńska H, Pogorzelski A, Sulikowska-Rowińska A, Rutkiewicz E, Witt M. Mutations in radial spoke head genes and ultrastructural cilia defects in East-European cohort of primary ciliary dyskinesia patients. *PLoS ONE* 2012;7:e33667.

89 Kott E, Legendre M, Copin B, Papon JF, Dastot-Le Moal F, Montantin G, Duquesnoy P, Piterboth W, Amram D, Bassinet L, Beucher J, Beydon N, Deneuville E, Houdouin V, Journel H, Just J, Nathan N, Tamalet A, Collot N, Jeanson L, Le Gouez M, Vallette B, Vojtek AM, Epaud R, Coste A, Clement A, Housset B, Louis B, Escudier E, Amselem S. Loss-of-function mutations in RSPH1 cause primary ciliary dyskinesia with central-complex and radial-spoke defects. *Am J Hum Genet* 2013;93:561–70.

90 Onoufriadis A, Shoemark A, Schmidts M, Patel M, Jimenez G, Liu H, Thomas B, Dixon M, Hirst RA, Rutman A, Burgoyne T, Williams C, Scully J, Bolard F, Lafitte JJ, Beales PL, Hogg C, Yang P, Chung EM, Emes RD, O'Callaghan C; UK10K, Bouvagnet P, Mitchison HM. Targeted NGS gene panel identifies mutations in RSPH1 causing primary ciliary dyskinesia and a common mechanism for ciliary central pair agenesis due to radial spoke defects. *Hum Mol Genet* 2014;23:3362–74.

91 Knowles MR, Ostrowski LE, Leigh MW, Sears PR, Davis SD, Wolf WE, Hazucha MJ, Carson JL, Olivier KN, Sagel SD, Rosenfeld M, Ferkol TW, Dell SD, Milla CE, Randell SH, Yin W, Sannuti A, Metjian HM, Noone PG, Noone PJ, Olson CA, Patrone MV, Dang H, Lee HS, Hurd TW, Gee HY, Otto EA, Halbritter J, Kohl S, Kircher M, Krischer J, Bamshad MJ, Nickerson DA, Hildebrandt F, Shendure J, Zariwala MA. Mutations in RSPH1 cause primary ciliary dyskinesia with a unique clinical and ciliary phenotype. *Am J Respir Crit Care Med* 2014;189:707–17.

92 Olbrich H, Schmidts M, Werner C, Onoufriadis A, Loges NT, Raidt J, Banki NF, Shoemark A, Burgoyne T, Al Turki S, Hurles ME; UK10K Consortium, Köhler G, Schroeder J, Nürnberg G, Nürnberg P, Chung EM, Reinhardt R, Marthin JK, Nielsen KG, Mitchison HM, Omran H. Recessive HYDIN mutations cause primary ciliary dyskinesia without randomization of left-right body asymmetry. *Am J Hum Genet* 2012;91:672–84.

93 Chilvers MA, Rutman A, O'Callaghan C. Ciliary beat pattern is associated with specific ultrastructural defects in primary ciliary dyskinesia. *J Allergy Clin Immunol* 2003;112:518–24.

94 Daniels ML, Leigh MW, Davis SD, Armstrong MC, Carson JL, Hazucha M, Dell SD, Eriksson M, Collins FS, Knowles MR, Zariwala MA. Founder mutation in RSPH4A identified in patients of Hispanic descent with primary ciliary dyskinesia. *Hum Mutat* 2013;34:1352–6.

95 Lucas JS, Adam EC, Goggin PM, Jackson CL, Powles-Glover N, Patel SH, Humphreys J, Fray MD, Falconnet E, Blouin JL, Cheeseman MT, Bartoloni L, Norris DP, Lackie PM. Static respiratory cilia associated with mutations in Dnahc11/DNAH11: a mouse model of PCD. *Hum Mutat* 2012;33:495–503.

96 Knowles MR, Daniels LA, Davis SD, Zariwala MA, Leigh MW. Primary ciliary dyskinesia. Recent advances in diagnostics, genetics, and characterization of clinical disease. *Am J Respir Crit Care Med* 2013;188:913–22.

97 Lucas JS, Burgess A, Mitchison HM, Moya E, Williamson M, Hogg C; National PCD Service, UK. Diagnosis and management of primary ciliary dyskinesia. *Arch Dis Child* 2014;99:850–6.

98 Movassagh T, Bui K, Sakakibara H, Oiwa K, Ishikawa T. Nucleotide-induced global conformational changes of flagellar dynein arms revealed by in situ analysis. *Nat Struct Mol Biol* 2010;17:761–8.

99 Tanner C, Rompolas P, Patel-King R, Gorbatyuk O, Wakabayashi K, Pazour G, King S. Three members of the LC8/DYNLL family are required for outer arm dynein motor function. *Mol Biol Cell* 2008;19:3724–34.

100 Barber C, Heuser T, Carbajal-González B, Botchkarev VJ, Nicastro D. Three-dimensional structure of the radial spokes reveals heterogeneity and interactions with dyneins in Chlamydomonas flagella. *Mol Biol Cell* 2012;23:111–20.

101 O'Toole E, Giddings TJ, Porter M, Ostrowski L. Computer-assisted image analysis of human cilia and Chlamydomonas flagella reveals both similarities and differences in axoneme structure. *Cytoskeleton (Hoboken)* 2012;69:577–90.

J Med Genet: first published as 10.1136/jmedgenet-2014-102755 on 28 October 2014. Downloaded from http://jmg.bmj.com/ on April 24, 2024 by guest. Protected by copyright.