**ORIGINAL ARTICLE**

**Delineation of CCDC39/CCDC40 mutation spectrum and associated phenotypes in primary ciliary dyskinesia**

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**ABSTRACT**

**Background** CCDC39 and CCDC40 genes have recently been implicated in primary ciliary dyskinesia (PCD) with inner dynein arm (IDA) defects and axonemal disorganisation; their contribution to the disease is, however, unknown. Aiming to delineate the CCDC39/CCDC40 mutation spectrum and associated phenotypes, this study screened a large cohort of patients with IDA defects, in whom clinical and ciliary phenotypes were accurately described.

**Methods** All CCDC39 and CCDC40 exons and intronic boundaries were sequenced in 43 patients from 40 unrelated families. The study recorded and compared clinical features (sex, origin, consanguinity, laterality defects, ages at first symptoms and at phenotype evaluation, neonatal respiratory distress, airway infections, nasal polyposis, otitis media, bronchiectasis, infertility), ciliary beat frequency, and quantitative ultrastructural analyses of cilia and sperm flagella.

**Results** Biallelic CCDC39 or CCDC40 mutations were identified in 30/34 (88.2%) unrelated families with IDA defects associated with axonemal disorganisation (22 and eight families, respectively). Fourteen of the 28 identified mutations are novel. No mutation was found in the six families with isolated IDA defects. Patients with identified mutations shared a similar phenotype, in terms of both clinical features and ciliary structure and function. The sperm flagellar ultrastructure, analysed in 4/7 infertile males, showed evidence of abnormalities similar to the ciliary ones.

**Conclusions** CCDC39 and CCDC40 mutations represent the major cause of PCD with IDA defects and axonemal disorganisation. Patients carrying CCDC39 or CCDC40 mutations are phenotypically indistinguishable. CCDC39 and CCDC40 analyses in selected patients ensure mutations are found with high probability, even if clinical or ciliary phenotypes cannot prioritise one analysis over the other.

**INTRODUCTION**

Primary ciliary dyskinesia (PCD) (MIM 244400) is a rare inherited disorder characterised by abnormal ciliary motility, and affecting 1 in 15 000 to 30 000 individuals. Ciliary dysfunction, which in most cases results from structural defects, is responsible for impaired mucociliary transport leading to early upper and lower respiratory tract infections. In addition, given the key role of motile cilia in the establishment of left–right asymmetry during embryogenesis, ~50% of patients display a situs inversus (thereby defining Kartagener syndrome (MIM 244400)). Infertility is also frequently observed in male patients, due to functional and structural abnormalities in sperm flagella, whose axonemal structure is very similar to that of motile cilia.

The axoneme, which represents the core of motile cilia and flagella, consists of nine peripheral microtubules connected by nexin links and radial spokes, and a central complex composed of two single microtubules surrounded by the central sheath. Attached to the peripheral doublets, the inner and outer dynein arms (IDAs and ODAs, respectively) are multiprotein ATPase complexes that are essential for normal ciliary and flagellar movements. Another important regulator of motor activity is the dynein regulatory complex (DRC) that, as shown recently, corresponds to the nexin links. In theory, mutations in each of the genes encoding key components of the axoneme could lead to PCD. As expected, various axonemal ultrastructural defects have been reported in this disorder. As a second level of heterogeneity is attested to by the fact that mutations in distinct genes may result in the same ultrastructural defect. This, for example, is the case for DNAI1, DNAI2, DNAH5, TXNDC3 and DNAI1 mutations in patients with a PCD phenotype characterised by an absence of ODAs, and RPCR, DNAAF1/LRRC50, and DNAAF2/KTU mutations in patients with an absence of ODAs and IDAs. Other genes have been involved in central complex defects (RSPH9 and RSPH4A) or in Kartagener syndrome with normal ciliary ultrastructure (DNAH11). As for the so-called IDA defects, they can be isolated or associated with axonemal disorganisation in several
ciliary sections. In our experience, this latter phenotype accounts for about 16% of PCD cases; it was found to represent 14% to up to 29% of PCD cases in other PCD cohorts.\textsuperscript{4} \textsuperscript{21} \textsuperscript{22} Note-worthy, no gene has been found to be implicated in the isolated absence of IDAs. However, just recently, mutations in two genes—CCDC39 and CCDC40—have been found in patients with a complex ultrastructural defect characterised by the absence of IDAs and defects of nexin links and radial spokes, leading to axonemal disorganisation.\textsuperscript{23} \textsuperscript{24} Absence of IDAs and defects of nexin links and radial spokes, with a complex ultrastructural defect characterised by the genes absence of IDAs. However, just recently, mutations in two genes—CCDC39 and CCDC40—have been found in patients with a complex ultrastructural defect characterised by the absence of IDAs and defects of nexin links and radial spokes, leading to axonemal disorganisation.\textsuperscript{23} \textsuperscript{24} These latter data raise the larger question of the overall contribution of CCDC39 and CCDC40 to PCD related to an absence of IDAs associated or not with axonemal disorganisation. Another so far open question is to know whether patients with CCDC39 mutations are phenotypically distinguishable from those with CCDC40 mutations. Answering these two questions also has important consequences for the general strategy to follow in order to better guide the molecular analysis to be performed in patients with a suspicion of PCD. To address these issues, we screened CCDC39 and CCDC40 for mutations in a cohort of patients with a PCD phenotype characterised by an absence of IDAs associated or not with axonemal disorganisation. Genotype–phenotype correlations were subsequently performed in 45 patients, 15 of whom had previously been identified with mutations in CCDC39.\textsuperscript{23} After an extensive description of their clinical and ciliary features that were also assessed by quantitative studies and statistical analyses.

**PATIENTS AND METHODS**

**Patient recruitment**
The patients were recruited through the French National Center for Rare Respiratory Diseases located in Armand-Trousseau Children’s Hospital, Paris, France, where, since 1985, ciliary and genetic investigations are part of the PCD diagnostic procedure in patients with recurrent respiratory tract infections. A definitive diagnosis of PCD is established on the association of suggestive clinical symptoms (eg, sinopulmonary syndrome and laterality defect), exclusion of other pathologic conditions such as cystic fibrosis or immunodeficiency, and evidence of abnormal ciliary structure and/or function.

The ethical review board of our institution approved the use of the database of the French National Center for Rare Respiratory Diseases for this study (CCTIRS, n°08.015bis). The patients and/or their parents were informed of the goal of the investigations and gave their written consent.

**Ciliary investigations**
In the French National Center for Rare Respiratory Diseases, all ciliary investigations are performed when patients are free of airway tract infection or respiratory exacerbation for at least 6 weeks and, if necessary, at the end of an antibiotic course. Ciliary beat frequency (CBF) is evaluated on ciliated cells obtained by nasal or bronchial brushing, as described previously.\textsuperscript{25} The ciliary ultrastructure is analysed on airway biopsies obtained from either the inferior turbinate or the bronchi, immersed in glutaraldehyde, as described previously.\textsuperscript{25} Results are expressed as a percentage of abnormal cilia over the total number of analysed cilia (at least 50 per biopsy). Each axonemal abnormality is quantified and expressed as a percentage of each ultrastructural defect over the total number of abnormal cilia. Dynein arms are considered to be absent when missing from at least five of the peripheral microtubules. Since 2002, in case of questionable IDA defects on micrographs obtained by transmission electron microscopy (TEM), computerised analyses of cilia are systematically performed in order to improve IDA visualisation.\textsuperscript{26} Ciliary orientation is systematically evaluated by comparing the position of the central pairs from adjoining cilia; disorientation is defined as an angle >25°.\textsuperscript{27} The presence of compound cilia is also noted. For this study, ciliary length was evaluated on each sample (10 measures per patient) by means of optic microscopy with the Image-Pro Express 6.0 software (Media Cybernetics Inc, USA). Nasal nitric oxide is measured when feasible, according to patient’s age, using a chemiluminescence analyser (Aerocrine, Solna, Sweden), with a transnasal flow rate of 0.3 l/min, following American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines.\textsuperscript{28}

**Study design**
Mutation analysis of CCDC39 and CCDC40 was performed in 40 unrelated families (45 patients) with a PCD phenotype characterised by IDA defects. 34 families (37 patients) with an absence of IDAs associated with axonemal disorganisation, and six families (six patients) with an isolated absence of IDAs. We recorded and compared the clinical and ciliary phenotypes of patients with mutations identified in CCDC39 (CCDC39 group) or in CCDC40 (CCDC40 group). Fifteen patients from the CCDC39 group (table 1) had previously been reported in the study describing the very first mutations in CCDC39;\textsuperscript{23} these 15 patients were included in the current study given that its main objective was phenotype–genotype correlations based on deep phenotyping. The recorded clinical features included sex, geographic origin, familial consanguinity, laterality defects, age at first symptoms and at phenotype evaluation, history of unexplained neonatal respiratory distress, upper and lower respiratory tract infections, nasal polyposis, otitis media with effusion, bronchiectasis confirmed by computed tomographies, and infertility when appropriate. The ciliary phenotype was assessed through the CBF and qualitative and quantitative analyses of the ciliary ultrastructure. In case of male infertility, the flagellar ultrastructural abnormalities, when available, were compared to the ciliary ones.

**Mutation analysis**
Genomic DNA was obtained from whole blood samples by use of a FlexiGene kit (Qiagen, France). All CCDC39 and CCDC40 coding exons and flanking intronic sequences were amplified by PCR. The resulting PCR products were subsequently sequenced (ABI 3730XL, Applied Biosystems, USA) on both strands with primers located outside the known polymorphisms referenced in Ensembl (http://www.ensembl.org/Homo_sapiens/Info/Index) or dbSNP (http://www.ncbi.nlm.nih.gov/ snp/) databases. The previously described CCDC39 c.1167+1261A>G mutation located in intron 9 and creating a pseudoeXon was also searched.\textsuperscript{23} The molecular analysis was stopped when two unambiguous molecular defects were identified in the homozygous or compound heterozygous state. Primer sequences are available upon request.

**Statistical analyses**
Qualitative data were described with frequencies and quantitative data were described with medians, ranges, and interquartile ranges (IQR). Phenotype, age at first symptoms, clinical features, and ciliary ultrastructure were compared between the two genotypes with tests adapted to small samples. Qualitative data were compared with Fisher’s exact test and quantitative data were compared with Wilcoxon’s test. Family ties were not taken into account. Statistical analyses were performed with SAS V.9.2 software (SAS Institute, Cary, North Carolina, USA).
The R software (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org) was used for plots. All tests were two-sided, and a value of p<0.05 was considered significant.

RESULTS

**CCDC39 and CCDC40 mutation spectrum**

Biallelic mutations in CCDC39 and CCDC40 were identified in 30/40 (75%) unrelated families with PCD characterised by IDA defects. The mutations were observed in 30/34 (88.2%) unrelated families exhibiting an absence of IDAs associated with axonemal disorganisation (figure 1): 22/34 (64.7%) have mutations in CCDC39, and 8/34 (23.5%) have mutations in CCDC40. No mutation was found in the six remaining families with an isolated absence of IDAs. The genotypes of patients with CCDC39 and CCDC40 mutations are displayed in tables 1 and 2. Among the 17 CCDC39 mutations identified (three nonsense, nine frameshift, four splice, and one missense mutations), five are novel (figure 2A). The 11 CCDC40 mutations identified (four nonsense, six frameshift, and one splice mutations) include nine that are novel (figure 2B).

Phenotype:genotype correlation study

The phenotype was compared between the following two groups of patients: one consisting of 26 patients from 22 unrelated families with CCDC39 mutations (CCDC39 group), and the other comprising eight unrelated patients with CCDC40 mutations (CCDC40 group). There was no statistically significant difference in origin, sex ratio, familial consanguinity, laterality defects and infertility between these two groups (tables 1 and 2). The clinical phenotypic features of all the PCD patients from families with identified mutations, which are displayed in table 3, were found to be similar. The age at phenotype evaluation (median age, range) was found to be similar between the CCDC39 group (20.9 years, 2–68 years). Nasal nitric oxide measurements, available in eight patients with CCDC39 mutations, were found to be dramatically low for all patients, with a median (range) of 13.5 (2–46.3) parts per billion (ppb).

CBF analysis was performed in 28/34 patients with identified mutations. All cilia were found to be immotile, except in five unrelated patients with CCDC39 mutations (DCP481, DCP640, DCP692, DCP414, DCP323) in whom few beating cilia were observed (CBF ranging from 5–18 Hz).

Ciliary length and orientation, and presence of compound cilia, were found to be similar between CCDC39 and CCDC40 groups (table 4). The various axonemal structures could be precisely quantified in all but two patients (DCP854 from CCDC39 group, DCP815 from CCDC40 group) for whom micrographs were not available. All cilia from patients with identified mutations exhibited IDA defects. Associated axonemal
Defects are displayed in Figure 3: the absence of radial spokes and nexin links, leading to axonemal disorganisation, was documented in all the patients with identified mutations, but was observed only in about half of the analysed axonemal sections; defects of ODA, central complex and peripheral microtubules, which were much less frequent, did not concern all the patients. The ultrastructural analyses showed similar defects in patients with mutations in \textit{CCDC39} or \textit{CCDC40}.

The sperm flagellar ultrastructure was analysed in 2/5 and 2/2 infertile male patients with \textit{CCDC39} (DCP749 and DCP801) and \textit{CCDC40} mutations (DCP143 and DCP102), respectively. In all of them, the axonemal defects were found to be similar at the ciliary and flagellar levels (figure 1).

**DISCUSSION**

In our experience, the PCD phenotype characterised by IDA defects is present in about 16% of PCD patients.

Among them, 81% display an absence of IDAs associated with axonemal disorganisation, which were much less frequent, did not concern all the patients. The ultrastructural analyses showed similar defects in patients with mutations in \textit{CCDC39} or \textit{CCDC40}.

The sperm flagellar ultrastructure was analysed in 2/5 and 2/2 infertile male patients with \textit{CCDC39} (DCP749 and DCP801) and \textit{CCDC40} mutations (DCP143 and DCP102), respectively. In all of them, the axonemal defects were found to be similar at the ciliary and flagellar levels (figure 1).

**Table 2** \textit{CCDC40} mutations in PCD patients with an absence of IDAs associated with axonemal disorganisation

| Patients | Origin     | Sex | Consanguinity | Lateral defects | Sperm defects | Allele 1 | Allele 2 |
|----------|------------|-----|---------------|----------------|--------------|----------|----------|
| DCP102   | France     | M   | Y             | Kartagener     | AZS          | c.1345C>T (p.Arg449X) | c.1345C>T (p.Arg449X) |
| DCP915*  | France     | F   | N             | N              | —            | c.246delC (p.Glu82ValfsX84) | c.246delC (p.Glu82ValfsX84) |
| DCP353   | France     | M   | N             | Kartagener     | NA           | c.246delC (p.Val83ValfsX84) | c.2191G>T (p.Glu707X) |
| DCP143   | Sicilia    | M   | N             | Kartagener     | AZS          | c.344delC (p.Ala115ArgfsX52) | c.574C>T (p.Glu192X) |
| DCP79    | North Africa | F   | N             | N              | —            | c.1464delC (p.Ser489AlafsX18) | c.1464delC (p.Ser489AlafsX18) |
| DCP852   | France     | F   | N             | Kartagener     | —            | c.2591_2592delCaInsACGG | c.2712G>T |
| DCP591*  | Portugal   | M   | N             | —              | —            | c.2920C>T (p.Glu974X) | c.1989+3del |
| DCP619*  | Algeria    | F   | Y             | Kartagener     | —            | c.3242_3245dupGCCG | c.3242_3245dupGCCG |

Novel mutations are in bold characters; other mutations have been reported previously.25

*Homozygosity or compound heterozygosity was confirmed by parental DNA analysis or cloning of PCR products.

†The two parents originate from the same village.

AZS, asthenozoospermic; F, female; IDA, inner dynein arms; M, male; N, no; NA, not available; PCD, primary ciliary dyskinesia; Y, yes.
previously reported, and eight carry mutations in CCDC40. Overall, this study identified 14 new mutations: five in CCDC39 and nine in CCDC40. Four recurrent mutations (c.1072delA, c.2190delA, c.357+1G>C, and c.610-2A>G) that had previously been described were also identified in CCDC39. For each recurrent mutation, the patients originate from the same geographic area, in keeping with founder effects. Regarding the c.610-2A>G mutation, newly described as a recurrent one, a founder effect has been confirmed by microsatellite analysis for two patients (DCP640 and DCP692), whereas in one case (DCP274) the mutation could have arisen independently (data not shown). Most families with mutations in CCDC39 (13/22) were native from North Africa, while most of those with mutations in CCDC40 (1142 amino acids) contains two domains homologous to common bacterial type SMC protein.

Figure 2 Location of CCDC39 (A) and CCDC40 (B) mutations at the nucleotide and protein levels. Empty boxes represent the 20 coding exons of each gene, and grey boxes represent 5’ and 3’ untranslated regions. Solid and dashed lines indicate exonic and intronic mutations, respectively. Novel mutations are in bold characters. CCDC39 (941 amino acids) contains a domain homologous to the N terminus of SMC (structural maintenance of chromosomes) proteins (red box), as well as a domain homologous to common bacterial type SMC protein (purple box). CCDC40 (1142 amino acids) contains a domain homologous to the N terminus of SMC (structural maintenance of chromosomes) proteins (red box), as well as a domain homologous to common bacterial type SMC protein (purple box). CCDC40 (1142 amino acids) contains a domain homologous to common bacterial type SMC protein. CCDC40 (red box), as well as a domain homologous to common bacterial type SMC protein.

Table 3 Clinical phenotypic features of PCD patients with identified CCDC39 and CCDC40 mutations (CCDC39 and CCDC40 groups). Results are expressed in number (n) and percentage (%) of patients

| Feature                          | CCDC39 group n (%) | CCDC40 group n (%) | p Value |
|----------------------------------|--------------------|--------------------|---------|
| Total number of patients         | 26 (100)           | 8 (100)            | NS      |
| Age at first symptoms            |                    |                    |         |
| <1 year old                      | 14 (53.8)          | 7 (87.5)           | NS      |
| 1–15 years old                   | 12 (46.2)          | 1 (12.5)           |         |
| Clinical features                |                    |                    |         |
| Laterality defect                | 12 (46.2)          | 4 (50)             | NS      |
| Neonatal respiratory distress    | 12 (46.2)          | 5 (62.5)           | NS      |
| Rhinosinusitis                   | 25 (96.2)          | 8 (100)            | NS      |
| Nasal polyposis                  | 5 (19.2)           | 2 (25)             | NS      |
| Otitis media with effusion       | 21 (80.8)          | 4 (50)             | NS      |
| Chronic productive cough         | 26 (100)           | 8 (100)            | NS      |
| Bronchiectasis                   | 19 (73.1)          | 6 (75)             | NS      |
| Pneumonia                        | 12 (46.2)          | 5 (62.5)           | NS      |

Table 4 Ciliary ultrastructural analysis of PCD patients with identified CCDC39 and CCDC40 mutations (CCDC39 and CCDC40 groups)

| Feature                          | CCDC39 group | CCDC40 group | p Value |
|----------------------------------|--------------|--------------|---------|
| Ciliary length (µm) median        | 5.59 (5.47; 5.86) | 5.45 (5.38; 5.75) | NS      |
| Ciliary orientation, n (%)       |              |              | NS      |
| Normal                           | 3 (12)       | 2 (28.6)     |         |
| Abnormal                         | 22 (88)      | 4 (57.1)     |         |
| Non-evaluable                    | 0 (0)        | 1 (14.3)     |         |
| Compound cilia, n (%)            |              |              | NS      |
| Absent                           | 19 (76)      | 4 (57.1)     |         |
| Present                          | 6 (24)       | 3 (42.9)     |         |

NS, non-significant; PCD, primary ciliary dyskinesia.
unambiguous molecular defect (c.1363-3delC) and a potentially mild mutation (p.Thr594Ile). However, the four remaining patients with residual beating are homozygous or compound heterozygous for two unambiguous deleterious mutations. In addition, for two of these patients (DCP414 and DCP523) who belonged to two independent families, the affected siblings (DCP413 and DCP759, respectively) displayed a typical phenotype characterised by immotility of all observed cilia.

The clinical phenotypic features described in our cohort are in line with those previously reported in PCD (ie, sinopulmonary syndrome, bronchiectasis, laterality defect, neonatal respiratory distress, male infertility).32 Our phenotype-genotype correlation study revealed that, in the case of PCD related to an absence of IDAs associated with axonemal disorganisation, the ultrastructural phenotype allows the selection of two genes with a very high expected mutation rate. Indeed, according to our data obtained in a large cohort of patients, mutations in CCDC39 or CCDC40 account for 88.2% of cases related to this ultrastructural phenotype. Our detailed analysis revealed that patients with mutations in CCDC39 or CCDC40 are phenotypically indistinguishable, thereby precluding the choice of the first gene to be studied. However, the higher frequency of CCDC39 mutations in our cohort, a persistence of some beating cilia, or a North African origin would rather prompt us to start with CCDC39 analysis.

Given the similarity of the phenotypes of patients with mutations in CCDC39 or CCDC40, it is tempting to hypothesise that the CCDC39 and CCDC40 proteins could participate in the same function and/or structure. As shown previously,23 24 mutations in CCDC39 or CCDC40 result in the mislocalisation of GAS11, one of the subunits of the DRC, which is no longer in the axoneme but accumulates in the apical cytoplasm. On the other hand, in the alga Chlamydomonas reinhardtii, mutations in DRC4, the orthologue of GAS11, result in structural defects involving the DRC and IDAs.24 And as shown for DRC4 in Chlamydomonas, mutations in a single DRC subunit result in the lack of other DRC subunits.34—36 (CCDC39 and CCDC40, two proteins containing coiled-coil domains, could therefore play an important role in stabilising the DRC. Overall, even if their respective role in the proper assembly of ciliary axonemes remains unexplained, it is clear that these structurally related proteins are also functionally related. This is further supported by the reported mislocalisation of CCDC39 in respiratory cells from patients with CCDC40 mutations; in those cells, CCDC39 was shown to be absent from the axoneme and enriched in the apical cytoplasm at the ciliary base.24 However, the study of the molecular pathology of CCDC39 and CCDC40 reveals that, in spite of these structural and functional similarities, these two proteins are not redundant.

Ten PCD patients with an absence of IDAs, associated (n=4) or not (n=6) with axonemal disorganisation, did not carry any mutation in CCDC39 or in CCDC40. No obvious difference, in terms of clinical phenotypic features, was evident between those 10 individuals and patients with CCDC39 or CCDC40 mutations (data not shown), raising two non-exclusive hypotheses: the possible mutations in intronic or regulatory regions of CCDC39 or CCDC40, and the existence of at least another gene implicated in PCD related to IDA defects. Even though no mutation has yet been identified in patients with an isolated absence of IDAs, the small number of such patients already studied, together with the absence of a gene implicated in this phenotype, should encourage continuing analysis of CCDC39 and CCDC40 in these patients.

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Figure 3 Quantitative analysis of associated axonemal defects: the axonemal structures could be precisely quantified in 25 and seven patients with CCDC39 and CCDC40 mutations, respectively. The results are expressed as percentage of abnormal cilia with radial spoke defect (RS), nexin link defect (NL) in the upper panel; and with outer dynein arm defect (ODA), abnormal central complex (CC), and abnormal peripheral microtubules (PMT) in the lower panel. Triangles are medians and short horizontal lines are 25th—75th interquartile range.
Genotype-phenotype correlations

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Competing interests

None.

Ethics approval

The ethical review board of the French National Center for Rare Respiratory Diseases (CCTIRS, n.2002-06), the Committee on Human Research of the Swiss National Center for Rare Diseases (CSC, n.2002-06), the Committee on Human Research of the French National Center for Rare Respiratory Diseases (CCTIRS, n.2002-06), the Assistance Publique-Hôpitaux de Paris (PHRC AOM06053, P062045), the Fondation pour la Recherche Médicale, the Legs Poix from the Chancellerie des Universités, and the Milena Carvajal—ProKartagener Foundation.

Contributors

Involvement in the conception, hypotheses delineation, and design of the study: SB, ML, ACo, JdB, ACi, EE, SA. Acquisition of the data or the analysis and interpretation of such information: SB, ML, BC, PD, GM, EK, FD, LJ, MC, AR, DE, EE, SA. Writing the article or substantial involvement in its revision before to submission: SB, ML, AR, DE, EE, SA.

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REFERENCES

1. Ateljevic IA. A human syndrome caused by immotile cilia. Science 1978;193:317–19.

2. Bossh A, Chodhari R, Collins N, Copeland N, Fai P, Harrouf J, Hariri M, Hogg C, Lucas J, Mitchison HM, O’Callaghan C, Phillips G. Primary ciliary dyskinesia: current state of the art. Arch Dis Child 2007;92:1136–40.

3. Ateljevic IA. The immotile-cilia syndrome: a microtubule-associated defect. Adv Cell Biochem 1989;19:63–87.

4. Noone PG, Leigh MW, Sanni A, Minnix SL, Carson JL, Hazzuca M, Zarwala M, Knowles MR. Primary ciliary dyskinesia: diagnostic and phenotypic features. Am J Respir Crit Care Med 2004;169:959–67.

5. Escudier E, Ercoli D, Piron A, Boucharet M, Bemaudin JF, Fleury-Feith J. Disseminated expression of axonal anomalies in respiratory cilia and sperm flagella in infertility men. Am Rev Respir Dis 1990;142:674–9.

6. Satir P, Christensen ST. Overview of structure and function of mammalian cilia. Annu Rev Physiol 2007;69:377–86.

7. Hens N, T. Raychett M, Kroll J, Porter ME, 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.

8. Papon JF, Coste A, Roudot-Thoraval F, Boucharet 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;35:1067–63.

9. Escudier E, Dusquenoy P, Papon JF, Amselem S. Ciliary defects and genetics of primary ciliary dyskinesia. Paediatr Respir Rev 2009;10:51–4.

10. Pennarun G, Escudier E, Chapelin C, Aimard C, Vojtek AM, Amselem S, Escudier E. A common variant in combination with a nonsense mutation in a member of the thioredoxin family causes primary ciliary dyskinesia. Proc Natl Acad Sci U S A 2007;104:3338–41.

11. Loges NT, Olbrich H, Becke-Heck A, Hoffner K, Heer A, Reinhardt C, Schmidts M, Kispert A, Zarwala MA, Leigh MW, Knowles MR, Zentgraf H, Seife H, Nurnberg G, Nurnberg 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;85:860–9.