Considerable variability in the clinical and ciliary features of primary ciliary dyskinesia in patients with *DNAH5* mutations

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Research Article

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Abstract

Background: Primary ciliary dyskinesia (PCD) is a clinically and genetically heterogeneous disorder, but the relationship between genotype and phenotype is poorly established. We aimed to characterize the detailed clinical characteristics, ciliary phenotype and mutational spectrum of PCD patients with *DNAH5* mutations.

Methods: Whole exome sequencing followed by targeted copy number variation (CNV) analysis was used to screen 114 Chinese patients with highly suspected PCD. In cases with *DNAH5* mutation, detailed demographic and clinical data, as well as ciliary beat pattern and ciliary ultrastructure, were comprehensively reviewed.

Results: Thirty patients (median, 15.3 years; range, 0.6 to 43 years), who carried *DNAH5* mutation, were identified. We characterized 38 novel and 7 known *DNAH5* mutations (10 nonsense, 5 frameshift, 10 splicing, 16 missense mutations, 2 in-frame deletions and 2 large deletions). Despite bearing the same genotype, patients presented a highly variable phenotypic picture, including milder respiratory symptom, normal nasal nitric oxide levels (2/21, 9.5%) and “nontypical” ciliary beat pattern (4/21, 19.0%).

Conclusions: *DNAH5*-associated PCD disease demonstrates dramatic phenotypic heterogeneity, contributing to the challenges of diagnosis. A composite work-up and cautious interpretation of the results are necessary during the PCD diagnosis process.

Introduction

Primary ciliary dyskinesia (PCD) is a rare, heterogeneous ciliopathy resulting in mucociliary clearance failure. Patients typically present with newborn respiratory distress, daily wet cough, chronic nasal congestion, and laterality defects. PCD diagnosis can be achieved by following the diagnostic algorithms that include nasal nitric oxide (nNO) measurements, high-speed video microscopy analysis (HSVA), immunofluorescence (IF), transmission electron microscopy (TEM), and molecular testing^[1]. Currently, around 50 genes are known to be associated with PCD^[2].

The relationship between genotype and phenotype in PCD has been considered a matter of critical importance^[3], as better understanding of genotype-phenotype correlations helps to promote more accurate prognostic assessments. It has been reported that patients with a *CCDC39* or *CCDC40* mutation have lower body mass indices and worse lung function that declines more rapidly compared with those with *DNAH5* mutations^[4]. Conversely, patients with *RSPH1* and *DNAH9* mutations appear to have a milder respiratory phenotype^[5,6].

The ciliary ultrastructural and beat patterns correlate with specific genotypes. Specifically, mutations in *CCDC39* or *CCDC40* cause axonemal disorganization and loss of inner dynein arms, conferring extremely stiff cilia with reduced beat amplitude^[7]. Mutations in genes that encode cytoplasmic preassembly factors, such as *DNAAF3*, result in complete cilia immotility, with combined outer and inner dynein arm defects^[8]. The consistency across ciliary beating anomalies and specific genotypes makes HSVA a promising approach to assess the pathogenicity of VUS variants. However, extensive variability in the clinical and ciliary phenotype within a single mutation cohort has also been reported. In a study of *CCDC103* p.His154Pro patients by Shoemark et al., the cilia were found to be static in half of the individuals, but completely or nearly normal in the others^[10]. Variable diagnostic test results in nasal nitric oxide levels and ultrastructural images were also reported in patients with p.His154Pro mutation. Incomplete knowledge of phenotype could easily lead to underdiagnosis or false negatives. A larger number of patient subjects and detailed information was clearly needed to facilitate a better understanding of the subtle genotype-phenotype correlations in PCD.

First reported in 2002^[11], *DNAH5* is the most frequent disease-causing gene in PCD patients. *DNAH5* is an axonemal heavy chain dynein protein that functions as a force-generating protein to produce cilia bending. It has been reported that pathogenic variants in the *DNAH5* gene are responsible for nearly 15% to 29% of all PCD cases^[12]. Several previous studies focused on patients with *DNAH5*, revealing that mutations in *DNAH5* lead to absent or shorter dynein arms in respiratory cilia that are also mostly immotile or only show flickering movements. Patients usually have the typical clinical phenotype of PCD. In this study, we systematically evaluated the phenotype and genetic characteristics of 30 PCD patients carrying *DNAH5* mutations. Intriguingly, mild respiratory phenotype, normal nasal nitric oxide and diverse ciliary beat pattern were identified in our cohort group. For specified patients, IF was performed to investigate outer dynein arm (ODA) perturbations in nasal ciliated cells. It is hoped that the detailed phenotype information identified in our study will help to better delineate the genotype-phenotype relationship of *DNAH5*.

Materials And Methods

Patients and Clinical Materials

The project was approved by the ethics committee of the Ethics board of The Children's Hospital of Fudan University, and written informed consent was obtained from all family members participating in this study. Inclusion criteria were as follows: 1) at least 2 out of 4 key clinical features (laterality defects, unexplained neonatal respiratory distress, year-round daily cough, or nasal congestion) or siblings of confirmed patients; and 2) HSVA suggestive of PCD and/or low nNO level. nNO and HSVA were measured repeatedly in cases with equivocal results. Exclusion criteria were confirmed diagnosis other than PCD (e.g., immune deficiency or cystic fibrosis). Eventually, a total of 114 patients belonging to 104 unrelated nuclear families with symptoms suggestive of PCD and positive screening test (nNO and/or HSVA) results were enrolled between January 1, 2016 and June 1, 2021 at the Children's Hospital of Fudan University. The clinical data, including lung function, high resolution CT (HRCT), and bacterial cultures were performed at enrollment. The diagnosis of PCD was based on the clinical findings, nNO, HSVA, TEM, and genetic testing, in accordance with the guidelines of European Respiratory Society^[1].

Nasal Nitric Oxide Measurements

Measurements of nNO were performed with the use of an EcoMedics CLD88 chemiluminescence NO analyzer (Duernten, Switzerland); the aspiration-sampling rate of 330 nL/min was verified before and after each subject tested. The measurement of nNO in cooperative children was performed by breath hold maneuver for at least 10s to close their velum as described previously^[13]. For children that were uncooperative (less than 5 years old), nasal sampling was performed for 60 seconds during tidal breathing. Results were reported in nL/min with the following equation: nNO (nL/min) = NO (ppb) × sampling rate (nL/min).

Electron Microscopy and High-Speed Video Microscopy Analysis

Nasal tissue was collected using a Rhino-Probe (Arlington Scientific, Springville, UT). To avoid secondary ciliary dyskinesia, nasal samples were taken in patients who free from acute airway infection for at least 4 weeks. The tissue was suspended in L-15 medium (Invitrogen, CA) immediately for analysis using a Leica inverted microscope (Leica DMI300B, Solms, Germany) with a 63× oil objective under differential interference contrast optics. Cilia beats were recorded at 200 frames/sec at room temperature (25°C), using a 680 PROSILICA GE camera (Allied Vision, PA). At least ten videos were derived from each sample. The digital recordings were evaluated by two experienced but blinded investigators.

The ciliary ultrastructure was analyzed on nasal tissue, fixed in 2.5% glutaraldehyde, as described previously^[13]. For each specimen, at least 30 transverse ciliary sections of different cells were used to evaluate the internal axonemal structure. Ciliary abnormalities were defined as the presence of defects in >50% of cilia.

Whole Exome Sequencing (WES) and Copy Number Variation (CNV) Analyses

Blood samples were obtained from proband and available family members. Genomic DNA was extracted using the Gene Blood DNA Rapid Extraction Kit (Qiagen, China). WES was performed by Gimple Biotech Co. Ltd (Shanghai, China). Whole exome libraries were constructed using KAPA platform and KAPA Hyper Prep kit (Roche KAPA, Switzerland). Exomes were captured using SeqCap EZ MedExome (Roche NimbleGen) and sequenced by a HiSeq X Ten instrument (Illumina, San Diego, CA). Raw data was evaluated using FASTQC (version 0.11.5), and the linker sequences were removed by Cutadapt (version 1.10). The software BWA (version 0.7.15) was used to align the reads to the human reference genome GRCh37/hg19(UCSC). For analyzing the phenotype-associated mutation sites, the mutation data were annotated and categorized according to the American College of Medical Genetics and Genomics (ACMG) guidelines^[14]. Sanger sequencing was performed to validate the candidate variants, and segregation analyses were performed in family members. The panelcn.MOPS software was introduced to detect CNVs in WES data, which is a new algorithm that builds upon copy number estimation by Mixture of Poissons (cn.MOPS) to detect CNVs with high sensitivity and specificity^[15].

The RefSeq accession numbers of the transcript and corresponding protein isoform we used for mutation nomenclature were NM_001369.2 and NP_001360.1.

Quantitative Real-Time Polymerase Chain Reaction (qPCR) Analysis

To confirm CNVs detected by WES, qPCR was performed using DNA from patients, their parents and controls. DNA was obtained as described previously. qPCR was prepared using KAPA SYBR FAST qPCR Kit Master Mix(2X) Universal (Roche KAPA, Switzerland) on a CFX96™ Real-Time System instrument (BIO-RAD) and was performed on a LightCycler 480 System (Roche diagnostic) in triplicate. The reaction conditions were set at 95 °C for 3 min, followed by 40 cycles of 95 °C for 5 sec and 55 °C for 30 sec. The primer pair sequences were designed by NCBI (<https://www.ncbi.nlm.nih.gov/>). The comparative $\Delta\Delta C_t$ method was used to calculate relative expression with data normalized to the mean level of an internal standard.

Whole Genome Sequencing (WGS)

Genomic DNA was obtained and sheared to fragments of an average length of 350 bp to construct libraries using KAPA platform and KAPA Hyper Prep kit (Roche KAPA, Switzerland). The libraries were then subjected to sequencing on the Illumina Hiseq X platform and 150 bp paired-end reads were generated. The same bioinformatics pipeline as described above for the exome sequencing analysis was used to analyze *DNAH5* mutations and precise breakpoints. The variants identified were confirmed by Sanger sequencing.

IF Analysis

Nasal tissue, obtained by nasal brushing biopsy, was suspended in cell culture medium and spread onto glass slides, then air dried. Samples were treated with 4% paraformaldehyde, 0.2% Triton-X 100 and 1% BSA before incubation with primary (overnight at 4 °C) and secondary antibodies (2 h at room temperature). Rabbit polyclonal anti-DNAH5 (HPA037470, Sigma, Sweden) and mouse monoclonal anti-alpha tubulin antibodies (ab24610, Abcam, UK) were used at a dilution of 1:1000. Highly cross-adsorbed secondary antibodies, goat anti-mouse Alexa Fluor 488 (1:1000) and goat anti-rabbit Alexa Fluor 647 (1:1000), were obtained from Abcam. Confocal images were taken using a Leica TCS SP8 confocal laser scanning microscope (Leica, Jena, Germany).

Results

Clinical Phenotype of Individuals with *DNAH5*

In the present study, we identified 30 Chinese patients with *DNAH5* from 27 unrelated families. The age of affected individuals ranged from 0.6 to 43 years old and 60.0% (18/30) were males. PCD classical symptoms in our cohort included: Year-round wet cough (96.7%, 29/30), neonatal respiratory distress (47.6%, 10/21), nasosinusitis (93.1%, 27/29), and chronic otitis media (95.2%, 20/21). Further examination revealed that 80.0% of patients (24/30) had situs inversus totalis and 7% of patients (1/30) had heterotaxy; median forced expiratory volume in 1 s (FEV1) was estimated to be 74.6%. In general, lung function,

especially the forced expiratory flow at 25 and 75 % of the pulmonary volume (FEF 25-75%), gradually declined with age (Supplementary Figure 1). Surprisingly, as a 19-year-old boy, P40 had a mild clinical disease with normal lung function. Consistent with this, only small pieces of patchy exudation in his lower pulmonary lobes were identified by high resolution CT scans (Supplementary Figure 2). Patients P100 and P101 were from a Han Chinese consanguineous family (Figure 1A). No situs inversus totalis and bronchiectasis were identified in their mother (Figure 1B) and father (Figure 1C). While situs inversus totalis, bronchiectasis and nasosinusitis were found in P100 (Figure 1D-F), P101 (Figure 1G-I) and their older brother (not available). Surprisingly, the two older brothers had much earlier onset and more frequent lung infections than P100. Progressively lung failure led to P101 death shortly after diagnosis. Another met with lung failure and death at the age of 38, according to their parents. HRCT indicated that P100 (Figure 1E) had a milder destruction of lung than P101 (Figure 1H) at enrollment.

nNO testing of 19 individuals revealed a median nNO production rate of 22.8 nL/min (minimum, 5.6 nL/min; maximum, 99.0 nL/min). Interestingly, two siblings (P72 and P97) had nNO values that were above the disease-specific cut off value (77 nL/min). The detailed clinical characterizations and nNO values of our cohort are summarized in Table 1

Ciliary Structural and Functional Analysis

Ultrastructural analysis of 21 subjects with biallelic mutations in *DNAH5* showed completely missing or shortened ODA. HSVA revealed that most *DNAH5* mutant cilia were completely immotile or exhibited only occasional residual movement (Supplementary Video 1). However, four individuals (P24, P57, P69 and P97) with *DNAH5* mutations exhibited diverse CBP. CBP of P24 was relatively stiff, with a decreased proximal bending (Supplementary Video 2). Cilia of P57 showed uncoordinated beating activity (Supplementary Video 3), while HSVA of P69 (Supplementary Video 4) and P97 (Supplementary Video 5) displayed a subtle reduction of the beating amplitude. As siblings, P72 and P97 carried the same mutations, but their ciliary beat patterns were distinctive. To further confirm the pathogenicity of the mutations, we studied P24, P57, P97 and P100 for DNAH5 localization in respiratory cells by IF, and found complete absence of DNAH5 in ciliary axonemes (Figure 2A-E). The details of ciliary structural and functional analysis are shown in Table 2.

Genetic Characteristics and cDNA Analysis

Collectively, 45 biallelic mutations were identified, of which 16 were missense variants, 5 were frameshift, 10 were nonsense variants, 2 were CNV, 2 were in-frame deletions and 10 were splicing variants, while 37 variants (82.2%) were novel (Table 3). Interestingly, the previously reported mutation c.5563dupA was found in four families. To find out whether the mutation was derived from a common ancestor (founder), we had sequenced 21 single-nucleotide polymorphisms (Supplementary Table 1) in the 200 kb *DNAH5* locus to deduce the haplotypes. However, no identical haplotype was found in these families.

The WES results were routinely analyzed with an algorithm to detect CNVs. The positions of the CNV in the *DNAH5* gene are shown in Figure 3A. A CNV causing heterozygous deletions corresponding to exons 71 to 72 of *DNAH5* was suspected in P57 by analysis. Using qPCR, this deletion was recognized in P57 and her father (Figure 3B). As WES only covers exonic regions, WGS was performed to specify the precise breakpoints. Eventually, chr5:13717909-13722659del was detected and confirmed (Figure 3D). Two CNVs causing homozygous deletions spanning exon 69-71 and exon 77-79 were suspected in P100 and P101; qPCR verified that exon 69-71 and exon 77-79 were homozygous deletions in P100, P101 and heterozygous deletions in their parents (Figure 3C). This indicated an autosomal recessive inheritance pattern conforming to the genetic disposition of PCD. To specify the precise breakpoints of the two CNVs, WGS was performed. Ultimately, chr5:13720087_13733030delinsGTTTTC and chr5:13649539_13707643del were detected and confirmed (Figure 3E).

Discussion

In the present study, the utility of NGS screening with additional CNV analysis was evaluated in a large cohort of 114 patients with highly suspected PCD from China. Overall, *DNAH5* was the most prevalent mutant gene, and 45 different allelic mutations were identified in affected individuals from 27 (24.7%) families. It suggested that the genetic profile in China was similar with those of the Europeans^[16]. Multiple aspects of PCD characteristics, including clinical manifestations, ciliary phenotype, as well as genetic characterization were robustly obtained.

Several studies have systematically investigated associations between genotype and phenotype in PCD, which is a genetically heterogeneous disorder. It has been reported that lung function was significantly worse in individuals with *CCDC39* and *CCDC40* mutations and better in those with *DNAH11* and *DNAH5* mutations^[17]. Consistent with the report of Shoemark et al, highly phenotypically diverse were also observed in our cohort^[10]. For instance, P40 still retained nearly normal lung function in his adulthood with only a mild clinical phenotype. By contrast, P101 had severe clinical symptoms, and died of respiratory failure at middle age. Phenotypic diversity was in full display even among siblings. Despite frequent respiratory infections, P100 still had an apparently normal life at 38 years old with only mild to moderate impairment of pulmonary function. In contrast, his older brothers who carried identical mutations and were in the same living environment were not so fortunate; they all had severe bronchiectasis, and died from progressive lung failure at middle age. Similar heterogeneity was also observed in the pair of close-in-age sisters, P69 and P95. It is worth mentioning that data on mortality of PCD are extremely limited. To the best of our knowledge, the two respiratory deaths in our cohort are the first reported in *DNAH5*-associated PCD.

It has been widely reported that cilia beat patterns characterized in PCD patients correlate highly with the implicated gene^[9]. The ODA generate the main mechanical force to produce cilia bending. It is generally recognized that mutations in genes encoding ODA and ODA docking components (e.g. *DNAH5*) cause absent or deficient ODA in cilia, leading to immotile cilia. Intriguingly, as reported here, we observed substantial heterogeneity of ciliary beat pattern in our patients. During HSVA, the CBP differed markedly in the range of static, restricted to stiff movement. The ciliary beat functions of P24(c.13126-2A>G, c.7789A>G), P57(c.13724-1G>A, 4.7kB del), P69(c.5563dupA, c.4355+5G>A) and P97(c.8311C>T, c.6646_6657del) were largely preserved. Similar phenomena were also observed by Blanchon et al, who identified 50% of cilia with normal motion in a patient carrying a *DNAH5* missense mutation (p.R2771C)^[18]. It appears that the different mutations have diverse effects on the protein, and that the alleles with severe effects on the ciliary beating parameters generated drastic structural and functional alterations in the protein. However, it is difficult to explain the difference of beat patterns observed in siblings. Similarly,

variable ciliary beat pattern of PCD patients who carried identical mutation is also reported by Shoemark et al^[10]. Further research is needed to better understand the mechanisms and influential factors of ciliary beat. It should be noted that HSVA provides important clues for the determination of pathogenicity of mutations. In recent years, genetic testing has rapidly advanced and become a viable diagnostic tool for PCD diagnosis^[1,19]. In theory, a molecular genetic diagnosis of PCD can be established through the identification of biallelic pathogenic mutations in a known PCD-associated gene. In practice, however, frequent variants of unknown significance can make diagnosis equivocal, and in these situations, the concordance between genotype and phenotype becomes helpful. Overall, an insufficient knowledge of the ciliary phenotype for a specific genotype may lead to misdiagnosis.

CNVs have been shown to be associated with complex diseases such as cancer, neuropsychological disorders, Crohn disease, autism spectrum disorders, or multiple congenital anomalies^[20]. As technology developed, roles of CNVs in the pathogenesis of PCD were gradually decoded. Deletions in *SPAG1*, *ARMC4*, *DYX1C1*, *LRR50*, *ZYMD10* and *DNAH5* were observed successively^[21,22], suggesting that CNV analysis should be used in the molecular evaluation of patients with PCD. Marshall reported that WES followed by targeted CNV analysis identification of 4 of 45 (8.8%) PCD patients harbored clinically significant CNVs^[22]. In a study by Fassad et al, CNVs accounted for 4% of variants overall in PCD patients^[16]. In the present study, the CNVs were initially predicted by analysis with WES data plus panelcn.MOPS, a reliable algorithm to detect CNVs with high sensitivity and specificity, then confirmed by qPCR and Sanger sequencing. Through this process, we identified 2 of 27 (7.4%) families with clinically significant CNVs. To further evaluate the pathogenicity, we performed high-resolution IF analyses, and confirmed that DNAH5 were absent in the cilia. A considerable proportion of CNV in PCD suggests that routine CNV analysis in the molecular diagnosis of PCD is clinically beneficial.

Conclusions

In conclusion, by utilizing state-of-the-art diagnostic techniques, we have provided a detailed description of the high diversity in DNAH5-associated PCD, which included clinical manifestations, ciliary phenotypes, and genetic spectrum. We hope that the diversity of phenotypes and genotypes identified by this study will lead to a better diagnosis of PCD and recognize the heterogeneous nature of this orphan disease.

Abbreviations

ACMG American College of Medical Genetics and Genomics

CNV Copy number variation

FEV1 Forced expiratory volume in the first second

HRCT High-resolution computed tomography

HSVA High-speed video analysis

IF Immunofluorescence

ODA Outer dynein arms

PCD Primary ciliary dyskinesia

qPCR Quantitative Real-Time Polymerase Chain Reaction

TEM Transmission electron microscopy

WES Whole Exome Sequencing

WGS Whole Genome Sequencing

Declarations

Ethics approval and consent to participate

The project was approved by the ethics committee of the Ethics board of The Children's Hospital of Fudan University, and written informed consent was obtained from all family members participating in this study.

Consent for publication

Parents or subjects gave informed written consent.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZYG and WCC performed the research, analyzed and interpreted the data, and drafted the manuscript. MRL performed experiments and prepared Figures 2 and Table 3. LBW and LLQ conceived and designed the study, revised the manuscript. The authors read and approved the manuscript to be published.

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Tables

Table 1. Detailed clinical characteristics of the PCD patients with *DNAH5*

Case	Sex	Age at	Age at	nNO	RDS	Chronic	Sputum	Pulmonary function % pred			HRCT	nasosinusitis	otitis
No		Enrollment	Diagnosis			cough	pathogens	FEV ₁	FVC	FEF25–75			media
P5	F	10	10	10.4	No	Yes	No	82.9	61.6	27.1	Diffuse nodules; Lobar atelectasis; Bronchiectasis	Yes	NA
P9	F	16	14.2	10.3	No	Yes	No	87.6	75.0	41.0	Diffuse nodules; Lobar atelectasis; Bronchiectasis	Yes	Yes
P11	M	1	1	9.1	Yes	Yes	NA	NA	NA	NA	Patchy exudation	Yes	Yes
P12	M	6	6	12.2	Yes	Yes	MC	84.7	80.1	41.6	Lobar atelectasis	Yes	Yes
P14	F	7	7	6.6	Yes	Yes	No	94.3	105.4	63.7	Lobar atelectasis;	Yes	Yes
P21	M	17	17	5.6	NA	Yes	NA	85.2	70.6	34.0	NA	Yes	NA
P24	M	5	5	29.5	No	No	HI	NA	NA	NA	Patchy exudation	Yes	Yes
P31	F	10.9	10.4	12.3	Yes	Yes	NA	96.0	65.0	41.2	Diffuse nodules	Yes	Yes
P35	M	17	17	NA	No	Yes	NA	83.4	106.6	53.0	NA	Yes	NA
P36	M	0.9	0.9	NA	Yes	Yes	NA	NA	NA	NA	Patchy exudation; Lobar atelectasis	NA	NA
P40	M	17	17	13.5	Yes	Yes	No	97.2	109.8	89.9	Patchy exudation; Lobar atelectasis	Yes	Yes
P42	M	13.7	11.7	14.7	No	Yes	No	86.0	60.7	33.8	Diffuse nodules;	Yes	Yes
P49	M	10.3	10.3	13.2	No	Yes	NA	81.4	77.5	53.2	Diffuse nodules ; Lobar atelectasis; Bronchiectasis;	Yes	Yes
P57	F	0.6	0.6	8.1	Yes	Yes	NA	NA	NA	NA	NA	Yes	No
P58	M	8	8	17.0	No	Yes	HI	81.8	83	59.7	Lobar atelectasis;	Yes	Yes
P69	F	28	28	42.9	NA	Yes	Mab	54.8	76.6	67.5	Lobar atelectasis; Bronchiectasis;	Yes	NA
P70	M	7	7	6.6	No	Yes	No	71.6	76.3	45.0	Diffuse nodules ; Lobar atelectasis; Bronchiectasis;	Yes	Yes
P72	M	9.8	9.8	99	No	Yes	HI	65.2	63.3	37.3	Diffuse nodules ; Lobar atelectasis; Bronchiectasis;	No	Yes
P77	F	38	38	NA	NA	Yes	KP	50.5	70.5	20.4	NA	Yes	NA
P78	M	25	22	NA	NA	Yes	NA	35.8	73.6	18.9	Diffuse nodules ; Lobar atelectasis; Bronchiectasis;	Yes	Yes
P79	F	1.7	1.7	25.1	Yes	Yes	No	NA	NA	NA	Normal	Yes	NA
P92	M	29	29	NA	NA	Yes	No	81.4	112.0	34.0	Lobar atelectasis; Bronchiectasis;	Yes	Yes

P94	F	4	4	NA	No	Yes	NA	NA	NA	NA	Patchy exudation	Yes	Yes
P95	F	29	29	NA	NA	Yes	HI	36.2	74.2	6.3	NA	Yes	NA
P96	M	24	24	14.8	NA	Yes	NA	NA	NA	NA	NA	Yes	NA
P97	F	6.0	6.0	82.5	Yes	Yes	HI, MC	72.9	81.7	43.0	Lobar atelectasis; Bronchiectasis;	No	Yes
P100	M	38	38	9.9	NA	Yes	NA	62.2	62.7	27.0	Lobar atelectasis; Bronchiectasis;	Yes	Yes
P101	M	43	43	NA	NA	Yes	NA	NA	NA	NA	Lobar atelectasis; Bronchiectasis;	Yes	Yes
P102	M	2.5	2.5	3.6	No	Yes	SA	NA	NA	NA	Normal	Yes	Yes
P112	F	35	26	NA	Yes	Yes	NA	NA	NA	NA	Bronchiectasis;	Yes	Yes

AB, acinetobacter baumannii; ASD, atrial septal defect; CA, central apparatus; DCRV, double chambered right ventricle; F, female; FEF_{25-75%}, forced expiratory flow at 25 and 75% of the pulmonary volume; FEV₁, forced expiratory volume in the first second; FVC, forced vital capacity; HI, haemophilus influenza; HRCT, high-resolution computed tomography; HTX, heterotaxy; IDA, inner dynein arms; KP, Kleber pneumoniae; LP, legionella pneumophila; M, male; Mab, Mycobacterium abscessus; MC, moraxella catarrhalis; MTD: microtubular disarrangement; NA, not available; ODA: outer dynein arms; PA, pseudomonas aeruginosa; RDS, respiratory distress; SA, staphylococcus aureus; SIT, situs inversus totalis; SS, situs solitus; VSD, ventricular septal defect.

Table 2. Detailed ciliary feature in patients with *DNAH5*

Case	TEM	CBP	CBF(Hz)
P05	Inconclusive	Minimal residual movements	1.4
P09	ODA/IDA	Immotile	0
P11	ODA/IDA	Minimal residual movements	0
P12	ODA	Immotile	0
P14	ODA	Minimal residual movements	0
P21	ODA	Immotile	0
P24	ODA	Restricted	5.1
P31	Inconclusive	Immotile	0
P35	ODA	NA	NA
P36	ODA	NA	NA
P40	ODA	Immotile	0
P42	ODA	Immotile	0
P49	Inconclusive	Minimal residual movements	0
P57	Inconclusive	Restricted	2.1
P58	ODA	Minimal residual movements	0
P69	NA	Restricted	2.2
P70	ODA	Minimal residual movements	0
P72	Inconclusive	Minimal residual movements	0
P77	NA	NA	NA
P78	NA	NA	NA
P79	NA	Minimal residual movements	0
P92	NA	NA	NA
P94	NA	NA	NA
P95	NA	NA	NA
P96	NA	NA	NA
P97	Inconclusive	Restricted	4.0
P100	ODA	Minimal residual movements	NA
P101	ODA	Immotile	0
P102	ODA	Minimal residual movements	0
P112	NA	NA	NA

IDA, inner dynein arms; NA, not available; ODA, outer dynein arms;

Table 3 *DNAH5* mutations in primary ciliary dyskinesia

Patient	Location	cDNA position	Zygosity	Type of variants	Effect on protein	Ref(PMID)	GnomAD, All	ACMG Guideline	
								Class	Evidence
P05	<i>Exon4</i>	c.278-1_295delinsACAACAACAA	Comp.	Splice-site	/	/	None	P	PVS1;PM2
	<i>Exon38</i>	c.6304C>T	Het	Missense	p.R2102C	/	0.00002439	LP	PM1;PM2
P09	<i>Exon54</i>	c.7579_7590del	Comp.	Inframe	p.2527_2530del	/	None	LP	PM2_Suppl
	<i>Exon32</i>	c.5146C>T	Het	Missense	p.R1716W	19357118	0.000008125	LP	PM1;PM2
P11	<i>Exon40</i>	c.6647delA	Comp.	Frameshift	p.K2216Rfs*20	/	None	P	PVS1;PM2
	<i>Exon45</i>	c.7523T>C	Het	Missense	p.L2508P		None	VUS	PM2_Suppl
P12	<i>Exon37</i>	c.6139C>T	Comp.	Nonsense	p.Gln2047*	/	None	P	PVS1;PM2
	<i>Exon14</i>	c.1852C>T	Het	Nonsense	p.Arg618*	29363216	0.000004102	P	PVS1;PM
P14	<i>Exon45</i>	c.7469G>A	Comp.	Nonsense	p.W2490*	/	None	P	PVS1;PM
	<i>Exon55</i>	c.9235C>T	Het	Nonsense	p.R3079*	/	None	P	PVS1;PM
P21	<i>Exon27</i>	c.4193A>G	Hom	Missense	p.Q1398R	/	None	VUS	PM2_Suppl
P24	<i>Intron75</i>	c.13126-2A>G	Comp.	Splice-site	/	/	None	LP	PVS1_Mo
	<i>Exon47</i>	c.7789A>G	Het	Missense	p.T2597A	/	None	LP	PM1;PM2;
P31	<i>Exon34</i>	c.5563dupA	Comp.	Frameshift	p.I1855Nfs*6	31118369	0.00006099	P	PVS1;PM3
	<i>Intron79</i>	c.13724-1G>C	Het	Splice-site	/	/	None	LP	PVS1_Mo
P35	<i>Exon60</i>	c.9897+1G>A	Comp.	Splice-site	/	/	None	VUS	PVS1_Mo
	<i>Exon50</i>	c.8265C>G	Het	Missense	p.F2755L	/	None	VUS	PM1;PM2
P36	<i>Exon49</i>	c.8030G>A	Comp.	Missense	p.R2677Q	24150548	0.00002035	LP	PM1;PM2
	<i>Exon32</i>	c.5150T>A	Het	Missense	p.F1717Y	/	None	LP	PM1;PM2
P40	<i>Exon51</i>	c.8498 G>A	Comp.	Missense	p.R2833H	27637300	0.0000122	LP	PM1;PM2
	<i>Exon12</i>	c.1616_1617delAG	Het	Frameshift	p.E539Vfs*6	/	None	P	PVS1;PM2
P42	<i>Exon34</i>	c.5563dupA	Comp.	Frameshift	p.I1855Nfs*6	31118369	0.00006099	P	PVS1;PM3
	<i>Exon27</i>	c.4147_4148delAinsTCC	Het	Frameshift	p.I1383Sfs*22	/	None	P	PVS1;PM2
P49	<i>Exon56</i>	c. 9502 C>T	Comp.	Nonsense	p.R3168*	/	0.0000122	P	PVS1;PM2
	<i>Exon48</i>	c. 7912 A> T	Het	Nonsense	p.K2638*	/	None	P	PVS1;PM2
P57	<i>Intron79</i>	c.13724-1G>A	Comp.	Splice-site	/	/	None	LP	PVS1_Mo
	<i>Exon71-72</i>	4.7kB del	Het	CNV	/	/	None	LP	PVS1_Mo
P58	<i>Intron53</i>	c.8821-2A>C	Comp.	Splice-site	/	/	None	LP	PVS1_Mo
	<i>Exon7</i>	c.877dupA	Het	Frameshift	p.R293fs	/	0.000004065	P	PVS1;PM2
P69	<i>Exon34</i>	c.5563dupA	Comp.	Frameshift	p.I1855Nfs*6	31118369	0.00006099	P	PVS1;PM3
	<i>Intron27</i>	c.4355+5G>A	Het	Splice-site	/	/	0.0000449	VUS	PM3;PP1;I
P70	<i>Intron10</i>	c.1320+1G>A	Comp.	Splice-site	/	/	None	LP	PVS1_Mo
	<i>Exon34</i>	c.5557A >T	Het	Nonsense	p.K1853*	25525159	0.000008133	P	PVS1;PM2
P72	<i>Exon50</i>	c.8311C>T	Comp.	Missense	p.R2771C	/	0.00003658	P	PM1;PM2
	<i>Exon40</i>	c.6646_6657del	Het	inframe	p.K2216_Y2219del	/	None	P	PM2_Suppl
P77	<i>Exon38</i>	c.6305G>A	Comp.	Missense	p.R2102H	/	0.000004065	VUS	PM1;PM2
	<i>Exon19</i>	c.2980C>T	Het	Missense	p.R994W	/	0.0002	VUS	PP4
P78	<i>Exon79</i>	c.13746C>G	Comp.	Nonsense	p.Y4582*	/	None	VUS	PVS1_Mo
	<i>Exon48</i>	c.7979A>G	Het	Splice-site	/	/	None	VUS	PM1;PM2
P79	<i>Exon45</i>	c.7579_7590del	Comp.	inframe	p.T2527_D2530del	/	None	LP	PM2_Suppl
	<i>Exon31</i>	c.5047G>T	Het	Nonsense	p.E1683*	/	None	P	PVS1;PM2

P92	<i>Exon34</i>	c.5563dupA	Comp.	Frameshift	p.I1855Nfs*6	31118369	0.00006099	P	PVS1;PM3
	<i>Exon63</i>	c.10616G>A	Het	Missense	p.R3539H	22416021	0.00002038	LP	PM1;PM2
P94	<i>Intron76</i>	c.13339-1G>A	Comp.	Splice-site	/	/	None	P	PVS1;PM2
	<i>Exon36</i>	c. 5971A>G	Het	Missense	p.K1991E	/	None	LP	PM1;PM2
P95	<i>Exon34</i>	c.5563dupA	Comp.	Frameshift	p.I1855Nfs*6	31118369	0.00006099	P	PVS1;PM3
	<i>Intron27</i>	c.4355+5G>A	Het	Splice-site	/	/	0.0000449	VUS	PM3;PP1;I
P96	<i>Exon68</i>	c.11664C>G	Comp.	Nonsense	p.Y3888*	/	None	P	PVS1;PM2
	<i>Exon34</i>	c.5563dupA	Het	Frameshift	p.I1855Nfs*6	31118369	0.00006099	P	PVS1;PM3
P97	<i>Exon50</i>	c.8311C>T	Comp.	Missense	p.R2771C	/	0.00003658	P	PM1;PM2
	<i>Exon40</i>	c.6646_6657del	Het	Nonframeshift	p.K2216_Y2219del	/	None	P	PM2_Supl
P100	<i>Exon69-71Exon77-79</i>	58.1kB, 12.9kB del	Hom	CNV	/	/	None	LP	PVS1_Mo
P101	<i>Exon69-71Exon77-79</i>	58.1kB, 12.9kB del	Hom	CNV	/	/	None	LP	PVS1_Mo
P102	<i>Exon63</i>	c.10616G>A	Comp.	Missense	p.R3539H	22416021	0.00002038	LP	PM1;PM2
	<i>Exon34</i>	c.5648G> C	Het	Missense	p.R1883P	/	None	VUS	PM2_Supl
P112	<i>Intron69</i>	c.11883+2T>C	Comp.	Splice-site	/	/	None	P	PVS1;PM2
	<i>Exon63</i>	c.10616 G>A	Het	Missense	p.R3539H	22416021	0.00002038	LP	PM1;PM2

PMID PubMed Unique Identifier; HGMD, Human Gene Mutation Database; NA, not available;

Figures

Figure 1

Pedigree structure and clinical examinations of P100's family

(A) Pedigree structure of P100's family. Arrow denotes the proband P100. Affected patients are presented by black symbols; half black symbol indicates heterozygous deletions of *DNAH5*; symbols with diagonal slashes denote deceased individuals. CXR images of the mother (B) and father (C) of proband shows normal distribution of organs. Clinical imaging show mirrored distributed viscera (D) and features of bronchiectasis (E) and paranasal sinusitis (F) of P100. Clinical imaging of P101 show distinctive situs inversus (G) and severer bronchiectasis (H) and paranasal sinusitis (I), compared to P100.

Figure 2

Subcellular localization of DNAH5 in respiratory epithelial cells.

Axoneme-specific antibodies against acetylated α -tubulin (red) were used as the control. In respiratory epithelial cells from healthy probands (A), DNAH5 (green) localized predominantly along the entire length of the axonemes, as well as to the apical cytoplasm. In respiratory epithelial cells from individuals P24 (B), P57 (C), P97 (D) and P100 (E), DNAH5 was absent from the axoneme and markedly reduced in the apical cytoplasm.

Figure 3

CNV analysis by qPCR and Sanger sequencing.

(A) Schematic of DNAH5 protein structure, the red slash indicating the position of the CNVs. qPCR analysis of relative DNA content in whole blood from the control subject, P57 (B), P100 (C) and their family member. Sanger sequencing confirmed the CNVs at Chr5:13717907-13722661 in P57 (D) and chr5:13720087_13733030, chr5:13649539_13707643 in P100 (E).

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