Pathogenic variants identified by whole-exome sequencing in Chinese patients with primary ciliary dyskinesia

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Abstract

Background: The genetic factors contributing to primary ciliary dyskinesia (PCD), a rare autosomal recessive disorder, remain elusive for approximately 20–35% of patients with complex and abnormal clinical phenotypes. Our study aimed to identify causative variants of PCD-associated pathogenic candidate genes using whole-exome sequencing (WES).

Methods: All patients were diagnosed with PCD based on clinical phenotype or transmission electron microscopy (TEM) images of cilia. WES and bioinformatic analysis were then conducted for patients with PCD. Identified candidate variants were validated by Sanger sequencing. Pathogenicity of candidate variants was then evaluated using in silico software and the American College of Medical Genetics and Genomics (ACMG) database.

Results: In total, 15 rare variants were identified in patients with PCD, among which were three homozygous causative variants (including one splicing variant) in the PCD-associated genes *CCDC40* and *DNAI1*. Moreover, two stop-gain heterozygous variants of *DNAAF3* and *DNAH1* were classified as pathogenic variants by the ACMG criteria.

Conclusions: This study identified novel potential pathogenic genetic factors associated with PCD. Noteworthy, the PCD patients carried multiple rare causative gene variants, thereby suggesting that known causative genes along with other functional genes should be considered for such heterogeneous genetic disorders.

Background

Primary ciliary dyskinesia (PCD) is an autosomal recessive disorder characterized by respiratory distress, tympanitis, sinusitis, and bronchiectasis [1]. Kartagener syndrome (MIM# 244400) is a subtype of PCD exhibiting the situs inversus phenotype. The estimated prevalence of PCD ranges from 1 in 10,000 to 1 in 15,000 among Europeans [2]. PCD is diagnosed by measuring nasal nitric oxide and through brush biopsy [3]. Moreover, transmission electron microscopy (TEM) can be used to visualize the nasal mucosa cilia ultrastructure and achieve a definitive diagnosis. Given the main feature of this disorder (ciliary dyskinesia), male PCD patients commonly present with infertility due to abnormally immotile or dyskinetic sperm flagella [4].

Genetic screenings have improved our understanding of the pathogenesis of inherited PCD [5]. Previous genetic and functional studies revealed a series of PCD-associated genes causing congenital ultrastructural abnormalities that resulted in dysfunctional immotile cilia [6]. Inherited genetic variations are present in approximately 60% of PCD cases, indicating a high genetic heterogeneity of this disorder [3]. Mutations in inner dynein arm (IDA) and outer dynein arm (ODA) complex-coding genes cause ciliary abnormalities, but some patients with confirmed PCD clinical features also show normal ciliary motility and ultrastructure [7]. Interestingly, some studies have reported that PCD with heterotaxy and airway ciliary dysfunction only involves heterozygous mutations in PCD-associated genes [8]. Additionally, an autosomal dominant mutation of *FOXJ1* was also reported to cause a distinct motile ciliopathy related to defective ciliogenesis, with a similar clinical phenotype as that of PCD [9]. Taken together, these findings suggest the potential involvement of unknown genes and mechanisms in pathologies associated with ciliary function.

Next generation sequencing has advanced the identification of candidate causative genes in PCD [10]. This study aimed to identify pathogenic gene variants of sporadic PCD in Chinese patients, thereby allowing to identify genetic risk factors associated with PCD that could be relevant for the diagnosis and counseling of this disorder.

Results

Demographic and clinical characteristics of PCD patients

The patients were diagnosed with PCD using typical methods, as described in the Materials and Methods section, and all had clinical history of recurrent cough and expectoration for several years (Table 1).Chest CT or MR imaging revealed bronchiectasis in all patients. Moreover, situs inversus was identified in four patients (Fig. 1a,b), leading to the diagnosis of Kartagener syndrome(MIM# 244400), a subtype of PCD. TEM examination showed the presence of ODA and IDA defects, as well as microtubular disorganization of the cilia, in two patients of KT7 and KT8 (Fig. 1c,d).

Table 1
Clinical characteristics of primary ciliary dyskinesia patients

PCD ID	Age	Gender	Recurrent cough	Situs inversus	Bronchiectasis	Bronchitis
KT6	30	Female	Yes	Yes	Yes	Yes
KT7	32	Female	Yes	Yes	Yes	Yes
KT8	48	Male	Yes	Yes	Yes	Yes
KT9	48	Female	Yes	Yes	Yes	No
KT10	12	Male	Yes	No	Yes	Yes

Identification Of Rare Variants By Bioinformatics Analysis

To identify candidate PCD-causative gene variants in the five Chinese PCD patients, the identified variants were filtered to obtain rare genetic alterations based on function and frequency. In total, 15 variants were identified in candidate genes with MAF < 1% in the East Asian population, according to the 1000

Genomes, ExAC, and gnomAD databases (Table 2). Six novel variants were identified in five genes that were not recorded in the gnomAD and dbSNP databases. One homozygous *DNAI1* variant and one frameshift insertion homozygous *CCDC40* variant identified in KT9 and KT10 patients, respectively, were believed to be potential PCD-contributing factors (Table 2). No rare heterozygous genetic alterations were found along with the identified two potential pathogenic heterozygous stop-gain variants in KT7 and KT8, indicating that the genetic defects of these patients were not caused by compound heterozygosity. No rare pathogenic variants were identified in KT6, except for a likely benign rare variant in *DNAH1*.

Table 2
Pathogenicity assessment of the identified candidate variants

Chr †	Start	refGene	Alteration	KTS Carrier	Variant type	gnomeAD_EAS ‡	ACMG Classification	Causative
chr7	21781653	DNAH11	NM_001277115:exon49:c.A8023G:p.I2675V	KT6	Missense	0.0006	PM1 + PP5 + BS1 + BP4	Likely Benign
chr19	55672134	DNAAF3	NM_001256714:exon9:c.G1126T:p.E376X	KT7	**Stop-gain**	NA	PVS1 + PM2 + PP3	**Pathogenic**
chr17	40086999	TTC25	UNKNOWN	KT7	Missense	NA	PM1 + BP4	VUS
chr3	52392666	DNAH1	NM_015512:exon25:c.C4179A:p.N1393K	KT7	Missense	NA	PM1 + BP4	VUS
chr5	13911508	DNAH5	NM_001369:exon12:c.C1631T:p.T544I	KT8	Missense	0.0093	PM1 + PP5 + BS1 + BP4	Likely Benign
chr3	52388915	DNAH1	NM_015512:exon21:c.C3537G:p.Y1179X	KT8	**Stop-gain**	NA	PVS1 + PM2 + PP3	**Pathogenic**
chr2	84861727	DNAH6	NM_001370:exon30:c.C4615G:p.Q1539E	KT8	Missense	0.0019	PM1	VUS
chr14	50100519	DNAAF2	NM_001083908:exon1:c.G1349T:p.S450I	KT8	Missense	0.0006	BP4	VUS
chr17	78059800	CCDC40	NM_001243342:exon14:c.2236-2A>-	KT8	**Homozygote Splicing**	NA		**Likely Pathogenic**
chr9	34506853	DNAI1	NM_001281428:exon13:c.A1304C:p.H435P	KT9	**Homozygote Missense**	NA	PM1 + PM2 + PP3	**Likely Pathogenic**
chr17	11556249	DNAH9	NM_001372:exon14:c.G2525A:p.R842Q	KT9	Missense	NA	NA	VUS
chr3	50382932	ZMYND10	NM_001308379:exon1:c.A79G:p.M27V	KT9	Missense	0.0012	PM2	VUS
chr17	78023917	CCDC40	NM_001243342:exon7:c.994dupT:p.Y332fs	KT10	**Homozygote frameshift insertion**	NA		**Pathogenic**
chr7	21726746	DNAH11	NM_001277115:exon33:c.A5651G:p.H1884R	KT10	Missense	NA	PM1 + PM2	VUS
chr3	52430923	DNAH1	NM_015512:exon73:c.C11650T:p.R3884C	KT10	Missense	NA	PM1	VUS

†Chr: Chromosome; ‡EAS: East Asian population. VUS: variant of uncertain significance.

Acmg Guidelines And Pathogenic Variants Classification

Candidate variants were assessed using *in silico* tools and the ACMG guidelines (Table 2). All potential causative novel variants were validated by Sanger sequencing (Fig. 2a). The frameshift insertion in *CCDC40* (c.994dupT) was identified as a pathogenic homozygous variant causative of PCD in the patient KT10. A homozygous *DNAI1* variant was found in the patient KT9, without additional rare pathogenic genetic variants; thus, the *DNAI1* variant could be the potential cause of PCD, even though it was considered as a variant of uncertain significance (VUS) according to the ACMG criteria. Further evidence is needed to confirm the association between this homozygous variation in *DNAI1* and the observed PCD phenotype. Two stop-gain heterozygous variants of *DNAAF3* and *DNAH1* were identified in patients KT7 and KT8. TEM examination confirmed the presence of cilia ODA and IDA defects in these patients, which was consistent with the identified mutations (Fig. 1). The ACMG criteria classified these *DNAAF3* and *DNAH1* stop-gain heterozygous variants as pathogenic, with very strong evidence of pathogenicity (PVS1). No other rare variants of these two genes were identified in the patients KT7 and KT8, indicating that these two PCD cases were not only caused by heterozygous but also by trans-heterozygous gene interactions.

Confirmation of the pathogenicity of the *CCDC40* splicing variant

The homozygous deletion c.2236-2A>- in *CCDC40* (NM_017950) found in the patient KT8 was found to alter the nucleotide sequence at the splicing site near exon 14. To confirm whether this variation could cause abnormal mRNA transcription, cDNA sequencing was conducted using samples from the patient KT8 and healthy control. Genomic DNA sequencing revealed the splicing deletion in KT8 (Fig. 2c), which was further confirmed by PCR and sequencing data, showing that the portion of the cDNA between exons 13 and 14 was lost in the patient KT8 (Fig. 2b). cDNA-based PCR sequencing showed normal *CCDC40* cDNA products in the healthy control (Fig. 2b,d). These results indicated that the splicing deletion variant of *CCDC40* (c.2236-2A>-) resulted in the production of a shorter mRNA transcript compared with the normal control. Together with the clinical phenotype resulting from the *CCDC40* defect, these findings suggest that the *CCDC40* splicing variant c.2236-2A>- is likely to be pathogenic.

Two rare variants were predicted as likely benign, and the others were classified as VUS (Table 2). These variants were considered not to be associated with PCD based on the ACMG guidelines. In summary, five rare variants were considered to potentially cause PCD based on the ACMG guidelines (Table 2).

Methods

Subjects and PCD diagnosis

Five PCD patients, together with control individuals, were enrolled in the study from 2008 to 2020. High-resolution computed tomography (CT), magnetic resonance (MR) imaging, and TEM examinations were conducted to investigate the clinical phenotype of PCD, including chronic sinusitis, bronchiectasis, situs inversus, and ciliary ultrastructural defects. Patients were diagnosed with PCD according to the classic characterization and guidelines [11]. The control subjects included in the study were the healthy siblings or parents of the patients, and unrelated healthy individuals. Ethical approval was obtained from the ethics committee of the Shenzhen People's Hospital (Guangdong, China), as per the ethical standards recommended in the 1964 Declaration of Helsinki. Informed consent was obtained from each subject enrolled in the study.

Identification Of Gene Variants By Wes And Bioinformatics Analysis

The genome of all subjects were sequenced using the NovaSeq platform (Illumina, San Diego, CA, USA). Variants revealed using WES data were analyzed using the Genome Analysis Toolkit and Annovar [12]. Subsequently, all variants were filtered based on minor allele frequency (MAF < 0.1%), using the following databases: 1000 Genomes (https://www.internationalgenome.org), CG69, and gnomAD (http://gnomad.broadinstitute.org/). Sorting Intolerant From Tolerant, MutationTaster, Polymorphism Phenotyping v2 (PolyPhen-2), and Combined Annotation Dependent Depletion tools were used to assess the pathogenicity of identified variants. Further, an in-house filtering pipeline was used to identify candidate variants.

Pathogenicity assessment and validation of candidate variants in PCD-associated genes

In total, 46 pathogenic genes highly associated with PCD were selected as candidate gene variants, as described previously [13]. Gene function- and frequency-based advanced filtering analyses were conducted for candidate genes variants. Variant pathogenicity assessment was conducted using bioinformatics software and ACMG [14]. The classification and interpretation of the variants followed the ACMG guidelines. Identified candidate causative variants were validated in patients with PCD and control subjects using the Sanger sequencing platform. The Chromas software was used to analyze the sequencing results (Technelysium Pty Ltd., South Brisbane, Australia).

Polymerase chain reaction (PCR) and sequencing confirmation of the *CCDC40* splicing variant

Total RNA was extracted from whole blood of the PCD patients and reverse-transcribed using the RR036A kit (TAKARA Bio, Kusatsu, Japan). Primers for CCDC40 exons were designed using the IDT PrimerQuest tool (https://sg.idtdna.com/Primerquest/Home/Index). cDNA segments transcribed from *CCDC40* exons were amplified using standard PCR. The primer sets for exon 13–14 (primer4) and exon 14–15 (primer6) transcripts were as follows: F- 5′ GGACCAGGACGTGAAGAAAG 3′, and R- 5′ CTGTGTCACCTTGACCATCTC; and F- 5′ GATCGACGAGCACGATGG 3′, and R- GAGCTTCTTCAGGTCGTTGT 3′, respectively. Transcript products of the splicing variant in patient KT8 were confirmed by Sanger sequencing and agarose gel electrophoresis.

Discussion

In the present study, WES was conducted to identify candidate PCD-causative pathogenic gene variants in five patients. Five causative variations in *CCDC40*, *DNAH1*, *DNAAF3*, and *DNAI1* were considered pathogenic. Three homozygous variants, including a splicing variant, were identified in *CCDC40* and *DNAI1*. Further analyses showed that the splicing variant of *CCDC40* could result in the production of a shortened protein, which may contribute to PCD pathogenesis. Two stop-gain heterozygous variants of *DNAAF3* and *DNAH1* were classified as pathogenic variants with strong evidence according to the ACMG criteria. Altogether, this study identified five potential genetic factors involved in the PCD pathogenesis.

To date, strong functional evidence has demonstrated the contribution of over 50 genes to PCD pathogenesis, most of which mainly cause structural abnormalities of motile cilia, such as complete or partial deletion of IDAs or ODAs [6]. Defects in *CCDC40* are responsible for IDA defects and microtubular disorganization [15]. Herein, two homozygous variants of *CCDC40* were identified and the carrier showed the classic PCD phenotype involving situs inversus and bronchiectasis. TEM examination further revealed that the abnormal cilia structure in agreement with the previously described effects of *CCDC40* defects. Moreover, a splicing deletion in *CCDC40* was also found to potentially produce a shorter mRNA transcript. Based on this evidence, the splicing deletion variant of *CCDC40* may likely be a PCD-causative variant. Nevertheless, the exact function and mechanisms of the *CCDC40* splicing variant in PCD pathogenesis warrants further investigations.

One novel PCD-causative homozygous variant was identified in *DNAI1*. ACMG predicted this variant to be a VUS, but the variant was predicted to be pathogenic by all *in silico* tools. *DNAI1* encodes the intermediate chain of ODAs [16], and mutations in *DNAI1* result in situs inversus and PCD [17]. The carrier of the *DNAI1* variant exhibited the classic PCD phenotype and had no other rare gene variants; hence, this *DNAI1* variant was recognized as the main genetic factor contributing to PCD in this patient. However, appropriate functional studies would be helpful to further confirm the pathogenicity of this *DNAI1* variant.

A novel stop-gain heterozygous variant of *DNAAF3* was also identified, in a patient that did not harbored any other pathogenic variants. *DNAAF3* mutations were reported to cause ODA and IDA defects in cilia [18]. Two rare VUS mutations of *DNAH1* and *TTC25* were also identified, but whether the observed trans-heterozygous interactions between these variants could play a role in PCD remains to be confirmed. Identification of ciliary structural defects is helpful to confirm the association between a gene mutation and PCD phenotype. Unfortunately, some patients in our study refused to undergo TEM examination.

As PCD is an autosomal recessive disorder, homozygous or compound heterozygous mutations are commonly considered contributing genetic factors. However, the present and previous studies demonstrated that heterozygous pathogenic mutations are frequent in patients with PCD [13], especially frameshift insertions or deletions and stop-gain or -loss mutations. Nevertheless, none of the other candidate pathogenic variants were copresent. Interestingly, the inheritance model of the candidate variants was very similar to that for autosomal dominant disorders. Some studies have shown that autosomal dominant variants can also cause defects in ciliogenesis with a similar clinical phenotype to that of PCD [9]. However, further investigation is required to determine whether these heterozygous variants can cause PCD on their own. Additional evidence is required to confirm the pathogenicity of various types of genetic variants in PCD, given the heterogeneous nature of this genetic disorder.

Conclusions

In conclusion, five candidate PCD-causative gene variants in *CCDC40*, *DNAH1*, *DNAAF3*, and *DNAI1* were identified. Moreover, the collected data demonstrates that heterozygous pathogenic mutations alone are frequent in patients with PCD and that rare mutations can occur in several causative genes in the same PCD patient. Thus, this genetic disorder may be caused by heterozygous as well as trans-heterozygous mutations. Confirmation of the causative genetic factors and clinical ciliary defect type in PCD are important steps toward developing personalized clinical diagnosis and genetic counseling strategies.

List Of Abbreviations

PCD: Primary ciliary dyskinesia; WES: whole-exome sequencing; TEM: transmission electron microscopy; CT: computed tomography; MR: magnetic resonance; ACMG: the American College of Medical Genetics and Genomics; IDA: inner dynein arm; ODA: outer dynein arm; MAF: minor allele frequency; VUS: variants of uncertain significance.

Declarations

Ethics approval and consent to participate

The project was approved by the ethics committee of the Ethics Committee of Shenzhen People's Hospital. All procedures performed in studies involving human participants in accordance with the 1964 Declaration of Helsinki ethical standards.

Consent for publication

Written Informed consent for publication was obtained from all participants.

Availability of data and materials

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

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' contributions

Yongjian Yue and Yingyun Fu prepared the project proposal and study design. Yongjian Yue and Lipeng Chen conducted bioinformatics and statistical analysis of sequencing data. Qijun Huang, Fang Yuan, Chunxian Liang, and Kaixue Zhuang conducted sample collection and Sanger sequencing validation. Qijun Huang, Xiangxia Zhang, and Yutian Ye conducted clinical diagnosis of PCD. Lu Liu, Jie Li and Rongchang Chen assisted with the prepared and revised manuscript. All the authors have read and approved the final manuscript.

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References

1. Horani A, Ferkol TW: **Advances in the Genetics of Primary Ciliary Dyskinesia: Clinical Implications**. *Chest* 2018, **154**(3):645-652.

2. Bush A, Chodhari R, Collins N, Copeland F, Hall P, Harcourt J, Hariri M, Hogg C, Lucas J, Mitchison HM *et al*: **Primary ciliary dyskinesia: current state of the art**. *Arch Dis Child* 2007, **92**(12):1136-1140.
3. Lucas JS, Paff T, Goggin P, Haarman E: **Diagnostic Methods in Primary Ciliary Dyskinesia**. *Paediatric respiratory reviews* 2016, **18**:8-17.
4. Liu C, Miyata H, Gao Y, Sha Y, Tang S, Xu Z, Whitfield M, Patrat C, Wu H, Dulioust E *et al*: **Bi-allelic DNAH8 Variants Lead to Multiple Morphological Abnormalities of the Sperm Flagella and Primary Male Infertility**. *Am J Hum Genet* 2020, **107**(2):330-341.
5. Khalid F, Hannah WB, Gaston BM: **Rapid Advances in Primary Ciliary Dyskinesia Research. A Brief Update for Pulmonologists**. *Am J Respir Crit Care Med* 2019, **199**(2):136-138.
6. Lucas JS, Davis SD, Omran H, Shoemark A: **Primary ciliary dyskinesia in the genomics age**. *Lancet Respir Med* 2020, **8**(2):202-216.
7. Wallmeier J, Al-Mutairi DA, Chen CT, Loges NT, Pennekamp P, Menchen T, Ma L, Shamseldin HE, Olbrich H, Dougherty GW *et al*: **Mutations in CCNO result in congenital mucociliary clearance disorder with reduced generation of multiple motile cilia**. *Nat Genet* 2014, **46**(6):646-651.
8. Wilson CW, Nguyen CT, Chen MH, Yang JH, Gacayan R, Huang J, Chen JN, Chuang PT: **Fused has evolved divergent roles in vertebrate Hedgehog signalling and motile ciliogenesis**. *Nature* 2009, **459**(7243):98-102.
9. Wallmeier J, Frank D, Shoemark A, Nothe-Menchen T, Cindric S, Olbrich H, Loges NT, Aprea I, Dougherty GW, Pennekamp P *et al*: **De Novo Mutations in FOXJ1 Result in a Motile Ciliopathy with Hydrocephalus and Randomization of Left/Right Body Asymmetry**. *Am J Hum Genet* 2019, **105**(5):1030-1039.
10. Fassad MR, Patel MP, Shoemark A, Cullup T, Hayward J, Dixon M, Rogers AV, Ollosson S, Jackson C, Goggin P *et al*: **Clinical utility of NGS diagnosis and disease stratification in a multiethnic primary ciliary dyskinesia cohort**. *J Med Genet* 2020, **57**(5):322-330.
11. Shapiro AJ, Davis SD, Polineni D, Manion M, Rosenfeld M, Dell SD, Chilvers MA, Ferkol TW, Zariwala MA, Sagel SD *et al*: **Diagnosis of Primary Ciliary Dyskinesia. An Official American Thoracic Society Clinical Practice Guideline**. *Am J Respir Crit Care Med* 2018, **197**(12):e24-e39.
12. Wang K, Li M, Hakonarson H: **ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data**. *Nucleic Acids Res* 2010, **38**(16):e164.
13. Yue Y, Huang Q, Zhu P, Zhao P, Tan X, Liu S, Li S, Han X, Cheng L, Li B *et al*: **Identification of Pathogenic Mutations and Investigation of the NOTCH Pathway Activation in Kartagener Syndrome**. *Frontiers in genetics* 2019, **10**:749.
14. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E *et al*: **Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology**. *Genet Med* 2015, **17**(5):405-424.
15. Becker-Heck A, Zohn IE, Okabe N, Pollock A, Lenhart KB, Sullivan-Brown J, McSheene J, Loges NT, Olbrich H, Haeffner K *et al*: **The coiled-coil domain containing protein CCDC40 is essential for motile cilia function and left-right axis formation**. *Nat Genet* 2011, **43**(1):79-84.
16. Zariwala MA, Leigh MW, Ceppa F, Kennedy MP, Noone PG, Carson JL, Hazucha MJ, Lori A, Horvath J, Olbrich H *et al*: **Mutations of DNAI1 in primary ciliary dyskinesia: evidence of founder effect in a common mutation**. *Am J Respir Crit Care Med* 2006, **174**(8):858-866.
17. Guichard C, Harricane MC, Lafitte JJ, Godard P, Zaegel M, Tack V, Lalau G, Bouvagnet P: **Axonemal dynein intermediate-chain gene (DNAI1) mutations result in situs inversus and primary ciliary dyskinesia (Kartagener syndrome)**. *Am J Hum Genet* 2001, **68**(4):1030-1035.
18. Mitchison HM, Schmidts M, Loges NT, Freshour J, Dritsoula A, Hirst RA, O'Callaghan C, Blau H, Al Dabbagh M, Olbrich H *et al*: **Mutations in axonemal dynein assembly factor DNAAF3 cause primary ciliary dyskinesia**. *Nat Genet* 2012, **44**(4):381-389, S381-382.

Figures

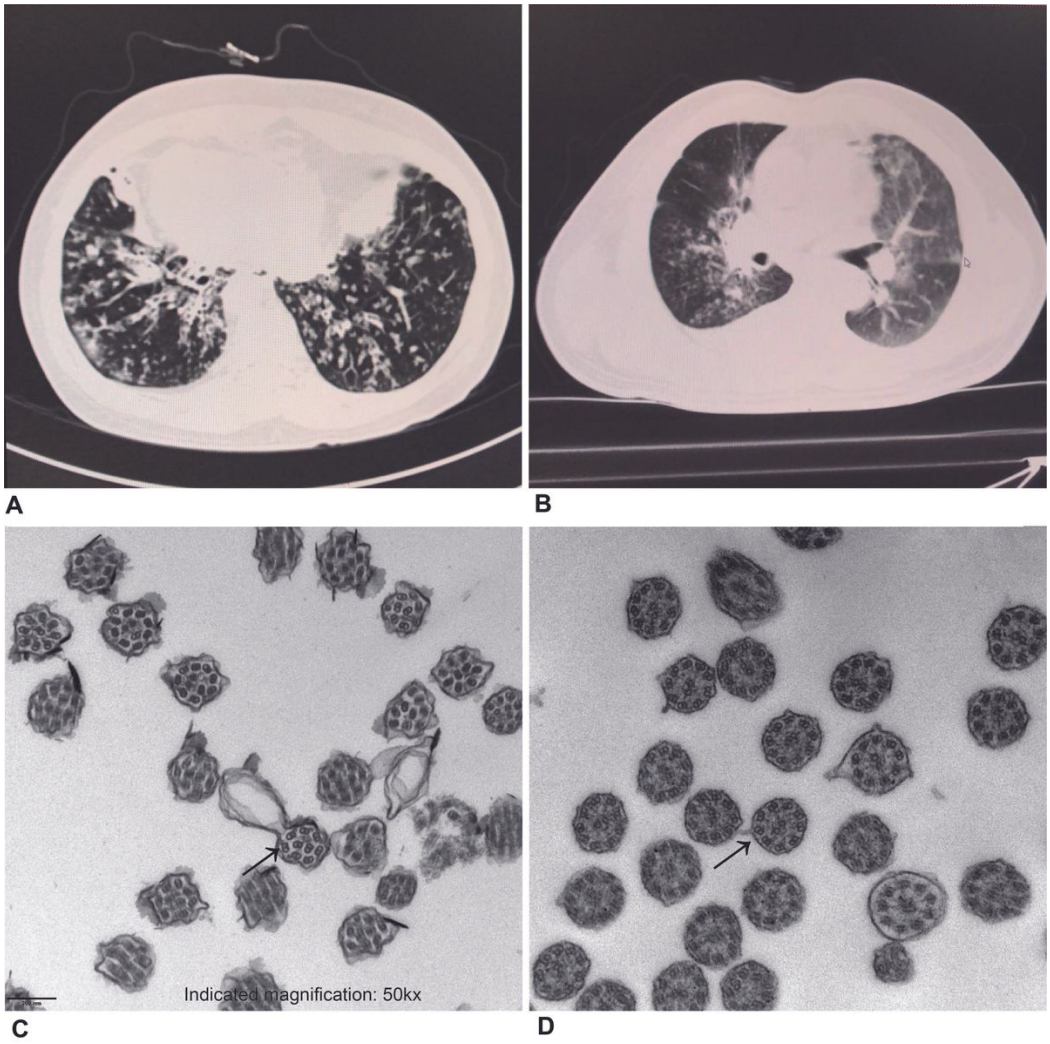

Figure 1

Primary ciliary dyskinesia diagnosis using traditional methods. Chest images showed bronchiectasis and situs inversus in patient KT9 (a) and bronchiectasis in patient KT10 (b). Transmission electron microscopy examination showed structural defects in the cilia of patients KT7 (c) and KT8 (d). The arrows represent the inner and outer dynein arms defects, and microtubular disorganization.

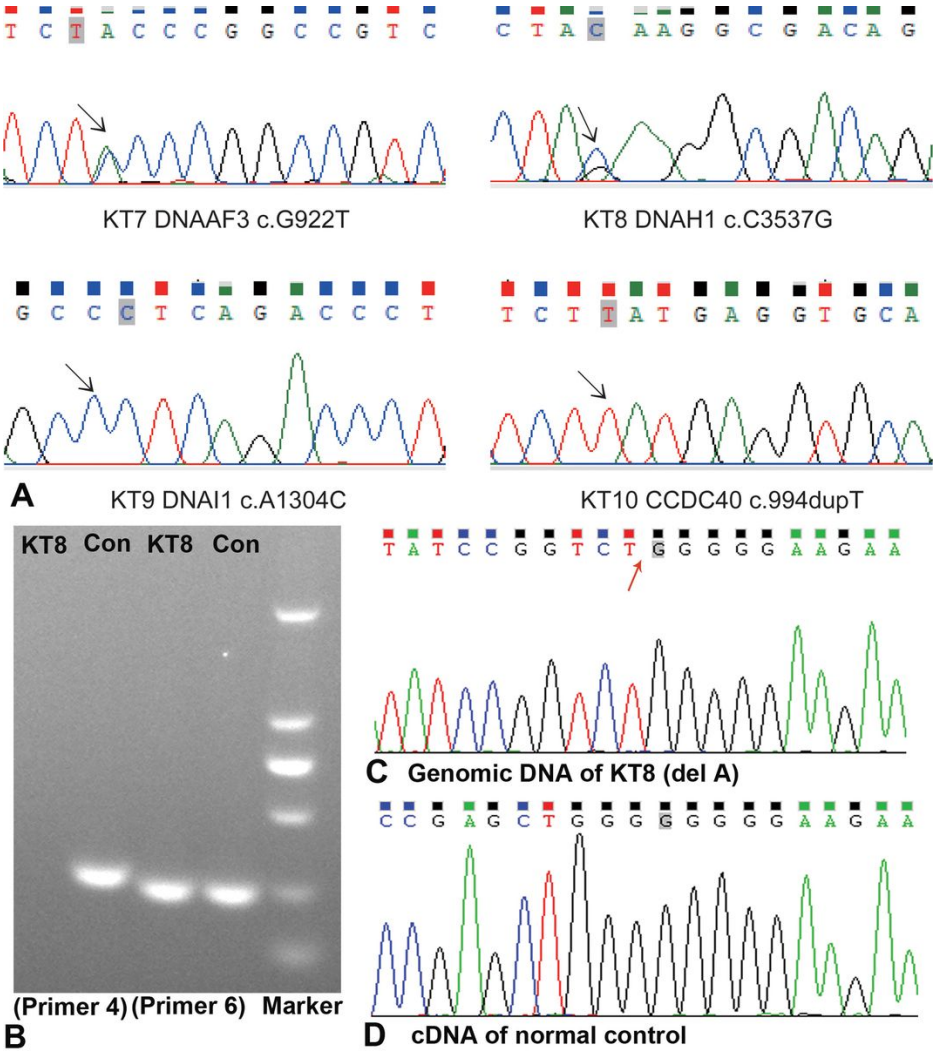

Figure 2

(a) Validation of causative candidate variants by Sanger sequencing. The variant positions are indicated by arrows. Sequencing data from healthy controls validation confirmed the absence of these variants(data not shown). (b) Agarose gel electrophoresis confirmed the CCDC40 splicing event in KT8 by the absence of exon 13–14 transcript product amplification (primer4). Primer6 represents the exon 14–15 transcript product amplification. (c) Splicing-promoted deletion identified in the patient KT8. (d) cDNA sequencing of a healthy control (con) showed a normal sequence between exon 13 and exon 14 of the transcript.