Clinical and genetic analysis of two patients with primary ciliary dyskinesia caused by a novel mutation of DNAAF2

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Abstract

Objective: To investigate the clinical manifestations, diagnosis and treatment processes of two siblings with PCD caused by the same compound heterozygous mutations in DNAAF2. **Methods**: With clinical diagnosis of PCD, the two siblings were recruited in the study. We collected their clinical histories, laboratory tests, bronchoscopy, otoscope images, and radiographic data. Whole blood of the siblings and their parents were separately harvested for whole-exome sequencing and Sanger sequencing. **Results**: The 7-year-old girl presented with recurrent respiratory tract infection, sinusitis and otitis media. Auxiliary examinations showed pneumonia, atelectasis, bronchiectasis, low nitric oxide concentration (nNO), and conducting hearing loss. The younger brother, 10-month boy, exhibited pneumonia, sinusitis, otitis media, intestine malrotation and with lower nNO, atelectasis in chest CT, obstructive ventilator dysfunction by pulmonary function and conductive hearing loss. Two compound heterozygous mutations in DNAAF2 were detected in both of the siblings, nonsense mutation c.156C>A and frameshift mutation c.177_178insA, and the c.177_178insA (p.E60Rfs*3) mutation is a novel mutation. **Conclusion**: The study enriches our knowledge of clinical manifestations and genetic information of PCD caused by DNAAF2.

Introduction

With an estimated incidence of one in 10,000–20,000 live births worldwide [1], primary ciliary dyskniesia (PCD) is a rare inherited autosomal recessive disease caused by impaired function of motile cilia, resulting in recurrent respiratory inflammation, bronchiectasis, sinusitis, otitis media, and neonatal respiratory distress. Approximately 50% of patients have situs inversus [2,8]. Besides the clinical features, nasal NO concentration, high speed video microscopy analysis (HSVMA) of cilia beat frequency and pattern, ciliary ultrastructure in cross section through transmission electron microscopy (TEM) are all helpful for the diagnosis of PCD[3]. Moreover, genetic testing can help to confirm the diagnosis of PCD. The pathogenic mechanism of PCD is cilia motility dysfunction, which depend on dynein arms composing of the outer dynein arms (ODAs) and the inner dynein arms (IDAs). The gene of dynein axonemal assembly factor 2 (DNAAF2), also known as KTU and PF13, has been reported to encode the cytoplasmic proteins required for the assembly of both ODAs and IDAs, lead to loss of cilia motility and cause PCD[6]. In this paper, we report the clinical manifestations,

diagnosis and treatment processes of two siblings with PCD caused by the same compound heterozygous mutations in DNAAF2, one of the mutations is novel.

Methods

After informed consent was obtained, we collected clinical histories, laboratory tests, bronchoscopy, otoscope images, and radiographic data of the two siblings. Identification of respiratory pathogens derived from cultured bronchoalveolar lavage specimens was conducted. Whole blood of the siblings and their parents were separately harvested for whole-exome sequencing and Sanger sequencing. Then ciliary ultrastructural defect was confirmed by Transmission Electron Microscopy (TEM).

We also searched the literature of all the DNAAF2 mutations since first reported. The clinical data of these patients were summarized.

Results

The probands, a 7-year-old girl and a 10-month-boy, were both born full term. Their family histories were not remarkable. The elder sister presented with wet cough and running nose for 2 months and had been diagnosed with pneumonia and treated for 15 days in a local hospital without improvement. Her computed tomography (CT) (Fig.1A, C) scanning of lungs and sinus showed atelectasis and potential bronchiectasis in the right middle lobe and sinusitis with hypertrophy of tonsils and adenoids. Before transferred to the respiratory medicine department of our hospital, she had suffered from recurrent respiratory tract infection for almost 5 years. On physical examination, pharyngeal congestion and swelling of tonsil were found, with normal growth and development. Her nasal nitric oxide concentration (nNO) was much lower (38.5ppb) than the PCD-specific nNO cutoff value (287ppb) [4]. While the pulmonary function tests were normal. During bronchoscopy, massive mucilage secretions were found in segments of lingula, left upper and right middle lobes (Fig.1B). The culture of bronchoalveolar lavage (BAL) showed positive for Haemophilus infuenzae (Hin). The pure-tone audiometry proved conductive hearing loss. The air-bone gaps were 10dB to 25dB for the right ear and 15dB to 35dB for the left one. The otoscope and inner ear CT indicated otitis media in both of her ears, which was responsible for the hearing loss (Fig.1D, E, F).

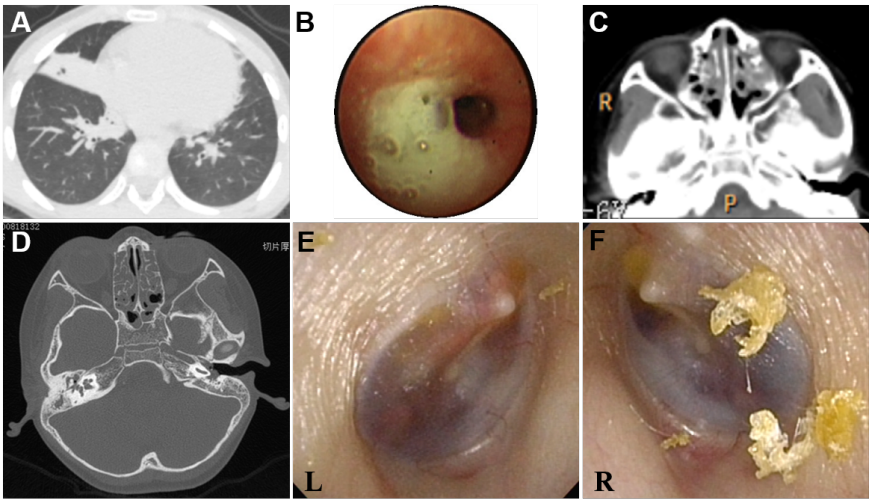

Figure 1 Clinical features of the elder sister. The chest high-resolution computed tomography (HRCT) scan showed bronchiectasis and atelectasis in the right middle lobe (A), sinusitis (C) and otitis media (D). Bronchoscopy indicated large amount of mucilage secretions in the right middle lobe. Otoscope findings of otitis media in left ear (E) and the right one (F).

Azithromycin was given to treat Hin and play the anti-inflammatory effect. Budesonide and acetylcysteine for inhalation were given for their anti-inflammation effect and to dilute sputum. As for pulmonary rehabilitation, mechanical vibration sputum expectoration, postural drainage and effective breathing techniques were performed to accelerate mucus clearance from the lungs. During follow-up, she had better symptoms but persistent atelectasis in right middle lung and recurrent sinusitis and otitis media.

The younger brother presented with cough and runny nose for one month. He was diagnosed with pneumonia in both lungs, atelectasis in lingular lobe, sinusitis, otitis media and hypertrophy of adenoids (Fig.2A-F) Physical examination found phlegm sounds by stethoscope, and his growth and development was normal. The pure-tone audiometry proved conductive hearing loss. The air-bone gaps were 40dB to 60dB for the right ear and 30dB to 55dB for the left one. Bilateral tympanotomy with tube placement and adenotomy were performed. His nNO was 32.8ppb, even lower than that of the elder sister. Pulmonary function tests showed obstructive ventilatory dysfunction. Transmission electron microscopy (TEM) of bronchial cilia exhibit complete loss of ODAs and IDAs (Fig.3). Besides, the echocardiography revealed residual left superior vena cava. With the history of emesis, he was diagnosed with intestine malrotation by abdominal ultrasound at 1.5 years old.

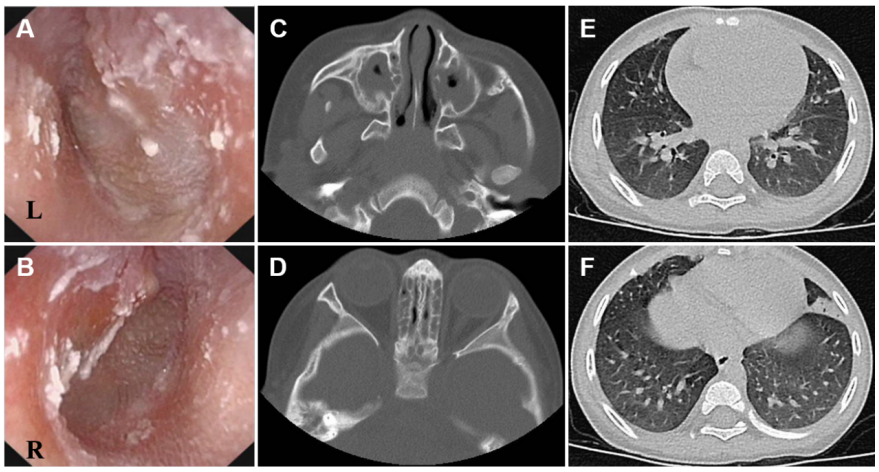

Figure 2 Clinical features of the younger brother. Otoscope findings of otitis media of left and right ears (A & B). The computed tomography (CT) scan of nasopharynx showed sinusitis (C&D). Chest HRCT scan illustrated atelectasis in lingular lobe (E&F).

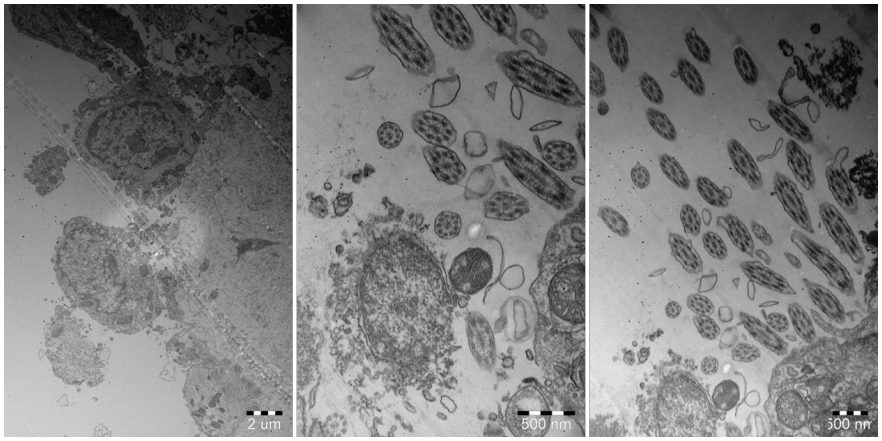

Figure 3 TEM analysis of bronchial mucosal biopsy specimens displayed complete loss of ODAs and IDAs.

After treated with azithromycin, budesonide inhalation solution, and adjuvant mechanical vibration sputum expectoration, he was discharged soon. He had better symptoms, better HRCT and well controlled sinusitis and otitis media during following-up.

Furthermore, both of the siblings did not have the histories of fatty diarrhea, malnutrition, meconium intestinal obstruction. Both of them had normal results of cellular and humoral immune function, normal blood amylase and lipase level, and negative results of tuberculosis infection test (T-SPOT. TB).

With the informed consent of the child's parents, 2ml whole blood of the siblings and their parents was separately collected for whole-exome sequencing (WES) and Sanger sequencing. Two compound heterozygous mutations in DNAAF2 were found in both siblings, one nonsense mutation of NM_018139: c.156C>A (p.Y52*) was inherited from the mother and one frameshift mutation NM_018139: c.177_178insA (p.E60Rfs*3) was inherited from the father (Fig.4 and 5). The mutation of c.177_178insA (p.E60Rfs*3) has not been reported before.

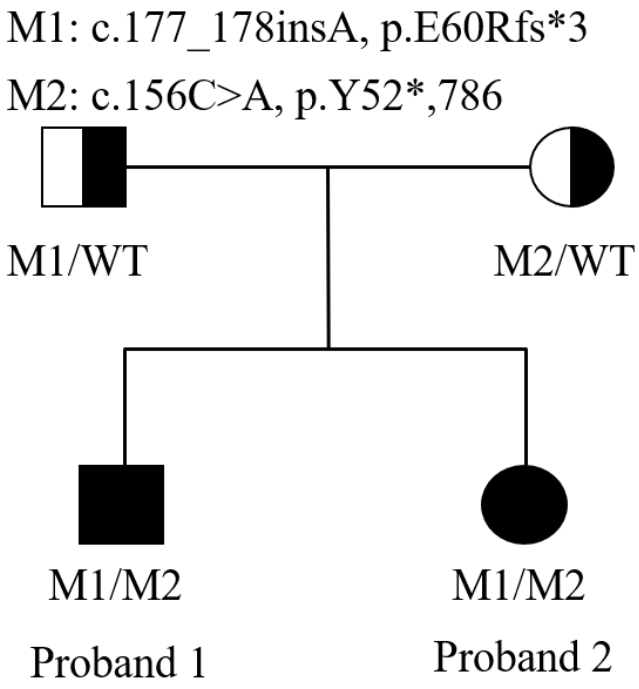

Figure 4 Pedigree of the family. Circles indicate females, squares indicate males, solid symbols indicate patients.

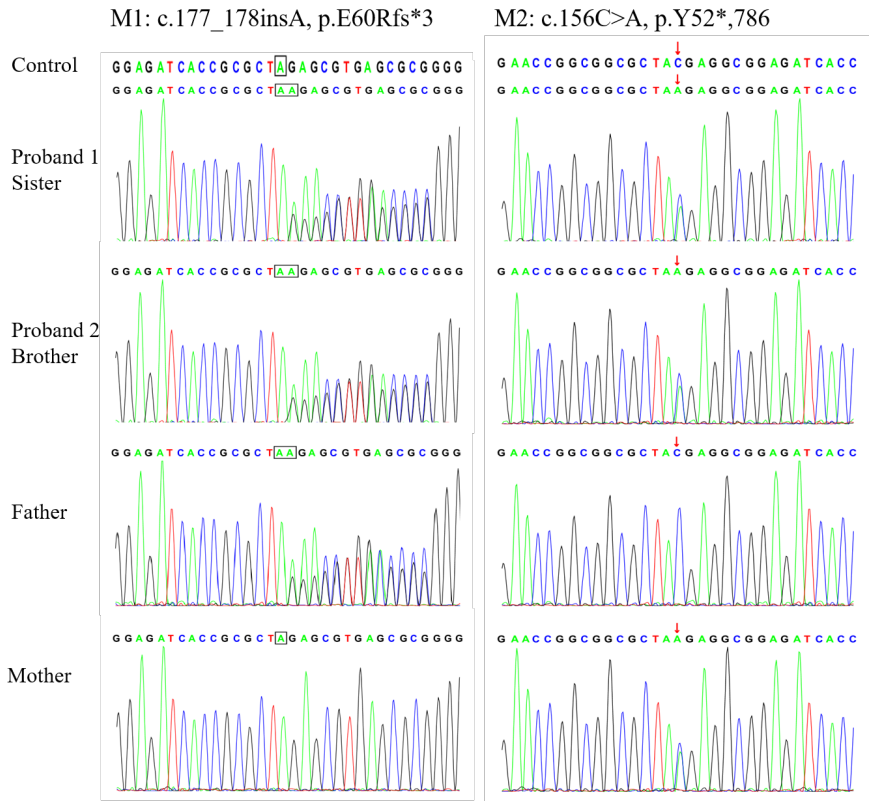

Figure 5 Sanger DNA sequencing chromatogram of probands and their parents. A novel mutation DNAAF2, c.177_178insA (p.E60Rfs*3) was identified in the probands, their father had a heterozygous mutation at the same location, whereas their mother did not. Another mutation DNAAF2, c.156C>A (p.Y52*,786) was also identified in both of the probands, their mother had a heterozygous mutation at the same location, whereas their father did not.

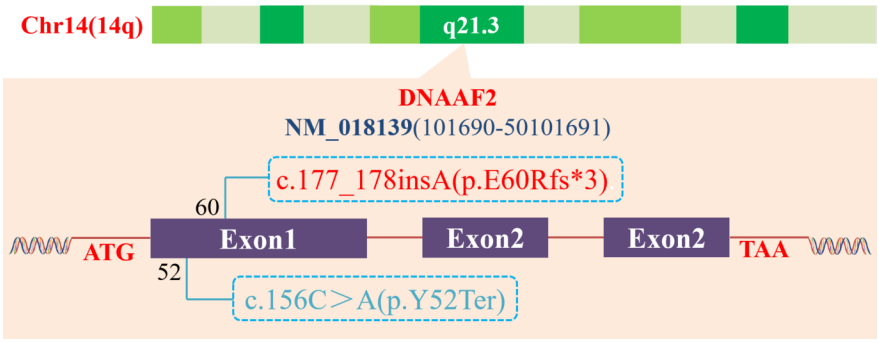

Figure 6 Mutations of DNAAF2 gene identified in the siblings with PCD. The mutation in red was newly found in this study.

cDNA change (Protein change)	Sex	Age(years)	Ethnicity	Clinical features	Ultrastructural defect
c.1160A>G(p.Glu387Gly)	F	-	Turkish	-	-
c.472G>T(p.Glu158Term)	F	7	-	VSD, Eisenmenger syndrome	-
c.23C>A(p.Ser8*)	M	-	-	Chronic otitis media and sinusitis, recurrent pneumonia, bronchiectasis, removed middle lobe, complete situs inversus, impaired fertility	ODA+IDA
c.1199_1214dupACGATACCTGCGTGGC (p.Gly406Argfs*89)	F	-	-	Chronic otitis media and sinusitis, recurrent pneumonia, bronchiectasis, complete situs inversus	ODA+IDA
c.564dupG(p.Lys189Glufs*5)	-	-	-	-	ODA+IDA
c.2147C>T(p.Ala716Val)	F	-	European	Developmental disorder	-
c.156C>A(p.Tyr52Term)	M	40	Asian	Recurrent bronchitis and pneumonia, chronic sinusitis, bronchiectasis, situs inversus, infertility	ODA+IDA
c.26C>A(p.Ser9Term)					
c.727G>T(Glu243Term)		-	Swedish	-	-
c.2027_2028delCT(p.S676Term)	-	-	-	-	-
c.962C>A(p.Ser321Term)	M	-	Greek-Cypriot	-	-
c.998C>T(p.Ala333Val)		-	Dutch	-	ODA+partial IDA
c.1901T>C(p.Phe634Ser)					

cDNA change (Protein change)	Sex	Age(years)	Ethnicity	Clinical features	Ultrastructural defect
c.796C>T(p.Gln266Term)	M	30	Asian	Kartagener Syndrom, producting cough, hemoptysis, naso-sinusitis, left-right laterality defects, azoospermia	-
c.1555delG(p.Glu519Lysfs)	M	-	-	-	-
c.1891G>A(p.Val631Ile)	M	-	-	Infetility	-
c.31delG(p.Glu11Argfs*5)	F	14	Italian	Neonatal RDS, bronchiectasis, sinusitis	ODA+IDA
c.491T>C (p.Leu164Pro)	F	26	Asian	Scoliosis, bronchiectasis, sinusitis, situsinversus and infertility	ODA
c.822del (p.Ala275Profs*10)	F	53	Asian	Bronchiectasis, sinusitis and infertility	-
c.177_178insA (p.E60Rfs*3) Novel	F	7	Asian	Recurrent respiratory tract infection, atelectasis, bronchiectasis, sinusitis and otitis media	-
	M	10/12	Asian	Pneumonia, atelectasis in lingular lobe, sinusitis, otitis media and hypertrophy of adenoids	ODA+IDA

Table 1 Cases of DNAAF2 mutations. RDS, respiratory distress syndrome; TEM, transmission electron microscopy; VSD, ventriculap septal defect; IDA, inner dynein arm; ODA, outer dynein arm; - not available.

Discussion

In this study, two compound heterozygous mutations of DNAAF2 were identified in two siblings with similar characteristic manifestations of PCD including recurrent pneumonia with atelectasis mainly in right middle and left lingular lobe, early bronchiectasis, sinusitis, otitis media, significant reduction in nNO level and complete loss of ODAs and IDAs of bronchial cilia found by the TEM. One of the mutations is a non-sense mutation of NM_018139: c.156C>A (p.Y52*) which has already been reported in a Chinese family and proved to be pathogenic[5]. Another mutation is a frameshift mutation (NM_018139: c.177_178insA (p.E60Rfs*3) , which is a novel mutation.

DNAAF2 is located on chromosome 14q21.3 and consists of three exons encoding cDNAs [6]. The mutation p.E60Rfs*3 locates in exon1 and near another mutation p.Y52*. Exon1 is consists of 621 amino acids, and the mutation of E60Rfs*3 leads to early transcription termination after the 63 amino acids (Fig.6). The p.E60Rfs*3 mutation has been predicted to be pathogenic with the evidences of PVS1+PM2+PM3+PP4 according to the standards and guidelines of American College of Medical Genetics and Genomics (ACMG). Multiple software, such as SIFT, Polyphen-2 and Mutation Taster, also predict the p.E60Rfs*3 mutation to be pathogenic. In addition, none of cystic fibrosis (CF) or primary immune deficiency (PID) related genes which may also lead to recurrent pneumonia, atelectasis and bronchiectasis were not detected. Based on the clinical manifestations and hereditary mode of compound heterozygous mutations from both parents, it's reasonable to highly suspected that the novel p.E60Rfs*3 mutation of DNAAF2 is a disease causing mutation and is responsible for the pathogenesis of PCD.

The movements of cilia can help to clear airway mucous, cerebrospinal fluid flow along the brain ventricular system, support the transport of the oocyte to the uterus, and male germ cell move along the female reproductive tract [6]. The normal structure of the cilia consists of the conserved basic 9+2 microtubule-based axonemes and other several functional modules[7]. Members of multi-subunit motor protein complexes, ODAs and IDAs are responsible for the generation and regulation of ciliary beating. Dynein arms are ATPase-based protein-complexes, via ATP hydrolyzed by dynein heavy chain, the paired microtubules slide relative to each other, leading to cilia curve over the deformed microtubules[8-9]. During the process, IDA causes the curvature of cilia, ODA accelerates active sliding of the outer microtubules, leading to effective propulsion produced by the cilia effectively paddling forward and reverting paddling backward. Appropriate rheological properties and the functional structure of cilia are the basis that maintain mucociliary clearance which is essential to the respiratory tract clearance defense[5].

Approximately 40 genes have been reported to be associated with PCD, including DNAH5, DNAH11, DNAI1, DNAI2, DNAL1, TXNDC3, DNAAF1, and DNAAF2 et al. [1, 6, 10-14]. In 2008, two heterozygous mutations c.C23A [pS8X] and c.1214-1215insACGATACCTGCGTGGC [p.G406Rfs89X mutations of DNAAF2 were first identified in two consanguineous families with PCD presented with recurrent respiratory tract infections, laterality defects and impaired fertility[6]. DNAAF2 works as a facilitator of dynein pre-assembly. The disfunction of DNAAF2 is associated with specific defects in the interaction of intermediate and heavy chains in the cytoplasm which leads to complete or partial loss of ODAs and IDAs and loss of motility[6]. After that, only 15 PCD cases have been reported caused by DNAFF2 with 19 mutations. The cases of DNAAF2 mutation are summarized in Tab.1[5-6, 15-27]. As for Chinese children, DNAFF2 gene is rare. As Guan et al reported reported in 81 Chinese children, genes with the highest incidence of mutations that caused PCD were DNAH11, followed by HYDIN, DNAH5, CCDC39, DNAH1 and CCNO, no DNAFF2 gene mutation was detected[28]. In Chinese adult patients, Sun et.at have identified two heterozygous mutations c.C156A [p.Y52X] and c.C26A [p.S9X] in the DNAAF2 gene which lead to the defect of outer dynein arms and inner dynein arms resulting in PCD with the manifestation of male infertility[5]. Lu C et.at detected another two heterozygous mutations c.491T>C [p.L164P] and c.822del [p.A275Profs*10] in two females presents with the bronchiectasis, sinusitis, situs inversus, and infertility, besides, the one with c.491T>C mutation had scoliosis[27].

Consequently, we made an early diagnosis of PCD in two siblings with a novel mutation of the rare DNAFF2 gene. The early stagy of PCD may be easily misdiagnosed as common pneumonia or sinusitis. When a child presented with recurrent pneumonia or atelectasis accompanied with recurrent sinusitis or otitis media, PCD should be considered. The characteristic HRCT manifestations including atelectasis in the right middle and left lingular lobe, thickened bronchial wall, and mild bronchiectasis can provide a diagnostic clue for PCD. Besides, nNO and TEM of bronchial cilia are useful for the further diagnosis , and genetic tests can help to confirm the diagnosis.

In conclusion, our study suggests that the p.E60Rfs*3 mutation of DNAAF2 gene is a novel disease causing mutation of PCD. This study enriches our knowledge of clinical manifestations and genetic information of PCD caused by DNAAF2.

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Conflicts of interest The authors declare that they have no competeing conflicts.

References

1. Mirra V, Werner C, Santamaria F. Primary Ciliary Dyskinesia: An Update on Clinical Aspects, Genetics, Diagnosis, and Future Treatment Strategies. Front Pediatr. 2017 Jun 9;5:135.
2. Kennedy MP, Omran H, Leigh MW, et al. Congenital heart disease and other heterotaxic defects in a large cohort of patients with primary ciliary dyskinesia. Circulation. 2007 Jun 5;115(22):2814-21.
3. Shapiro AJ, Dell SD, Gaston B, et al. Nasal Nitric Oxide Measurement in Primary Ciliary Dyskinesia. A Technical Paper on Standardized Testing Protocols. Ann Am Thorac Soc. 2020 Feb;17(2):e1-e12.
4. Zhang W, Li D, Wei S, et al. Whole-exome sequencing identifies a novel CCDC151 mutation, c.325G>T (p.E109X), in a patient with primary ciliary dyskinesia and situs inversus. J Hum Genet. 2019 Mar;64(3):249-252.
5. Sun M, Zhang Y, JiyunYang, et al. Novel compound heterozygous DNAAF2 mutations cause primary ciliary dyskinesia in a Han Chinese family. J Assist Reprod Genet. 2020 Sep;37(9):2159-2170.
6. Omran H, Kobayashi D, Olbrich H, et al. Ktu/PF13 is required for cytoplasmic pre-assembly of axonemal dyneins. Nature. 2008 Dec 4;456(7222):611-6.
7. Ibañez-Tallon I, Heintz N, Omran H. To beat or not to beat: roles of cilia in development and disease. Hum Mol Genet. 2003 Apr 1;12 Spec No 1:R27-35.
8. Takeuchi K, Kitano M, Ishinaga H, et al. Recent advances in primary ciliary dyskinesia. Auris Nasus Larynx. 2016 Jun;43(3):229-36.
9. Lucas JS, Davis SD, Omran H, Shoemark A. Primary ciliary dyskinesia in the genomics age. Lancet Respir Med. 2020 Feb;8(2):202-216.
10. Bartoloni L, Blouin JL, Pan Y, et al. Mutations in the DNAH11 (axonemal heavy chain dynein type 11) gene cause one form of situs inversus totalis and most likely primary ciliary dyskinesia. Proc Natl Acad Sci U S A. 2002 Aug 6;99(16):10282-6.
11. Pennarun G, Escudier E, Chapelin C, et al. Loss-of-function mutations in a human gene related to Chlamydomonas reinhardtii dynein IC78 result in primary ciliary dyskinesia. Am J Hum Genet. 1999 Dec;65(6):1508-19.
12. Mazor M, Alkrinawi S, Chalifa-Caspi V, et al. Primary ciliary dyskinesia caused by homozygous mutation in DNAL1, encoding dynein light chain 1. Am J Hum Genet. 2011 May 13;88(5):599-607.
13. Duriez B, Duquesnoy P, Escudier E, et al. 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 Feb 27;104(9):3336-41.
14. Duquesnoy P, Escudier E, Vincensini L, et al. 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 Dec;85(6):890-6.
15. Paff T, Kooi IE, Moutaouakil Y, et al. Diagnostic yield of a targeted gene panel in primary ciliary dyskinesia patients. Hum Mutat. 2018 May;39(5):653-665.
16. Emiralioğlu N, Taşkıran EZ, Koşukcu C, et al. Genotype and phenotype evaluation of patients with primary ciliary dyskinesia: First results from Turkey. Pediatr Pulmonol. 2020 Feb;55(2):383-393.
17. Zhu N, Welch CL, Wang J, et al. Rare variants in SOX17 are associated with pulmonary arterial hypertension with congenital heart disease. Genome Med. 2018 Jul 20;10(1):56.
18. Blanchon S, Legendre M, Bottier M, et al. Deep phenotyping, including quantitative ciliary beating parameters, and extensive genotyping in primary ciliary dyskinesia. J Med Genet. 2020 Apr;57(4):237-244.

19. Turner TN, Wilfert AB, Bakken TE, et al. Sex-Based Analysis of De Novo Variants in Neurodevelopmental Disorders. Am J Hum Genet. 2019 Dec 5;105(6):1274-1285.
20. Stranneheim H, Lagerstedt-Robinson K, Magnusson M, et al. Integration of whole genome sequencing into a healthcare setting: high diagnostic rates across multiple clinical entities in 3219 rare disease patients. Genome Med. 2021 Mar 17;13(1):40.
21. LaDuca H, Farwell KD, Vuong H, et al. Exome sequencing covers >98% of mutations identified on targeted next generation sequencing panels. PLoS One. 2017 Feb 2;12(2):e0170843.
22. Yiallouros PK, Kouis P, Kyriacou K, et al. Implementation of multigene panel NGS diagnosis in the national primary ciliary dyskinesia cohort of Cyprus: An island with a high disease prevalence. Hum Mutat. 2021 Jun;42(6):e62-e77.
23. Zhao X, Bian C, Liu K, et al. Clinical characteristics and genetic spectrum of 26 individuals of Chinese origin with primary ciliary dyskinesia. Orphanet J Rare Dis. 2021 Jul 1;16(1):293.
24. Capalbo A, Valero RA, Jimenez-Almazan J, et al. Optimizing clinical exome design and parallel gene-testing for recessive genetic conditions in preconception carrier screening: Translational research genomic data from 14,125 exomes. PLoS Genet. 2019 Oct 7;15(10):e1008409.
25. Alhathal N, Maddirevula S, Coskun S, et al. A genomics approach to male infertility. Genet Med. 2020 Dec;22(12):1967-1975.
26. Kim RH, A Hall D, Cutz E, et al. The role of molecular genetic analysis in the diagnosis of primary ciliary dyskinesia. Ann Am Thorac Soc. 2014 Mar;11(3):351-9.
27. Lu C, Yang D, Lei C, et al. Identification of Two Novel DNAAF2 Variants in Two Consanguineous Families with Primary Ciliary Dyskinesia. Pharmgenomics Pers Med. 2021 Nov 10;14:1415-1423.
28. Guan Y, Yang H, Yao X, et al. Clinical and Genetic Spectrum of Children With Primary Ciliary Dyskinesia in China. Chest. 2021 May;159(5):1768-1781.