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ORIGINAL ARTICLE

Novel Gene Variants Associated with Primary Ciliary Dyskinesia

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Abstract

Objectives To determine the demographic, clinical, and genetic profile of Turkish Caucasian PCD cases.

Methods Targeted next-generation sequencing (t-NGS) of 46 nuclear genes was performed in 21 unrelated PCD cases. Sanger sequencing confirmed of potentially disease-related variations, and genotype–phenotype correlations were evaluated.

Results Disease-related variations were identified in eight different genes (*CCDC39*, *CCDC40*, *CCDC151*, *DNAAF2*, *DNAAF4*, *DNAH11*, *HYDIN*, *RSPH4A*) in 52.4% (11/21) of the cases. The frequency of variations for *CCDC151*, *DNAH11*, and *DNAAF2* genes which were highly mutated genes in the cohort was 18% in 11 patients. Each of the remaining gene variations was detected once (9%) in different patients. The variants, p.R482fs*12 in *CCDC151*, p.E216* in *DNAAF2*, p.I317* in *DNAAF4*, p.L318P and p.R1865* in *DNAH11*, and p.N1505D and p.L1167P in *HYDIN* gene were identified as novel variations. Interestingly, varying phenotypic findings were identified even in patients with the same mutation, which once again confirmed that PCD has a high phenotypic heterogeneity and shows individual differences.

Conclusion This t-NGS panel is potentially helpful for exact and rapid identification of reported/novel PCD-disease–causing variants to establish the molecular diagnosis of ciliary diseases.

Keywords Primary ciliary dyskinesia · Targeted next-generation sequencing · Mutation analysis · Ciliary diseases

Introduction

Primary ciliary dyskinesia (PCD; OMIM #244400) is a rare genetic disorder characterized by the lack of activity or inactivity of the cilia resulting from the abnormality of ciliary structure or function [1]. PCD is inherited primarily as autosomal recessive, and rarely as an X-linked recessive pattern [2]. The prevalence of PCD has been estimated to range from 1 in 10,000 to 40,000 live births [3–6], and it increases especially in populations where consanguineous marriages are relatively common [1, 7]. Inactivation of ciliary movement particularly causes primarily chronic upper and lower respiratory tract infections and also infertility [6, 8, 9]. In addition, the inactivity of embryonic nodal cilia causes situs inversus, which is defined as the positioning of the internal organs in the opposite direction, in 50% of the patients [6].

The genetic etiology of the disease shows a heterogeneous structure. To date, more than 40 disease-related genes have been identified in the literature [10]. It has been updated that approximately 70% of the cases can carry mutations in PCD-related genes [10].

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The present study aimed to assess the prevalence of mutations in selected PCD-related genes to evaluate whether this approach can be useful as first-step genetic testing in PCD patients. For this, 46 nuclear genes related to PCD were screened by using targeted next-generation sequencing (t-NGS). Here, the highest molecular diagnosis rate at 52.4% for the Turkish Caucasian population is reported by using the authors' custom design clinical t-NGS panel, which also extends the genetic profile of Turkish pediatric PCD cases.

Materials and Methods

Clinical evaluations of pediatric patients suspected to have PCD were performed by following a clinical checklist which, included PCD-specific abnormalities observed in patients and/or transmission electron microscopy (TEM) findings. Unrelated 21 Turkish pediatric cases were selected for further genetic analysis. Inclusion criteria for PCD workup were determined according to European Respiratory Society guidelines. Patients having several of the following characteristics were included in the study: situs anomalies; persistent wet cough; persistent rhinitis; congenital cardiac defects; chronic middle ear disease with or without hearing loss; neonatal intensive care admittance; a history in term infants of neonatal upper and lower respiratory symptoms. PCD diagnosis criteria were having biallelic pathogenic variants in a PCD-associated gene and/or distinctive ciliary ultrastructural defect detected by TEM [9]. Initially, all patients were tested by dideoxy-sequencing analysis for hot-spot exons (exons #34, 50, 63, 76, 77) of *DNAH5* (NM_001369.2), which is mostly mutated in PCD cases and whole exons of *CFTR* (NM_000492.4) to exclude the cystic fibrosis. Patients who have the wild-type genotype for these genes were included in the study.

This study was approved by the Ethical Review Committee for the Protection of Human Subjects of Akdeniz University, Turkey (approval number 70904504/58). A written and signed informed consent was obtained from all patients included in the study or their parents.

The nasal mucosa samples were fixed in 4% phosphate-buffered glutaraldehyde for 2 h at room temperature. Samples were washed with 0.1% Sorenson Phosphate Buffer (SBP) three times for 10 min each, and fixed again with 1% osmium tetroxide for 2 h at room temperature. After dehydration, samples were cleared with propylene oxide and embedded into Araldite resin overnight. After polymerization thin and semithin sections were obtained from the plastic blocks. Sections were stained with uranyl acetate and analyzed using TEM (LEO 906E, Zeiss, Oberkochen, Germany). The defects in the structure of cilia were examined for the diagnosis of PCD.

Genomic DNA of the patients was isolated from peripheral blood samples by using the in-house, modified nonenzymatic method [11]. To investigate the possible genetic variations, a custom design On-Demand AmpliSeq panel (Thermo Fisher Scientific, MA, USA) was used, which includes 46 nuclear genes (Table 1) related to PCD. The wet-bench process was performed following the manufacturer's instructions and samples were sequenced in the Ion S5 System (Thermo Fisher Scientific, MA, USA). For aligning the sequence reads of the raw data to the hg19/GRCh37 reference genome, Torrent Mapping Alignment Program (TMAP; Ion Torrent Suite, Thermo Fisher, Carlsbad, CA, USA) was used. Mapping and variant calling were performed using Torrent Suite Software. The data were annotated and filtered with Ingenuity Variant Analysis (IVA; QIAGEN, CA, USA). Variants in the exonic, 5'and 3' untranslated regions (UTRs) and intronic regions close to exons and splice sites covering by the panel were analyzed. Variants in the deep intronic and noncoding regions, and synonymous changes were filtered out. In addition, variants listed in population databases like dbSNP, 1000 Genomes Project (1000G), and Exome Aggregation Consortium (ExAC) browser with > 1% for minor allele frequency (MAF) were excluded. Following the examination of remaining variants, dideoxy-sequencing analyses were performed using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, USA) in the ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Carlsbad, USA) to verify the detected variants (Table 2) related to the PCD phenotype of the patients. Variants that were not previously reported in any of the various databases, such as dbSNP, HGMD, LOVD, ClinVaror Exome Variant Server, were identified as "novel".

In silico predictions of the pathogenicity of the novel variants were performed using Mutation Taster (http://www.mutationtaster.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and SIFT (https://sift.bii.a-star.edu.sg) tools. Evolutionary conservation of regions harboring the mutations was examined by comparing these regions of the genome inhuman, primates, and varying species in UCSC Genome Browser (https://genome.ucsc.edu/cgi-bin/hgGateway). Conservation rates of the amino acids were estimated by using ConSurf Web Server (https://consurf.tau.ac.il). Color grades from 1 to 9 obtained through the ConSurf Server represent the relative degree of conservation (conservation score) of each amino acid position (1–4 variable, 5–6 average, and 7–9 conserved). Detailed information is given in Fig. 1.

Results

In this study, the clinical and genetic characteristics of a pediatric PCD population of 21 individuals in Antalya, Turkey are reported. This is the second genetic characterization

Table 1 Gene list included in clinical t-NGS panel

Gene name	Gene symbol	Chromosomal location	cDNA accession number	EXON #
Chromosome 21 open reading frame 59	*C21orf59 (CFAP298)*	21q22.11	NM_021254.2	7
Coiled-coil domain containing 103	*CCDC103*	17q21.31	NM_213607.2	4
Coiled-coil domain containing 114	*CCDC114*	19q13.33	NM_144577.3	19
Coiled-coil domain containing 151	*CCDC151*	19p13.2	NM_145045.5	14
Coiled-coil domain containing 39	*CCDC39*	3q26.33	NM_181426.1	20
Coiled-coil domain containing 40	*CCDC40*	17q25.3	NM_017950.4	29
Coiled-coil domain containing 65	*CCDC65*	12q13.12	NM_033124.4	8
Cyclin O	*CCNO*	5q11.2	NM_021147.4	3
Centrosomal protein 290	*CEP290 (NPHP6)*	12q21.32	NM_025114.3	60
Dynein axonemal assembly factor 1	*DNAAF1 (LRRC50)*	16q24.1	NM_178452.5	15
Dynein axonemal assembly factor 2	*DNAAF2 (KTU)*	14q21.3	NM_018139.3	3
Dynein axonemal assembly factor 3	*DNAAF3 (PF22/C19orf51)*	19q13.42	NM_001256714.1	12
Dynein axonemal assembly factor 4	*DNAAF4 (DYX1C1)*	15q21.3	NM_130810.4	11
Dynein axonemal heavy chain 11	*DNAH11*	7p15.3	NM_001277115.2	82
Dynein axonemal heavy chain 5	*DNAH5*	5p15.2	NM_001369.2	86
Dynein axonemal heavy chain 7	*DNAH7*	2q32.3	NM_018897.2	69
Dynein axonemal heavy chain 9	*DNAH9*	17p12	NM_001372.3	73
Dynein axonemal intermediate chain 1	*DNAI1*	9p13.3	NM_012144.3	24
Dynein axonemal intermediate chain 2	*DNAI2*	17q25.1	NM_023036.5	17
Dynein axonemal light chain 1	*DNAL1*	14q24.3	NM_031427.3	10
Dynein regulatory complex subunit 1	*DRC1 (CCDC164)*	2p23.3	NM_145038.4	17
Intraflagellar transport 140	*IFT140*	16p13.3	NM_014714.3	40
Intraflagellar transport 172	*IFT172*	2p23.3	NM_015662.2	52
Intraflagellar transport 80	*IFT80*	3q25.33	NM_020800.2	21
Intraflagellar transport 88	*IFT88*	13q12.11	NM_175605.4	33
Inversin	*INVS*	9q31.1	NM_014425.4	18
Kinesin family member 3A	*KIF3A*	5q31.1	NM_001300791.1	20
Kinesin-associated protein 3	*KIFAP3 (KAP3)*	1q24.2	NM_014970.3	24
Leucine rich repeat containing 6	*LRRC6*	8q24.22	NM_012472.5	17
OFD1, centriole and centriolar satellite protein	*OFD1*	Xp22.2	NM_003611.2	27
RPGRIP1 like	*RPGRIP1L (KIAA1005)*	16q12.2	NM_015272.4	37
Radial spoke head 1 homolog	*RSPH1*	21q22.3	NM_080860.3	9
Radial spoke head 4 homolog A	*RSPH4A*	6q22.1	NM_001010892.3	7
Radial spoke head 9 homolog	*RSPH9*	6p21.1	NM_001193341.1	7
Sperm associated antigen 1	*SPAG1*	8q22.2	NM_003114.4	21
T-complex-associated-testis-expressed 3	*TCTE3*	6q27	NM_174910.2	5
Transmembrane protein 216	*TMEM216*	11q12.2	NM_016499.5	6
Transmembrane protein 67	*TMEM67*	8q22.1	NM_153704.5	35
Zinc finger MYND-type containing 10	*ZMYND10*	3p21.31	NM_015896.3	12
Sperm-associated antigen 17	*SPAG17*	1p12	NM_206996.4	56
Dynein axonemal heavy chain 1	*DNAH1*	3p21.1	NM_015512.5	80
Dynein axonemal heavy chain 8	*DNAH8*	6p21.2	NM_001206927.2	97
Radial spoke head 3 homolog	*RSPH3*	6q25.3	NM_031924.6	11
Armadillo repeat containing 4	*ARMC4*	10p12.1	NM_001290020.2	30
HYDIN, axonemal central pair apparatus protein	*HYDIN*	16q22.2	NM_001270974.2	92
Growth arrest specific 8	*GAS8*	16q24.3	NM_001481.2	15

Table 2 Detected mutations and primers used in dideoxy sequencing for verification

Gene	Nucleotide change	Protein change	Mutation type	Exon #	Forward primer (5'->3')	Reverse primer (5'->3')	$MgCl_2$ (mM)	Tm °C
CCDC39	c.151C>T	p.R51*	Nonsense	2	AGCTGTAAA AATCGCAGA TG	TTCTTTGAT TTTAGGTCC TTCA	2	55
CCDC40	c.961C>T	p.R321*	Nonsense	7	CTGGTGTGT ATCCGTCCA GT	GCCTTCCCC TCCTAA AAAG	2	55
CCDC151	c.1445_1446delGC	p.R482fs*12	Frameshift	11	CTGCACCTC TTCTGGTTT CT	TATCTGCAG TTCTGGAGC AG	2	55
DNAAF2	c.646G>T, c.1199_1214dupACG ATACCTGCG TGGC	p.E216*; p.G406fs*90	Nonsense; frameshift	1	GCCACCAGG TGAGTT TTCT	AGACCCTGA AGGCCAAGT AT	2	55
DNAAF4 (DYX1C1)	c.948delC	p.I317*	Nonsense	8	CACCAGTTC CTTGTCCAT TAG	GGCAACGTT TACCCTAAA GA	2	55
DNAH11	c.953T>C	p.L318P	Missense	5	TATTTTCAA CTGGAAACC AGATA	CCATTTTGG CAGGTACTA CTAT	2	55
DNAH11	c.5593C>T	p.R1865*	Nonsense	32	AGCGTGCAA GAGATTTCC TA	TCATCGTTT CCTCTCATT CC	2	55
HYDIN	c.3500T>C	p.L1167P	Missense	23	GTGCTGGGG GTACCTTAT CT	GTTTCATGT ACCAGG GTGGA	2	55
HYDIN	c.4513A>G	p.N1505D	Missense	30	TGCACACAA CTCCTATGC AC	CACTTCCCT TCCCACCTT AC	2	55
RSPH4A	c.1105G>C	p.A369P	Missense	3	TGAAAAAGA CAAGGG AAGCA	CTGCATTTT TGGCTGTGT TT	2	55

study of a pediatric group of PCD patients in Turkey. The mean age of the cohort was approximately 6 ± 3.35 y. The girl-to-boy ratio of the present cohort was 1.3:1. It is also worth noting that 47.6% of the patients had parental consanguinity.

According to the clinical examinations, the most common diagnoses are sinusitis (90.5%), pneumonia (76.2%), otitis media with effusion (66.6%), situs inversus (62%), dextrocardia (62%), bronchiectasis (62%), hearing loss (57%), asthma (57%), and neonatal respiratory distress (28.5%).

None of the patients included in the study had any mutations in *CFTR* or in hot-spot exons of *DNAH5* genes. The NGS chip generated approximately 20.5 million total reads and 19.9 million of them (99.9%) were aligned. The mean read length of the chip was approximately 204 bp and the mean length of the alignment was 199 bp with a mean coverage depth of 1.3. The mean target base coverage at 100X was 97.7% and the mean uniformity of base coverage was 98%. As a result of t-NGS analysis in 21 unrelated pediatric cases, 11 different disease-causing variants were identified; of which, 4 were already reported [12–16] and 7 are novel alleles, in 8 different genes in 11 cases (52.4%). All of these variants were confirmed by dideoxy-sequencing analysis (Supplemantary Fig. S1). Detected mutations and detailed polymerase chain reaction conditions are given in Table 2. The status of the detected mutations in gene-variation databases, in silico analysis of novel ones, and their effects on ciliary functions are listed in Table 3.

Phenotypic profiles with TEM findings of the mutation-positive patients are given in Table 4. Sufficient cilia to interpret could not be obtained from 4 of these patients.

In silico analysis for predicting pathogenicity of novel variants revealed that the variants were all potentially associated with the PCD phenotype except for the c.4513A>G(p.N1505D) variation in the *HYDIN* gene (Table 3). According to the evolutionary conservation analysis, p.N1505D is a nonsynonymous variation affecting a moderately conserved amino acid (Cons. Score: 6) (Fig. 1g). Other nonsynonymous variations, c.953 T>C(p.L318P) in *DNAH11* and c.3500 T>C (p.L1167P) in *HYDIN* were shown to affect

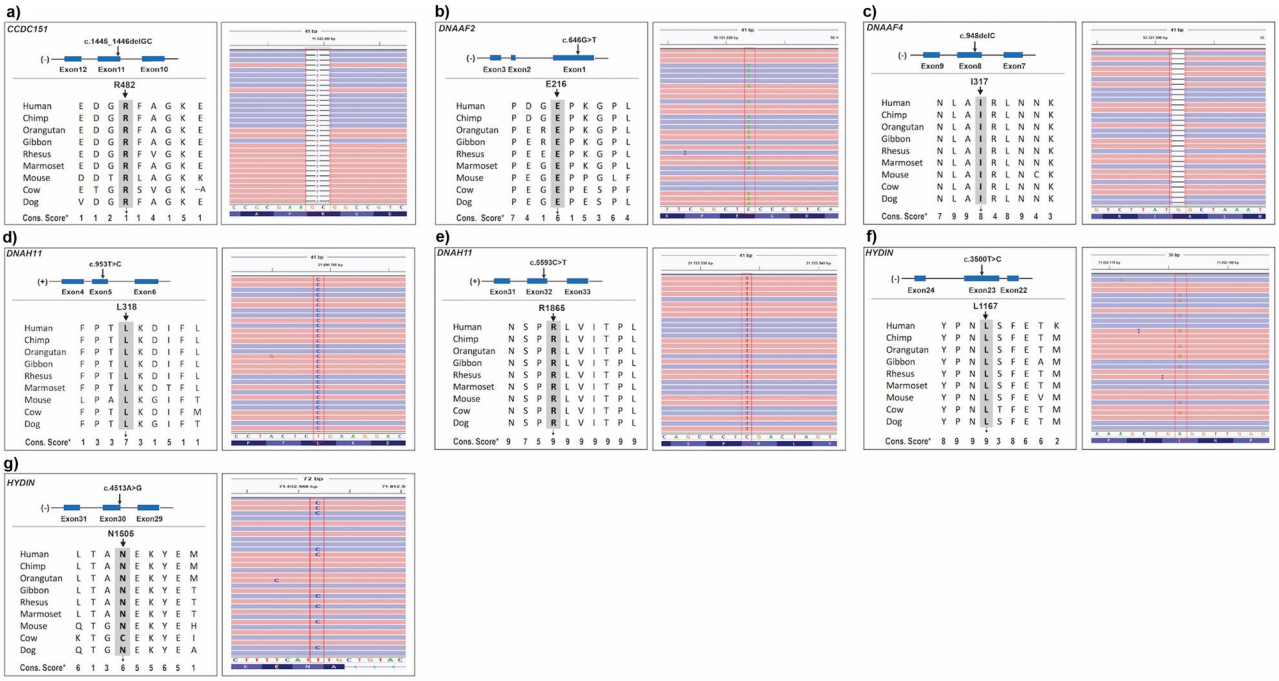

Fig. 1 Evolutionary conservation analysis of novel variations with amino-acid sequence alignments with conservation scores (*Cons. Score), locations of the mutations within the related genes and integrative genomics viewer (IGV) images. **(a)** *CCDC151*; p.R482fs*12 **(b)** *DNAAF2*; p.E216* **(c)** *DNAAF4*; p.I317* **(d)** *DNAH11*; p.L318P **(e)** *DNAH11*; p.R1865* **(f)** *HYDIN*; p.L1167P **(g)** *HYDIN*; p.N1505D

highly conserved amino acids with conservation scores of 9 (Fig. 1d, f). It was shown that the p.E216-*DNAAF2* residue is moderately conserved, and p.I317-*DNAAF4* and p.R1865-*DNAH11* residues are highly conserved amino acids (Fig. 1 b, c, and e). The first amino acid in the shifted region of c.1445_1446delGC(p.R482fs*12) mutation in *CCDC151* is not conserved between various species (Cons Score: 1), but is conserved throughout the 9 given vertebrates (Fig. 1a).

Discussion

Since its first introduction into clinical practices, NGS has become widely used as an emerging diagnostic tool, especially for the diagnosis of complex disorders [17]. As a genetically heterogeneous disorder, PCD does not show any racial or sexual predilection [18]. In addition, not only can different mutations in the same PCD-related gene result in disease phenotypic variability [18], but also the same mutations in two different patients may show phenotypic differences. To date, mutations in *DNAH5* (15%–21%), *DNAI1* (2%–9%), *DNAAF1* (4%–5%), *CCDC39* (2%–19%), *CCDC40* (2%–8%), *DNAH11* (6%), and *LRRC6* (3%) genes have been detected more commonly in PCD cases [12].

As a result of this study, 7 novel variations were detected. Six of these variants meet at least 4 of ACMG criteria (PVS1, PM2, PP3, PP4) for classifying pathogenic variants [19], except for c.4513A>G(p.N1505D) in the *HYDIN* gene that can be classified as a variant of unknown significance (VUS).

In a study from Italy, the molecular diagnosis rate was recorded as 43% of the PCD cases ($n=51$) by using a custom design panel for sequencing 24 PCD-related genes [20]. Based on their study, the highly mutated genes were reported as *DNAH5* (26.3%), *DNAH11* (15.8%), *LRRC6* (10.5%), and *ARMC4* (10.5%). The major clinical findings of the cohort were reported as situs solitus (57%), neonatal respiratory distress (33%), recurrent pneumonia (72%), bronchiectasis (64%), and sinusitis (55%) [20]. Takeuchi and colleagues also performed exome sequencing of 32 known PCD genes and found mutations in 10 Japanese patients (22%). According to this study, *DNAH5* was reported as the highly mutated gene, the other mutated genes identified in the cohort were *DNAI1*, *CCDC40*, and *RSPH4A* [21].

Recently, in Turkey, Emiralioglu and colleagues performed whole-exome sequencing analysis on a cohort of 265 patients with PCD. The researchers identified mutations in 11 known PCD genes (*DNAH5, CCDC40, RSPH4A, DNAH11, HYDIN, CCNO, DNAI1, ARMC4, TTC25, DNAH1,* and *CCDC39*) in 17.4% of the cases [22]. Interestingly, a pathogenic variant of the *DNAH5* gene was found most commonly (26.1%) in the study by Emiralioglu et al. Although, they were patients from the same geographic region as the patients in the present study, *DNAH5*

Table 3 Information about the detected mutations, in silico analysis of novel variants, and their functional effects on ciliary functions

Case #	Gene	Mutation	HGMD status (novel/reported)	LOVD status	Reference	MutationTaster	PolyPhen	SIFT	Functional effect
08	*CCDC39*	p.R51*/p.R51*	Reported	Reported	Antony et al. [12]				Defects of the 96 nm ruler machine
09	*CCDC40*	p.R321*/p.R321*	Reported	Reported	Becker-Heck et al. [13], Xiong et al. [14]				Defects of the 96 nm ruler machine
16	*CCDC151*	p.R482fs*12/p.R482fs*12	**Novel**	**Novel**		Disease causing	n/a	n/a	Defects in outer dynein arm docking and targeting
18	*CCDC151*	p.R482fs*12/p.R482fs*12	**Novel**	**Novel**		Disease causing	n/a	n/a	Defects in outer dynein arm docking and targeting
05	*DNAAF2*	p.E216*/p.E216*	**Novel**	**Novel**		Disease causing	n/a	n/a	Defects in cytoplasmic preassembly of dynein arms
06	*DNAAF2*	p.E216*/p.G406fs*90	reported - p.G406fs*90	**Novel**	Omran et al. [15]	p.E216*: Disease causing	n/a	n/a	Defects in cytoplasmic preassembly of dynein arms
12	*DNAAF4*	p.I317*/p.I317*	**Novel**	**Novel**		Disease causing	n/a	n/a	Defects in cytoplasmic preassembly of dynein arms
04	*DNAH11*	p.L318P/p.L318P	**Novel**	**Novel**		Disease causing	Probably damaging (Score: 0.989)	p.L1167P: Damaging (Score: 0.000)	Defects of outer dynein arm composure
21	*DNAH11*	p.R1865*/p.R1865*	**Novel**	**Novel**		Disease causing	n/a	n/a	Defects of outer dynein arm composure
02	*HYDIN*	p.N1505D/p.L1167P	**Novel (both)**	**Novel**		p.N1505D: Polymorphism p.L1167P: Disease causing	p.N1505D: Benign (Score: 0.483) p.L1167P: Probably damaging (Score: 1.000)	p.N1505D: Tolerated (Score:0.539) p.L1167P: Damaging (Score: 0.000)	Defects in central pair associated proteins
10	*RSPH4A*	p.A369P/p.A369P	Reported	**Novel**	Frommer et al. [16]				Defects in radial spoke components

HGMD Human Gene Mutation Database; *LOVD* Leiden Open Variation Database; *n/a* Not available; *SIFT* Sorting Intolerant From Tolerant program

Table 4 Phenotypic profiles of mutation-detected PCD patients

	Case #										
Anomalies	02	04	05	06	08	09	10	12	16	18	21
Perinatal history	+	−	+	−	+	−	−	−	−	n/a	+
Kartagener syndrome	−	+	+	+	+	+	−	+	+	+	+
Situs inversus	+	+	+	+	+	+	−	+	+	+	+
Dextrocardia	+	+	+	+	+	+	−	+	+	+	+
Wet cough	+	+	+	+	+	+	+	+	+	+	+
Pneumonia	−	+	+	+	+	+	+	+	+	+	+
Chronic rhinosinusitis	+	+	+	+	+	+	+	+	+	+	+
Asthma	−	+	−	+	+	+	+	+	+	−	−
Bronchiectasis	−	+	+	+	+	+	−	+	+	+	+
Otitis media with effusion	+	+	+	+	−	−	+	+	+	−	−
Hearing loss	+	+	−	+	−	−	+	+	+	+	−
Recurrent infections of the lower respiratory tract	+	+	+	+	+	+	+	+	+	+	+
Neonatal respiratory distress	−	−	+	+	+	−	−	+	+	+	−
TEM findings	Central pair defect	Normal ultrastructure	Inner dynein arm with microtubular disorganisation	n/a	n/a	Isolated outer dynein arm defect	Isolated outer dynein arm defect	Isolated outer dynein arm defect	n/a	Central pair defect	n/a
Videomicroscopy findings	−	n/a	+	+	+	n/a	+	n/a	+	n/a	+
Demografical findings											
Sex	F	M	F	M	M	F	F	F	F	F	M
Consanguineous marriage	−	+	+	+	+	+	+	+	+	+	+
NGS-panel analysis											
Gene	*HYDIN*	*DNAH11*	*DNAAF2*	*DNAAF2*	*CCDC39*	*CCDC40*	*RSPH4A*	*DNAAF4*	*CCDC151*	*CCDC151*	*DNAH11*

Table 4 (continued)

Anomalies	Case #												
	02	04	05	06	08	09	10	12	16	18	21		
Genotype	p.N1505D/	p.L318P/	p.E216*/	p.E216*/	p.R51*/	p.R321*/	p.A369P/	p.I317*/	p.R482fs*12/	p.R482fs*12/	p.R1865*/		
	p.L1167P	p.L318P	p.E216*	p.G406fs*90	p.R51*	p.R321*	p.A369P	p.I317*	p.R482fs*12	p.R482fs*12	p.R1865*		

(+) present; (−) absent

F Female; *M* Male; *n/a* Not available; *NGS* Next-generation sequencing; *TEM* Transmission electron microscopy

mutations were not detected in any of the patients in the present study. This might be due to a lower number of patients in the present cohort [22].

Disease-related variations were found in 52.4% of the cases by using the authors' custom design t-NGS panel, and as far as the authors know, this is one of the highest diagnostic rates for PCD cases. In spite of the higher rate of mutation-positive patients in the present cohort, genetic studies should be done in more patients with PCD to make an Anatolian region-spesific NGS panel. No pathogenic variation was found in 47.6% of the patients in the present study. Although intronic regions were analyzed in proximity (< 50 bp) to exons covering by the panel, it should be kept in mind that deep intronic or promoter region variations may be related to the disease. Epigenetic regulations and/or variations of other genes not included in the present t-NGS panel may also play roles in the development of PCD disease. Any large DNA deletions were not detected. Large heterozygous deletions might be missed due to the limitation of the t-NGS technique. Alternatively, microarray-based genomic copy number variation analysis can be performed to detect large deletions in patients with no mutations identified using NGS.

The highly mutated genes in the present cohort were detected as *CCDC151* (9.5%), *DNAAF2* (9.5%), and *DNAH11* (9.5%), which were related to the defects in outer dynein arms. The mutation-positive patients in the present study shared similar clinical features. Among these, situs inversus, dextrocardia, and pneumonia were observed in 91% of the mutation-positive cases, bronchiectasis was observed in 82%, and asthma and otitis media with effusion (OME), and hearing loss were observed in 64% of the cases with pathogenic mutations (Table 4). Wet cough and chronic rhinosinusitis were the common clinical features observed in all of the patients with mutations.

A novel c.646G>T(p.E216*) variant in *DNAAF2* was identified in two of the patients (#05, #06) and although they were not related, both families were from the same region of Anatolia, suggesting a subpopulation in which founder mutations can also occur in PCD cases. Since case #06 carries this variant as monoallelic, the clinical features of the patients may show different phenotypical findings (Table 4).

The other novel c.1445_1446delGC(p.R482fs*12) variant in *CCDC151* was also identified in 2 patients (#16, #18) in a homozygous state who are unrelated but from the same region of Anatolia, Turkey. It can be seen that these patients share more or less the same phenotypic features except for asthma and OME. This result makes one wonder if some other mechanisms, such as epigenetic control, may also play a key role in forming some clinical features, such as asthma. On the other hand, a relatively higher prevalence of two novel variations (p.E216* variant in *DNAAF2*, p.R482fs*12 variant in *CCDC151*) does show

evidence demonstrating the association of these variants with the disease phenotype [19].

As a result of the t-NGS–panel analysis, two novel biallelic mutations [c.953T>C (p.L318P); c.5593C>T(p.R1865*)] were identified in *DNAH11* in 2 unrelated patients (#04, #21). Based on the comparison of the genotype–phenotype relationship, both of the patients share the same phenotypic features except perinatal history, asthma, OME, and hearing loss.

The novel p.N1505D variant in the *HYDIN* gene has been predicted as a polymorphism in MutationTaster, benign by PolyPhen-2. Even if a novel variation is predicted to be benign by in silico analysis methods, additional research such as functional studies should be done to determine its pathogenicity. On the other hand, the patient (#02) harboring the variant p.N1505D had another variation in the *HYDIN* gene (p.L1167P) which was predicted to be damaging. The combined effect of these variations could cause the development of PCD phenotype. Previously, Xia et al. also reported a patient with situs abnormality having benign and damaging variations in the compound heterozgous state in the *DNAH11* gene [23]. Apart from these, patient #02 may have a heterozygous deletion in the *HYDIN* gene that might be missed due to the limitations of the NGS method.

Conclusions

As PCD is underdiagnosed in Turkey because of the variability of clinical and genetic heterogeneity, t-NGS panels should be used to reach a more reliable conclusion in larger patient cohorts. Taken together, the results of this study demonstrate that this t-NGS panel revealed reported/novel PCD-disease–causing variants with a high diagnostic rate. In addition, this t-NGS panel can be helpful for the exact and rapid identification of PCD-disease–causing variants. It is suggested that using t-NGS should be the first step in genetic analysis for early molecular diagnosis of the PCD disease.

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Authors' Contributions DDE, EY, and OMA designed the study; DDE, EY, GE performed molecular genetic studies; AEB, BN, EM, and AB did patient evaluation; EY, DDE, GE, and OMA performed data analyses; EY, DDE, and OMA wrote the paper. OMA will act as the guarantor for this paper.

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Data Availability On request.

Declarations

Ethics Approval All procedures carried out in the study comply with the ethical standards of the institutional and/or national research ethics committee [protocol number 70904504/58 dated Feb 02, 2015] and the 1964 Helsinki Declaration and its subsequent changes or comparable standards of ethics.

Consent to Participate Informed consent was obtained from all individuals included in this study.

Conflict of Interest None.

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