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RESEARCH ARTICLE

Evaluation of the Clinical and Genetic Characteristics of Primary Ciliary Dyskinesia Patients With Situs Inversus Totalis

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ABSTRACT

Background: Situs inversus totalis (SIT) is present in approximately 40%–50% of patients with primary ciliary dyskinesia (PCD). We evaluated the relationships between novel genetic results and the clinical and radiological characteristics of PCD patients with SIT.

Methods: The study included 48 patients diagnosed with PCD and SIT. Demographic and clinical features, disease-related scores (Bhalla, Primary Ciliary Dyskinesia Rule [PICADAR], and American Thoracic Society [ATS]), and genetic analyses were retrospectively assessed.

Results: The median age of patients was 13 (6.5–16) years, and parental consanguinity was observed in 43 (89.58%) patients. Bhalla score was available in 31 patients and "moderate and severe" score was observed in 19 (61.29%) patients. The median PICADAR score was 10 (8–11), and 34 (70.83%) patients had a high (≥ 10) PICADAR score. The ATS score was found to be 4 in 24 (50%) patients and 3 in 20 (43.75%) patients. Genetic data were available in 40 patients and mutations were found in 27 (67.5%) patients. The most common pathogenic variants were DNAH5 in 8 (20%), CCDC103 in 4 (10%), and CCDC39 in 3 (7.5%) patients. Subjects with any genetic variants may be older, have a greater frequency of parental consanguinity, higher Bhalla score, and higher ATS score ($p < 0.05$). DNAH5 mutation was associated with a lower likelihood of neonatal ICU stay and neonatal respiratory distress-related symptoms ($p = 0.036$ and 0.015, respectively).

Conclusions: Situs abnormalities may be a warning sign for the early diagnosis of PCD. Early diagnosis of PCD through genetic analysis is important for preventing chronic lung pathologies and predicting prognosis and may improve the quality of life.

1 | Introduction

Primary ciliary dyskinesia (PCD) is a rare disorder with genetic and clinical heterogeneity. It is characterized by inherited abnormalities that alter both the structure and the function of the motile cilia, resulting in a wide range of clinical manifestations, such as persistent or recurrent sinorespiratory tract infections, organ laterality disorders, and fertility problems (Raidt

et al. 2023). In approximately 50% of patients with PCD, the dysfunction of embryonic nodal cilia can lead to situs inversus (SI), a condition in which the lateral positioning of the thoracic and abdominal organs is reversed (Burwick, Govindappagari, and Sanchez-Lara 2021). The prevalence of PCD in the general populations of Europe and Asia is reported to vary between 1 in 10,000 and 40,000 live births, which may be an underestimation due to low awareness and nonspecific symptoms (or asymptomatic nature) (Asfuroglu et al. 2021). Incidence is reportedly higher in societies where consanguineous marriage is common (Best et al. 2019). Although PCD symptoms and findings may appear in the neonatal period and early infancy, diagnosis is often established later in life. Interestingly, the presence of SI is reported to allow earlier diagnosis (Coren et al. 2002). The European Respiratory Society and American Thoracic Society have reported evidence-based guidelines for the diagnosis of PCD, both recommending a combination of tests including high-speed video analysis of ciliary beat pattern, electron microscopy, nasal nitric oxide measurement, immunofluorescence staining of ciliary proteins, and genetic studies (Bal Topçu et al. 2020; Lucas et al. 2017; Shapiro et al. 2018). However, the costs and various technical difficulties limit the widespread use of these tests.

Early diagnosis and management in PCD may limit the progression of chronic lung disease and may positively impact quality of life. In recent years, there has been increasing focus in performing genetic analysis as the first method to confirm PCD diagnosis in individuals with a compatible PCD phenotype. To date, more than 50 disease-causing gene mutations have been identified (Bal Topçu et al. 2020; Lucas et al. 2017; Shapiro et al. 2018), but there are limited data concerning the relationships between genetic findings and the several hallmarks of the disease, including situs inversus totalis (SIT). Analyzing genetic data with respect to clinical findings may uncover new causative mutations, and possibly, could identify specific mutations or variations that explain the clinical heterogeneity of PCD and certain phenotypic features such as SIT. Using improved genetic testing tools such as whole exome sequencing may improve data in this regard (Demir Eksi et al. 2022).

Utilizing the increased availability of genetic testing, we sought to assess genotype/phenotype correlations to obtain data that may be transformative in the characterization and management of PCD. Thus, the aim of this study was to investigate genetic findings and identify associations with the clinical, laboratory, and radiological characteristics of PCD patients with SIT.

2 | Methods

2.1 | Setting, Design, and Participants

The study was designed as a single-center, retrospective cohort study and conducted between January 2018 and December 2020 in the Department of Pediatric Pulmonology of Bezmialem University, Istanbul, Turkey. The study initially included 66 patients with SIT, and patients without PCD findings were excluded from the study in later examinations, and the study was conducted on the remaining 48 patients. Although important methods for PCD diagnosis, such as electron video microscopy and nasal nitric oxide test, are not available in our center, patients were diagnosed with PCD according to the descriptive criteria of the guidelines by evaluating anamnesis, family history, physical examination, as well as laboratory, radiological, and/or genetic results. Eight patients without genetic results were included in the study because they had a clinical or radiological diagnosis of PCD. The diagnosis of SIT was performed by chest X-ray, computed tomography, and echocardiography. The inclusion criterion for the study was to have both SIT and PCD, based on the anamnesis, family history, physical examination, as well as laboratory, radiological, and genetic results. The exclusion criteria of the study were determined as having other chronic lung diseases, clinical instability and conditions that cause recurrent lower respiratory tract infections such as immune deficiency and cystic fibrosis. All patients were treated in terms of local standard care for PCD and SIT. All events were extensively reviewed and reported by the authors with strict measures to ensure data quality. All research procedures were evaluated and accepted by the Research Ethics Committee of Bezmialem University (approval number: E-54022451-050.05.04-21,194, 2021/188) and were conducted in agreement with the ethical standards specified in the Declaration of Helsinki.

2.2 | Collected Data and Analyses

Demographic and clinical features including age, sex, body mass index (BMI), personal and family history, pregnancy and neonatal period outcomes, current or prolonged medication use, and physical examination findings (including referrals from ear-nose throat and chest departments) were recorded. Furthermore, data obtained from cardiac evaluations and echocardiography, pulmonary functional tests (PFTs, most recent), thorax computed tomography (performed within the prior 2 years), and abdominal ultrasound (at any time) were analyzed. Finally, patient files were reviewed to record any relevant information concerning previous hospitalizations, surgical histories, and microbiological results, in addition to obtaining complete data from genetic analyses.

BMI was calculated as the ratio of body weight (kg) to height squared (m^2). Parental consanguinity was considered as marriages between second cousins or closer relatives (first cousins, once-separated first cousins, and second cousins). PFT could not be performed in 17 patients due to their age. Thirty-one patients had undergone PFT within the prior year in accordance with international standards, including measurement of forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), and FEV1/FVC ratio—depending on their age and compliance with the test.

2.3 | Genetic Analysis

Whole exome sequencing of 40 patients was available and was performed to identify pathogenic gene mutations, including those involving the ARMC4, CCDC103, CCDC114, CCDC151, CCDC39, CCDC40, DNAAF3, DNAAF4, DNAH5, DNAI1, DNAI2, and TTC25 genes. Approximately 36.5 Mb of

Consensus Coding Sequences (CCS) was targeted using the Twist Human Core Exome Plus kit, which was generated from fragmented genomic DNA covering more than 98% of Gencode v28 regions derived from the human genome. An Illumina Nextseq NGS platform (Illumina, San Diego, CA, USA) was used to achieve a minimum of 20× read depth for over 98% of the targeted bases. The bioinformatics filtering strategy included screening exonic and donor/acceptor splicing variants. In accordance with the pedigree and phenotype, priority was given to rare variants; <0.01% in public databased including 1000 Genomes Project, Exome Variant Server, and so on. which were fitting appropriate inheritance pattern in disease genes previously linked to PCD.

2.4 | Bhalla Score Calculation

The Bhalla CT scoring system was used to evaluate the extent and severity of pulmonary abnormalities for each lobe (Bhalla et al. 1991). The total score ranged from 0 to 31 and was obtained by summing the scores according to the number of affected lung lobes and segments, taking into account the lingula and middle lobe independently. A Bhalla score of 0 indicates a normal result. Scores ranging from 1 to 10 denote a mild condition, while scores between 11 and 21 suggest a moderate severity. A score falling within the range of 22–31 indicates a severe condition. Radiological evaluations were performed by the same radiologist in a blinded manner.

2.5 | Scores Used to Assess PCD

The PCD Rule (PICADAR) score was used to predict the likelihood of having PCD based on seven queries that is applied to patients with daily purulent cough beginning in the early childhood. It assesses SI, full/preterm gestation, neonatal chest symptoms, chronic rhinitis, neonatal intensive care unit admission, ear symptoms, and congenital cardiac defect (Behan et al. 2016). While the maximum score from PICADAR is 14 (corresponding to a 99.8% likelihood of PCD), patients with a score of 5 or above should be investigated for PCD, and if the score is 10 or above, the probability of PCD is over 90%.

Another scoring system developed to assess PCD was published by the American Thoracic Society in 2016 by identifying the four most sensitive main features to diagnose PCD (ATS score) (Leigh et al. 2016). These criteria include the presence of laterality defect, unexplained neonatal respiratory distress, early-onset (under 6 months) persistent nasal congestion, and early-onset productive cough. It has been shown that the specificity of the ATS score for PCD is 99% when all of these criteria are present together, 96% if three criteria are present, 72% if two criteria are positive, and 41% if only one criterion is positive.

2.6 | Statistical Analyses

IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The statistical significance value was accepted as $p<0.05$. Histogram and Q-Q plots were used to determine whether variables are normally distributed. Descriptive statistics were presented by using median (25th percentile–75th percentile) for continuous variables due to non-normal distribution, while frequency (percentage) summaries were used for categorical variables. Continuous variables were compared with the Mann–Whitney U test. Categorical variables were analyzed with appropriate chi-square tests or Fisher's exact test (or its Freeman–Halton extension).

3 | Results

Forty-eight PCD patients with SIT were included in the study. Clinical and demographic features of the study population are summarized in Table 1. The median age of patients were 13 (6.5–16) years and 54.17% of them were male. Median BMI values were 16 (14.73–19.06) kg/m^2. Parental consanguinity was observed in 43 (89.58%) patients. Thirty-six (75%) patients presented with symptoms of neonatal respiratory distress syndrome and 30 had been admitted for treatment in neonatal ICUs.

Bhalla score was available in 31 patients and "moderate and severe" score was observed in 19 (61.29%) patients. The median PICADAR score was 10 (8–11) and 34 (70.83%) patients exhibited a high (≥10) PICADAR score. ATS score was found to be 4 in 24 (50%) patients and 3 in 20 (43.75%) patients (since all patients had SIT, the lowest possible score was 1 in our study group). Genetic analysis results were available/accessible in 40 patients, showing known mutations in 27 (67.5%) (Table 2). Pathogenic mutations were detected in DNAH5 in 8 (20%) patients, in CCDC103 in 4 (10%) patients, in CCDC39 in 3 (7.5%) patients, in CCDC151, CCDC40, and DNAI2 in 2 (5%) patients each, and in ARMC4, CCDC114, DNAAF3, DNAAF4, DNAI1, and TTC25 in 1 (2.5%) patient each. We have identified four novel variants in previously associated genes. The other known variants' population frequencies are listed in Table 2. The patients whom the causative mutation could not be identified had undergone reanalysis including copy number variations (CNVs) algorithm but not included in this study since it is not completed.

Bronchiectasis was diagnosed in 31 (64.58%) patients. The findings of the patients included chronic sinusitis in 37 (77.08%) patients, pathological bronchoscopy results in 26 (54.17%) patients, persistent rhinitis in 22 (45.83%) patients, chronic otitis in 19 (39.58%) patients, snoring in 18 (37.5%) patients, reflux in 10 (20.83%) patients, hearing loss in 6 (12.5%) patients, dermatologic symptoms in 5 (10.42%) patients, and cardiac problems such as mitral and tricuspid valve disease in 5 (10.42%) patients. PFT was available in 31 patients and the median FEV1 was 75% (60%–82%), FVC was 72% (59%–87%), and FEV1/FVC was 103% (90%–112%).

The most common microorganisms detected in prior nasopharyngeal aspirate cultures were *Haemophilus influenza* (31, 64.58%), *Streptococcus pneumonia* (20, 41.67%), *Moraxella catarrhalis* (10, 20.83%), and *Pseudomonas aeruginosa* (9, 18.75%). Twenty-nine surgeries were performed on 20 of the patients (42%) for different reasons: 12 (41%) underwent tubulation, 11 (38%) underwent adenoid or tonsil surgery, and 3 underwent heart surgery. Two patients needed active noninvasive

TABLE 1 | Clinical and demographic characteristics of patients.

Variables	Results	Variables	Results
Age ($n=48$)	13 (6.5–16)	Cardiac problems ($n=48$)	5 (10.42%)
Sex ($n=48$)		Pathological bronchoscopy findings ($n=48$)	26 (54.17%)
Female	22 (45.83%)	Hospitalization ($n=48$)	29 (60.42%)
Male	26 (54.17%)	Surgery history ($n=48$)	20 (41.67%)
Body mass index ($n=48$)	16.00 (14.73–19.06)	Adenoidectomy	8 (16.67%)
Underweight	24 (50.00%)	Tubulation	12 (25.00%)
Normal	20 (41.67%)	Tonsillectomy	2 (4.17%)
Overweight	4 (8.33%)	Myringoplasty	1 (2.08%)
Asthma history in family ($n=48$)	32 (66.67%)	Cardiac surgery	3 (6.25%)
Parental consanguinity ($n=48$)	43 (89.58%)	Cholecystectomy	1 (2.08%)
Preterm birth ($n=48$)	1 (2.08%)	Inguinal hernia	1 (2.08%)
IUGR ($n=48$)	3 (6.25%)	FVC ($n=31$)	72 (59–87)
Stay in neonatal ICU ($n=48$)	30 (62.50%)	≥80	10 (32.26%)
Symptom in the neonatal period ($n=48$)	36 (75.00%)	60–79	13 (41.94%)
Bhalla score ($n=31$)	13 (8–16)	40–59	7 (22.58%)
Mild (1–10)	12 (38.71%)	<40	1 (3.23%)
Moderate (11–21)	18 (58.06%)	FEV1 ($n=31$)	75 (60–82)
Severe (22–31)	1 (3.23%)	≥80	10 (32.26%)
PICADAR ($n=48$)	10 (8–11)	60–79	14 (45.16%)
<10	14 (29.17%)	40–59	6 (19.35%)
≥10	34 (70.83%)	<40	1 (3.23%)
ATS score ($n=48$)		FEV1 to FVC ratio ($n=31$)	1.03 (0.90–1.12)
2	3 (6.25%)	NPA culture positivity ($n=48$)	42 (87.50%)
3	21 (43.75%)	*Haemophilus influenza*	31 (64.58%)
4	24 (50.00%)	*Streptococcus pneumoniae*	20 (41.67%)
Genetic mutation ($n=40$)	27 (67.50%)	*Moraxella catarrhalis*	10 (20.83%)
ARMC4	1 (2.50%)	*Pseudomonas aeruginosa*	9 (18.75%)
CCDC103	4 (10.00%)	*Staphylococcus aureus*	5 (10.42%)
CCDC114	1 (2.50%)	*Serratia marcescens*	3 (6.25%)
CCDC151	2 (5.00%)	*Streptococcus pyogenes*	2 (4.17%)
CCDC39	3 (7.50%)	*Acinetobacter baumannii*	1 (2.08%)
CCDC40	2 (5.00%)	*Stenotrophomonas maltophilia*	1 (2.08%)
DNAAF3	1 (2.50%)	*Klebsiella oxytoca*	1 (2.08%)
DNAAF4	1 (2.50%)	*Enterobacter cloacae*	1 (2.08%)
DNAH5	8 (20.00%)	*Citrobacter freundii*	1 (2.08%)
DNAI1	1 (2.50%)	*Escherichia coli*	1 (2.08%)
DNAI2	2 (5.00%)	Treatment ($n=48$)	
TTC25	1 (2.50%)	Inhaler steroids	36 (75.00%)

(Continues)

TABLE 1 | (Continued)

Variables	Results	Variables	Results
Bronchiectasis ($n=48$)	31 (64.58%)	Nasal steroids	20 (41.67%)
Persistent rhinitis ($n=48$)	22 (45.83%)	Theophylline	2 (4.17%)
Dermatologic findings ($n=48$)	5 (10.42%)	Beta-2 agonists	26 (54.17%)
Reflux ($n=48$)	10 (20.83%)	Azithromycin	31 (64.58%)
Snoring ($n=48$)	18 (37.50%)	Herbal medicine	7 (14.58%)
Chronic sinusitis ($n=48$)	37 (77.08%)	Noninvasive ventilation	2 (4.17%)
Chronic otitis ($n=48$)	19 (39.58%)	Physical therapy	18 (37.50%)
Hearing loss ($n=48$)	6 (12.50%)		

Note: Descriptive statistics were presented by using median (25th percentile—75th percentile) for continuous variables due to non-normally distribution and frequency (percentage) for categorical variables.
Abbreviations: ATS, American thoracic society; FEV1, Forced expiratory volume in 1s; FVC, Forced vital capacity; ICU, Intensive care unit; IUGR, Intrauterine growth retardation; NPA, Nasopharyngeal aspirate samples; PICADAR, Primary ciliary dyskinesia rule.

ventilation (NIV) support and 18 patients had been referred to physical therapy.

The 40 patients with complete genetic analyses were classified into two groups based on the presence/absence of known genetic variants: 27 patients were in the mutation group and 13 patients were in the nonmutation group. The median age in patients with genetic variants was 13 (9–17) years and was higher than the group without mutations ($p=0.018$). Parental consanguinity was more frequent in the mutation group (96.3% vs. 69.23%, $p=0.031$). The median Bhalla score was 13 (10–16) in those with mutation, significantly higher than that of patients without mutation [7.5 (3–8), $p=0.033$]. The percentage of patients with moderate or severe Bhalla score was significantly higher in the mutation group ($p=0.023$), as well as the frequency of patients with an ATS score of 3 or 4 ($p=0.043$). No significant differences were found between groups in terms of sex, BMI, stay in neonatal ICU, symptoms of respiratory distress syndrome in the neonatal period, clinical symptoms and treatment, PICADAR score, hospitalization, and microbiological results (all, $p>0.05$). The comparison of the mutation and nonmutation groups are detailed in Table 3.

Owing to the high frequency of the mutation, we also examined patients with respect to DNAH5 mutation status (Table 4). Stay in neonatal ICU and neonatal respiratory distress symptoms were lower among patients with DNAH5 mutation compared to those without ($p=0.036$ and 0.015, respectively). Age and the Bhalla, PICADAR, and ATS scores of these two groups were similar ($p>0.05$).

4 | Discussion

In our endeavor to explore the relationships of genetic data with the clinical and radiological characteristics of PCD patients with SIT, we detected the following known genetic variants in our cohort: DNAH5, CCDC103, CCDC39, CCDC151, CCDC40, DNAI2, ARMC4, CCDC114, DNAAF3, DNAAF4, DNAI1, and TTC25. The study suggested that patients with genetic variants may have a higher likelihood of parental consanguinity, higher rates of severe or moderate Bhalla score, and higher ATS score. We also recommended that DNAH5 mutation was associated with a lower likelihood of having a history of neonatal ICU stay and lower frequency of respiratory distress syndrome-related symptoms in the neonatal period.

Definitive diagnosis of PCD is challenging, and usually necessitates video microscopy analysis for cilia function and electron microscopy for ultrastructural cilia defects (Lucas et al. 2017). Even these advanced diagnostic tools, which are available in few centers, are not sufficient for diagnostic purposes in some cases, ultimately leading to the need for genetic analyses to confirm diagnosis. It is estimated that there are approximately 500,000 patients with PCD worldwide (Hannah et al. 2022). However, since diagnostic methods are limited and based on experience, the number of patients with a definitive diagnosis is much lower. Similar to other complex diseases and syndromes, suspicion in the presence of clinical findings is the first step in the diagnosis of PCD. Among the characteristic clinical features of PCD, daily productive cough, recurrent respiratory tract infections, chronic sinusitis, recurrent otitis, recurrent lung infections, and neonatal respiratory distress syndrome have been observed in approximately 80% of children. Laterality defects such as SIT are reported in almost 40%–50% of children, and other situs anomalies such as situs ambiguous, are found in a further 10%–12% of affected patients (Emiralioğlu et al. 2020). In the context of diagnostic approaches and features (other than SI/SIT), our study population revealed similar clinical findings to those reported in the literature. In the majority of cases, PCD symptoms become overt in the early childhood, but diagnosis may be delayed up to 4 years if SI is present, and up to 6 years if there is no SI (Coren et al. 2002). This indicates a critical role for SI/SIT in this population. Epidemiologic data in a multicenter study from 26 European countries reported a median age at diagnosis of 5.3 years (Kuehni et al. 2010). Similarly, the median age of patients at diagnosis was 5.5 years in our study. The delay in PCD diagnosis can be associated with many factors including late admission of patients to our center, which is a tertiary care hospital, and the difficulty in diagnosing the disease. However,

TABLE 2 | Genetic variants identified in PCD cases with SIT.

Gene	Transcript number	Nucleotide change	Protein change	HGVS name	Exon/ intron position	dbSNP	ClinVar ID
DNAI1	NM_001281428.2	c.1688G>A	p.Cys563Tyr	NM_001281428.2(DNAI1):c.1688G>A	Exon 17	rs1335386227	1,007,474
DNAH5	NM_001369.3	c.1432C>T, c.13132G>T	p.R478[a], p.E4378[a]	NM_001369.3(DNAH5): c.[1432C>T];[13132G>T]	Exon 11, exon 76	rs747900131/–	454,748/–
DNAH11	NM_001277115.2	c.3356G>A	p.S1119N	NM_001277115.2(DNAH11): c.3356G>A	Exon 17	rs754921505	575,674
CFAP53	NM_145020.5	c.1411C>T, c.1013A>T, c.319T>C	p.R471[a], p.E338V, p.L107=	NM_145020.5(CFAP53):c.1411C>T(;) 1013A>T(;)319T>C	Exon 8/ exon 6/ exon3	rs202140882/ rs369765061/–	1,030,233/–/–
TTC25	NM_031421.5	c.398-2A>G		NM_031421.5(TTC25):c.398-2A>G	Exon 4	n/a	n/a
DNAH5	NM_001369.3	c.6037C>T	p.Arg2013Ter	NM_001369.3(DNAH5):c.6037C>T	Exon 36	rs1273352530	454,789
ARMC4	NM_001290021.2	c.1250C>A	p.Ser417Ter	NM_001290021.2(ARMC4): c.1250C>A	Exon 11	rs587777049	66,047
CCDC39	NM_181426.2	c.206_209delCACA	p.Thr69SerfsTer28	NM_181426.2(CCDC39): c.206_209delCACA	Exon 2	n/a	n/a
DNAH5	NM_001369.3	c.10384C>T	p.Gln3462[a]	NM_001369.3(DNAH5):c.10384C>T	Exon 61	rs571919972	238,953
DNAH5	NM_001369.3	c.6037C>T	p.Arg2013Ter	NM_001369.3(DNAH5):c.6037C>T	Exon 36	rs1273352530	454,789
CCDC114	NM_144577.4	c.1391 + 5G>A		NM_144577.4(CCDC114): c.1391 + 5G>A	Exon 12	rs201133219	39,640
CCDC103	NM_001258395.2	c.461A>C	p.His154Pro	NM_001258395.2(CCDC103): c.461A>C	Exon 4	rs145457535	31,698
CCDC39	NM_181426.2	c.2447_2448del	p.Thr816Lysfs*3	NM_181426.2(CCDC39): c.2447_2448del	Exon 18	n/a	n/a
CCDC40	NM_017950.4	c.2440C>T	p.Arg814Ter	NM_017950.4(CCDC40):c.2440C>T	Exon 14	rs747233125	569,156
CCDC151	NM_145045.5	c.1445_1446delGC	p.Arg482LeufsTer12	NM_145045.5(CCDC151): c.1445_1446delGC	Exon 11	n/a	n/a
CCDC151	NM_145045.5	c.615delC	p.Met206CysfsTer13	NM_145045.5(CCDC151):c.615delC	Exon 5	n/a	n/a
CCDC103	NM_001258395.2	c.461A>C	p.His154Pro	NM_001258395.2(CCDC103): c.461A>C	Exon 4	rs145457535	31,698

(Continues)

TABLE 2 | (Continued)

Gene	Transcript number	Nucleotide change	Protein change	HGVS name	Exon/ intron position	dbSNP	ClinVar ID
DNAAF4	NM_130810.4	c.390_393del	p.Val132fs[a]	NM_130810.4(DNAAF4): c.390_393del	Exon 4	rs781156334	n/a
DNAH5	NM_001369.3	c.11758A>T	p.Lys3420Ter	NM_001369.3(DNAH5):c.11758A>T	Exon 68	n/a	n/a
CCDC40	NM_017950.4	c.2716C>T	p.Q906[a]	NM_017950.4(CCDC40):c.2716C>T	Exon 17	n/a	n/a
CCDC103	NM_001258395.2	c.568_569delAG	p.Ser190LeufsTer5	NM_001258395.2(CCDC103): c.568_569delAG	Exon 4	rs746242380	1,451,307
DNAH5	NM_001369.3	c.3514C>T	p.Gin1172Ter	NM_001369.3(DNAH5):c.3514C>T	Exon 23	n/a	n/a
DNAH5	NM_001369.3	c.6763C>T	p.Arg2255Ter	NM_001369.3(DNAH5):c.6763C>T	Exon 41	rs745918507	454,795
DNAI2	NM_023036.6	c.883C>T	p.Arg295Ter	NM_023036.6(DNAI2):c.883C>T	Exon 8	rs200708870	408,961
DNAH5	NM_001369.3	c.6037C>T	p.Arg2013[a]	NM_001369.3(DNAH5):c.6037C>T	Exon 36	rs1273352530	454,789
DNAAF3	NM_001256715.2	c.459G>A	p.W153[a]	NM_001256715.2(DNAAF3): c.459G>A	Exon 5	n/a	n/a
DNAH5	NM_001369.3	c.6037C>T	p.Arg2013Ter	NM_001369.3(DNAH5):c.6037C>T	Exon 36	rs1273352530	454,789
CCDC103	NM_001258395.2	c.461A>C	p.His154Pro	NM_001258395.2(CCDC103): c.461A>C	Exon 4	rs145457535	31,698
DNAI2	NM_023036.6	c.883C>T	p.R295[a]	NM_023036.6(DNAI2):c.883C>T	Exon 8	rs200708870	408,961

Gene	ClinVar classification	Zygosity	Variation classification	Disease	Disease inheritance	Gnomad exome freq %	Turkish variome %[a]
DNAI1	Pathogenic/likely pathogenic	Homozygous	CLASS3	PCD type1	AR	0,00010	n/a
DNAH5	Pathogenic, likely pathogenic	Compound Heterozygous	CLASS1, CLASS2	PCD type3	AR	0.0012/–	n/a
DNAH11	VUS	Heterozygous	CLASS3	PCD type7	AR	0,01190	0,2083
CFAP53	VUS/–/–	Heterozygous	CLASS2, CLASS3	Heterotaxy type6	AR	0.0038/0.0025/–	0.0595/0.0647/–
TTC25	No record, novel variant	Homozygous	CLASS2	PCD type35	AR	n/a	n/a
DNAH5	Pathogenic/likely pathogenic	Homozygous	CLASS1	PCD type3	AR	0,00020	n/a
ARMC4	Pathogenic	Homozygous	CLASS1	PCD type23	AR	n/a	n/a
CCDC39	No record, novel variant	Homozygous	CLASS2	PCDtype14	AR	n/a	n/a

(Continues)

TABLE 2 | (Continued)

Gene	ClinVar classification	Zygosity	Variation classification	Disease	Disease inheritance	Gnomad exome freq %	Turkish variome %[a]
DNAH5	Pathogenic	Homozygous	CLASS1	PCD type3	AR	0,00600	n/a
DNAH5	Pathogenic/likely pathogenic	Homozygous	CLASS1	PCD type3	AR	0,00020	n/a
CCDC114	Pathogenic/likely pathogenic	Homozygous	CLASS1	PCD type20	AR	0,06470	0,06470
CCDC103	Pathogenic/likely pathogenic	Homozygous	CLASS1	PCDtype17	AR	0,18,830	0,1049
CCDC39	n/a	Homozygous	CLASS2	PCD type14	AR	n/a	n/a
CCDC40	Pathogenic	Homozygous	CLASS1	PCD type15	AR	0,01830	0,0183
CCDC151	n/a	Homozygous	CLASS2	PCD type30	AR	0,00010	n/a
CCDC151	No record, novel variant	Homozygous	CLASS2	PCD type30	AR	n/a	n/a
CCDC103	Pathogenic/likely pathogenic	Homozygous	CLASS1	PCD type17	AR	0,11,940	0,1049
DNAAF4	Pathogenic	Homozygous	CLASS1	PCD type25	AR	0,00010	0,0354
DNAH5	n/a	Homozygous	CLASS2	PCD type3	AR	n/a	n/a
CCDC40	No record, novel variant	Homozygous	CLASS2	PCD type15	AR	n/a	n/a
CCDC103	Pathogenic	Homozygous	CLASS1	PCD type17	AR	0,00010	n/a
DNAH5	Pathogenic	Homozygous	CLASS2	PCD type3	AR	n/a	n/a
DNAH5	Pathogenic/likely pathogenic	Homozygous	CLASS2	PCD type3	AR	0,00200	n/a
DNAI2	Pathogenic/likely pathogenic	Homozygous	CLASS1	PCD type9	AR	0,00080	n/a
DNAH5	Pathogenic/likely pathogenic	Homozygous	CLASS1	PCD type3	AR	0,00020	n/a
DNAAF3	n/a	Homozygous	CLASS2	PCD type2	AR	n/a	n/a
DNAH5	Pathogenic/likely pathogenic	Homozygous	CLASS2	PCD type3	AR	n/a	n/a
CCDC103	Pathogenic/likely pathogenic	Homozygous	CLASS1	PCD type17	AR	0,11,940	0,1049
DNAI2	Pathogenic/likely pathogenic	Homozygous	CLASS1	PCD type9	AR	0,00160	n/a

Abbreviation: n/a, Absent in database.
[a]Presented according to the Turkey report (Kars et al. 2021).

TABLE 3 | Characteristics of the patients with regard to genetic mutation.

Variables	Genetic mutation		*p*
	Absent ($n=13$)	Present ($n=27$)	
Age ($n=40$)	7 (5–11)	13 (9–17)	0.018[a]
Sex ($n=40$)			
Female	7 (53.85%)	14 (51.85%)	1.000[b]
Male	6 (46.15%)	13 (48.15%)	
Body mass index ($n=40$)	14.91 (14.17–15.81)	17.33 (14.59–20.31)	0.059[a]
Underweight	6 (46.15%)	16 (59.26%)	0.695[d]
Normal	6 (46.15%)	8 (29.63%)	
Overweight	1 (7.69%)	3 (11.11%)	
Asthma history in family ($n=40$)	10 (76.92%)	17 (62.96%)	0.484[c]
Parental consanguinity ($n=40$)	9 (69.23%)	26 (96.30%)	0.031[c]
Preterm birth ($n=40$)	1 (7.69%)	0 (0.00%)	0.325[c]
IUGR ($n=40$)	0 (0.00%)	3 (11.11%)	0.538[c]
Stay in neonatal ICU ($n=40$)	8 (61.54%)	17 (62.96%)	1.000[c]
Symptom in the neonatal period ($n=40$)	9 (69.23%)	21 (77.78%)	0.700[c]
Bhalla score ($n=25$)	7.5 (3–8)	13 (10–16)	0.033[a]
Mild	5 (83.33%)	5 (26.32%)	0.023[d]
Moderate and severe	1 (16.67%)	14 (73.68%)	
PICADAR ($n=40$)	10 (7–11)	11 (10–11)	0.108[a]
<10	5 (38.46%)	6 (22.22%)	0.451[c]
≥ 10	8 (61.54%)	21 (77.78%)	
ATS score ($n=40$)			
2	3 (23.08%)	0 (0.00%)	0.043[d]
3	5 (38.46%)	11 (40.74%)	
4	5 (38.46%)	16 (59.26%)	
Bronchiectasis ($n=40$)	6 (46.15%)	19 (70.37%)	0.175[c]
Rhinitis ($n=40$)	4 (30.77%)	15 (55.56%)	0.258[b]
Dermatologic findings ($n=40$)	2 (15.38%)	2 (7.41%)	0.584[c]
Reflux ($n=40$)	2 (15.38%)	6 (22.22%)	1.000[c]
Snoring ($n=40$)	5 (38.46%)	10 (37.04%)	1.000[c]
Sinusitis ($n=40$)	8 (61.54%)	22 (81.48%)	0.246[c]
Otitis ($n=40$)	5 (38.46%)	12 (44.44%)	0.986[b]
Hearing loss ($n=40$)	2 (15.38%)	2 (7.41%)	0.584[c]
Cardiac problems ($n=40$)	1 (7.69%)	4 (14.81%)	1.000[c]
Pathological bronchoscopy findings ($n=40$)	7 (53.85%)	17 (62.96%)	0.836[b]
Hospitalization ($n=40$)	6 (46.15%)	17 (62.96%)	0.506[b]
Surgery history ($n=40$)	5 (38.46%)	13 (48.15%)	0.812[b]
FVC ($n=26$)	57 (54–75)	72 (67–87)	0.297[a]

(Continues)

TABLE 3 | (Continued)

Variables	Genetic mutation		p
	Absent ($n=13$)	Present ($n=27$)	
Normal (≥80)	1 (20.00%)	8 (38.10%)	0.628[c]
Low (<80)	4 (80.00%)	13 (61.90%)	
FEV1 ($n=26$)	66 (56–67)	78 (62–82)	0.282[a]
Normal (≥80)	1 (20.00%)	9 (42.86%)	0.617[c]
Low (<80)	4 (80.00%)	12 (57.14%)	
FEV1 to FVC ratio ($n=26$)	1.04 (1.03–1.05)	1.00 (0.91–1.12)	0.649[a]
NPA culture positivity ($n=40$)	10 (76.92%)	25 (92.59%)	0.307[c]
Treatment ($n=40$)			
Inhaler steroids	9 (69.23%)	22 (81.48%)	0.437[c]
Nasal steroids	8 (61.54%)	9 (33.33%)	0.177[b]
Theophylline	1 (7.69%)	1 (3.70%)	1.000[c]
Beta-2 agonists	7 (53.85%)	16 (59.26%)	1.000[b]
Azithromycin	6 (46.15%)	19 (70.37%)	0.175[c]
Herbal medicine	1 (7.69%)	6 (22.22%)	0.393[c]
Noninvasive ventilation	0 (0.00%)	1 (3.70%)	1.000[c]
Physical therapy	6 (46.15%)	9 (33.33%)	0.498[c]

Note: Descriptive statistics were presented by using median (25th percentile—75th percentile) for continuous variables due to non-normally distribution and frequency (percentage) for categorical variables.
Abbreviations: ATS, American thoracic society; FEV1, Forced expiratory volume in 1s; FVC, Forced vital capacity; ICU, Intensive care unit; IUGR, Intrauterine growth retardation; NPA, Nasopharyngeal aspirate samples; PICADAR, Primary ciliary dyskinesia rule.
[a]Mann–Whitney U test.
[b]Chi-square test.
[c]Fisher's exact test.
[d]Fisher–Freeman–Halton test.

since all patients in our study had SIT, an threshold for diagnosis could be expected. Furthermore, 75% of patients in our study population had history of respiratory distress syndromes during the neonatal period, and 60% had been hospitalized in the neonatal ICU, indicating that earlier diagnosis (before 5.5 years of age) could have been possible with greater awareness and clinical suspicion. However, our data also showed that the presence of DNAH5 mutation (the most common in the present study) was associated with lower odds for having a history of neonatal symptoms and ICU stay, which is a cohort-specific factor that could explain delayed diagnosis. In addition, neonatal respiratory distress syndrome may occur in 12–23 patients per 100,000 term infants, making routine screening for PCD difficult (Pham and Rubenfeld 2017). Specific testing should be considered when there are prolonged symptoms and other characteristics consistent with PCD, such as unexplained tachypnea, neonatal pneumonia, rhinitis, congenital heart disease, and particularly, in the presence of laterality defects.

PCD is usually inherited in an autosomal recessive pattern, but recent studies have described autosomal dominant and X-linked inheritance, with a high prevalence rate in some countries due to consanguinity (Boon et al. 2013). Although parental consanguinity was reported between 13% and 19.6% in previous studies, a consanguinity rate as high as 89.58% was demonstrated in PCD patients with SIT in our cohort (Boon et al. 2014). Similar exceptionally high percentages have been reported from our country, including studies by Kilinc et al. and Emiralioğlu et al., which found consanguinity in 64% and 80% of their patients, respectively (Emiralioğlu et al. 2020; Kilinc et al. 2021). In Turkey, the prevalence of consanguineous marriage has been reported to be as high as 23%, based on data from the Turkish Statistical Institute, and it is a well-established fact that hereditary disorders are more common in children born to consanguineous families than in children born to non-consanguineous families (Emiralioğlu et al. 2020). It is therefore crucial to re-iterate that questioning parental consanguinity may be a viable and costless approach to assess the likelihood of PCD in patients with suspicious findings and histories.

Few studies have investigated the association between mutations and the clinical findings and severity scores of subjects with PCD. Hornef et al. showed that the most common mutation in patients with PCD are those that involve DNAH5, which is found in 24% of patients (Hornef et al. 2006). Emiralioğlu et al., in a cohort of 265 patients with PCD, reported mutations in 11 PCD genes, including DNAH5, CCDC40, RSPH4A, DNAH11, HYDIN, CCNO, DNAI1, ARMC4, TTC25, DNAH1, and CCDC39, which were present in 17.4% of cases, and DNAH5 mutation, the most

TABLE 4 | Characteristics of participants with regard to DNAH5 mutation.

Variables	DNAH5 mutation		
	Absent ($n=32$)	Present ($n=8$)	p
Age ($n=40$)	12 (6.5–15.5)	12.5 (8.5–16.5)	0.576[a]
Stay in neonatal ICU ($n=40$)	23 (71.88%)	2 (25.00%)	0.036[b]
Symptom in the neonatal period ($n=40$)	27 (84.38%)	3 (37.50%)	0.015[b]
Bhalla score ($n=25$)	12 (8–15)	12 (6–18)	0.879[a]
Mild	7 (38.89%)	3 (42.86%)	1.000[c]
Moderate and Severe	11 (61.11%)	4 (57.14%)	
PICADAR ($n=40$)	10.5 (10–11)	9 (7.5–10.5)	0.117[a]
<10	7 (21.88%)	4 (50.00%)	0.182[b]
≥ 10	25 (78.13%)	4 (50.00%)	
ATS score ($n=40$)			
2	3 (9.38%)	0 (0.00%)	0.305[c]
3	11 (34.38%)	5 (62.50%)	
4	18 (56.25%)	3 (37.50%)	

Note: Descriptive statistics were presented by using median (25th percentile—75th percentile) for continuous variables due to non-normally distribution and frequency (percentage) for categorical variables.
Abbreviations: ATS, American thoracic society; ICU, Intensive care unit; PICADAR, Primary ciliary dyskinesia rule.
[a]Mann–Whitney U test.
[b]Fisher's exact test.
[c]Fisher–Freeman–Halton test.

common pathogenic variant, was found in 26.1% of these cases (Emiralioğlu et al. 2020). Similarly, in our cohort from Turkey, we detected the DNAH5 mutation in 20% of analyzed subjects. We identified 12 different gene mutations compatible with PCD. Of the mutations we identified, DNAH5, DNAI1, and DNAI2 are associated with outer dynein arm defects, CCDC114, ARMC4, CCDC151, TTC25, and CCDC103 with outer dynein arm docking and targeting defects, DNAAF3 and DNAAF4 with cytoplasmic precursor defects of dynein arms, and CCDC39 and CCDC40 with inner dynein arm and axonemal disorganization (Lucas et al. 2020). Davis et al. showed in a study of 118 patients with PCD that subjects with CCDC39 and CCDC40 mutations exhibited worse clinical presentations and earlier age at diagnosis. Also, particularly subjects with outer dynein arm mutations such as DNAH5 had relatively less-severe phenotypes and better lung function (Davis et al. 2015). Consistently, we demonstrated that genetic variation in DNAH5 was related with a lower likelihood of having a history of ICU stay and lower frequency of respiratory symptoms in the neonatal period. Kilinç et al. demonstrated in 14 children diagnosed with PCD that having the CCDC40 mutation was associated with lower BMI an earlier age at diagnosis, while patients with DNAH5 mutations had higher BMI (Kilinc et al. 2021). Our data reveal that patients with any genetic variants may be older, have a higher likelihood of parental consanguinity, a higher likelihood of severe or moderate Bhalla score, and a higher ATS score. Bhalla score is a method developed to detect structural lung disease with CT scans, and the relationship between the presence of mutation and Bhalla score in patients with PCD indicates that some of the mutations contribute to the severity and/or progression of the disease. Performing specific mutation analyses in the early period can therefore alleviate bronchiectasis development or its severity. Our results also indicate that the presence of a genetic mutation in PCD patients with SIT did not have a negative impact on other clinical, radiological, and microbiological results. However, these results may be attributed to the small sample size relative to the extreme levels of clinical and genetic heterogeneity in patients with PCD. In addition, it must be reiterated that our study focused on a specific subset of PCD patients (with SIT), and therefore, the relationships identified in this study and lack thereof may be attributed to this selection.

There are several limitations to our study. The study was conducted with a retrospective design from a single center, and although the sample size is respectable considering the rarity of PCD with SIT, it is evident that the heterogeneity of the disease creates limitations in terms of statistical analyses. Important methods for the diagnosis of PCD, such as electron video microscopy, nasal nitric oxide test, high-speed video microscopy analysis of ciliary beat frequency and pattern, and immunofluorescence staining of ciliary proteins, were not available in our center, so data from these methods could not be included in our analyses. Additionally, the lack of statistical correction for multiple testing was a limitation to the results of our study.

5 | Conclusion

The analyses of our cohort of PCD patients with SIT suggested that patients with any genetic variant may be older, have higher ATS and higher Bhalla score, and their parents may be more likely to have consanguinity. The presence of the DNAH5 mutation was associated with having a history of ICU stay and respiratory symptoms in the neonatal period. Clinical suspicion in patients with situs abnormalities may be a red flag for the diagnosis of PCD. Early diagnosis of PCD through genetic analysis is critical to alleviate the progression and severity of chronic lung pathologies and may contribute to prognostication and improve the quality of life.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

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