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Evaluation of Clinical and Genetic Characteristics of Primary Ciliary Dyskinesia

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Abstract

Primary ciliary dyskinesia (PCD) is a clinically and genetically heterogeneous condition characterized by defective motile cilia activity. There is no "gold standard" diagnostic test currently available. In this article, we summarize the clinical and genotypic features of 25 children with PCD who received therapy at a single location in Turkey. This study was done between October 2020 and July 2022 as a retrospective cohort study in which the medical records of Turkish and refugee patients with PCD were reviewed regarding their medical history, clinical and radiologic findings, and genetic data. We evaluated the outcomes of 25 patients whose genetic results were reported to be associated with known PCD genes. The mean age of patients with PCD was 10.5 (±5). PICADAR scores ranged from 2 to 10, with the mean score being 6.1 (± 2.2). Age at diagnosis was shown to be moderately negatively correlated with PICADAR. (r;-0,502, p;0,01).16% DNAH5 within four patients, 16% with CCDC40 in four patients,12% with DNAAF2 in three patients, 8% with DNAH11 in two patients, 8% with TTC25 in two patients, 8% with DNAAF4 in two patients, 8% with CCNO in two patients 4% with DYNC2H1 in one patient, 4% with DNAI1 in one patient, 4% with ARMC4 in one patient,4% with RSPH4A in one patient, 4% with HYDIN in one patient,4% with CCDC65 in one individual from each PCD gene. The association between the phenotype and genotype of PCD patients in the southeast Anatolian region of our nation was explored for the first time in this study. Additionally, PCD patients with PIBO were reported for the first time with CCNO defects. Genotype and phenotype studies will help us determine the prognosis of patients in the future. These findings should increase our knowledge of PCD pathogenic pathways, hence enhancing early illness diagnosis, disease treatment, and prognosis.

Keywords: Primary ciliary dyskinesia, genetic, genotype, phenotype

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Introduction

Primary ciliary dyskinesia (PCD) is a clinically and genetically heterogeneous condition characterized by defective motile cilia activity.[1] It is a rare disorder often inherited in an autosomal recessive and X-linked disease pattern.[2] Patients often seek therapy for persistent wet cough, sinusitis, bronchiectasis, otitis media, and infant respiratory distress, with approximately fifty percent of patients presenting situs inversus.[1] There is no "gold standard" diagnostic test currently available. There are unique guidelines for determining the diagnosis of PCD individuals with distinct clinical characteristics.[3,4] They suggested that diagnosis needed access to a variety of technically challenging approaches, such as nasal nitric oxide (nNO), high-speed video analysis (HSVA), transmission electron microscopy (TEM), and genetic testing.[5] However, reaching all of these diagnostic methods may not be possible. Despite identifying more than 40 disease-causing genes, 20% to 30% of patients with a confirmed PCD diagnosis have no known genetic etiology.[6] Patients may be diagnosed with PCD using improved genetic testing tools, and novel genes can be found.

Highlights

- The relationship between the phenotype and genotype of patients with PCD in the southeast Anatolian area of our country was investigated for the first time.
- PCD patients with PIBO were reported, for the first time, with CCNO genetics.
- Genotype and phenotype studies will help us determine the prognosis of patients in the future. These findings should increase our knowledge of PCD pathogenic pathways, enhancing early illness diagnosis, disease treatment, and prognosis.

This article summarizes the clinical and genotypic features of 25 children with PCD who received therapy at a single location in Turkey. Additionally, we examined the correlation between PICADAR score, clinical, radiological, and laboratory features of patients with PCD.

Material and Method

This study was done between October 2020 and July 2022 as a retrospective cohort study in which the medical records of Turkish and refugee patients with PCD were reviewed regarding their medical history, clinical and radiologic findings, and genetic data.

Over the last two years, our department has followed up with 38 PCD patients. All patients underwent genetic testing. Six patients with situs inversus and a Primary Ciliary Dyskinesia Rule (PICADAR) score ≥5 had normal genetic testing results, and the genetic results of seven patients have not yet been released. Therefore, these 13 patients were excluded from the study. Patients carrying 25 pathological genetic homozygote alleles for primary ciliary dyskinesia were enrolled in the study.

At their clinical follow-up, we recorded the age at diagnosis, PICADAR score, weight z-scores, height z-scores, BMI z-scores, duration of symptoms, history of birth (term or preterm), persistent wet cough, an abnormal situs, an abnormal heart, a history of pneumonia, chronic sinusitis, chronic otitis, bronchiectasis, persistent rhinitis, recurrent wheezing, hearing loss, hospitalization in the neonatal period, prior infections, parental consanguinity, duration of symptoms, pulmonary function test (PFT) results, echocardiographic finding, radiological evaluation, and sputum microbiology result.

PICADAR score estimates the probability of PCD. It contains seven predictive factors that may be employed in any patient with a persistent wet cough, including full-term gestation, chest symptoms in the neonatal period, admission to a neonatal care unit, chronic rhinitis, ear complaints, situs inversus, and a congenital heart abnormality. For a cut-off score of ≥5, the tool's sensitivity and specificity were 0.90 and 0.75, respectively, to diagnose PCD.[7] Patients were suspected of PCD if their Primary Ciliary Dyskinesia Rule (PICADAR) score was ≥5. All patients' PICADAR scores were determined at admission. The Spirometry PFTs were conducted in line with the norms of the American Thoracic Society.[8] The measures of forced expiratory flow in 1 second (FEV1) %, forced vital capacity (FVC)%, and forced expiratory flow (FEF) 25-75% are stated. Before the test, the patients were informed about the techniques. PFT was not applied to children under six and non-cooperative children. It was applied to over six years of age and cooperative children. BMI z scores were recorded at the time of their most recent clinical follow-up. The BMI was computed by dividing weight in kilos by the square of height in meters. The "z" score for BMI for age was derived using the Centers for Disease Control and Prevention software.

Genetic Analysis

Genomic DNA extraction was performed according to the manufacturer's instructions (Maxwell RSC Blood DNA kit, Promega, USA) using the Maxwell RSC Instrument (Promega, USA). 30 µl of Proteinase K (PK) Solution was added into a 200 µl blood sample. 300 µl of Lysis Buffer was added to the blood and PK mix and incubated at 56°C for 20 minutes. After this step, each blood lysate sample was transferred to the cartridges. At the end of the assay in the instrument, 50 µl of DNA was eluted. The concentration of DNA was determined spectrophotometrically by measurement of the absorbance at 260/280 nm using a Nanodrop 1000 apparatus (Thermo Fisher Scientific). The concentration of DNA samples for libraries was determined using Qubit 3.0 (Thermo Fisher Scientific). The sequencing libraries for exome sequencing were prepared according to the Twist Human Core Exome Kit protocol (Twist Bioscience, USA). Paired-end 150 bp spread sequencing was performed on a NovaSeq system (Illumina, USA). Whole-exome sequencing

(WES) was performed for all the affected patients with a suspected history of PCD. Nova Seq data was then uploaded to the Sophia DDM (Sophia Genetics-Lausanne-Switzerland) platform and made ready for analysis. In Silico prediction databases, by consensus, stated that the changes were "destructive". The variants were evaluated using the American College of Medical Genetics (ACMG) 2015 criteria.[9]

Statical Analysis

Statistical analysis was performed using SPSS (Statistical Package for Science Studies) version 22.0 for Windows. Firstly, descriptive statistics were performed with the data obtained. Then, the Shapiro-Wilk test was used to test whether the variables were normally distributed. Characteristic data are presented as number (%) for categorical variables and mean±SD or median (minimum-maximum) for continuous variables, where appropriate. The differences between groups were compared using the independent sample T-test and Mann-Whitney U tests for numerical values. Correlations between data not normally distributed were examined using Spearman's correlation test, and correlations between normally distributed data were analyzed using Pearson's correlation test. All tests were two-tailed, and p-values less than 0.05 were considered statistically significant in all cases.

The study was conducted according to the ethical norms and standards of the Declaration of Helsinki, and ethical approval was obtained from the local ethics committee.

Results

We evaluated the outcomes of 25 patients whose genetic results were reported to be associated with known PCD genes. The mean age of patients with PCD was 10.5 (±5). The mean age of the patients at the time of diagnosis was 9.2 (±4.9) years. Sixteen (64%) patients were female, and nine (36%) were male. There were 16 (64%) consanguineous marriages involved. There were eight (%32) immigrants among the patients.

PICADAR scores ranged from 2 to 10, with the mean score being 6.1 (±2.2). Age at diagnosis was shown to be moderately negatively correlated with PICADAR. (r;-0.502, p;0.01).The PICADAR score of five patients (20%) was less than five, whereas the score of twenty (80%) patients was more than five. The mean age at diagnosis of patients with a PICADAR score of >5 was 8 (±4.6), and for those with a PICADAR score of < 5, the mean age at diagnosis was 14.4 (±1.5). Patients with high and low PICADAR scores were compared. The age at diagnosis of patients with high PICADAR score was significantly lower (p=0.01). When the height z scores, weight z scores, and BMI z scores of those with low and high PICADAR scores were compared, no statistically significant difference was found (p>0.05).

The mean age of patients with situs inversus was 9 (min:1-max:16). The mean age of patients without situs inversus was 10 (min:4-max:16) years. The age at diagnosis of patients with and without situs inversus was compared. There was no statistically significant difference between them (p=0.330). When the height z scores, weight z scores, and BMI z scores of those with situs inversus and without situs inversus were compared, no statistically significant difference was found (p>0.05).

In terms of symptoms, most of the patients %96 (n=24), had previous pneumonia and wet cough. The other symptoms;44% (n=11) of patients had prenatal respiratory distress, 56 % (n=14) had recurrent sinusitis, 20% (n=5) had recurrent otitis, 16 % (n=4) had hearing impairment, 60% (n=15) had situs inversus,4 % (n=1), had clubbing, and 12 % (n=3) had congenital cardiac abnormalities (atrial septal defect, patent ductus arteriosus, mitral regurgitation and pulmonary hypertension). According to their prior medical histories, %, 8 (n=2) of the patients who had a lobectomy and 4% (n=1) had bronchiolitis obliterans (**Table 1**). Bronchiectasis was diagnosed on computed tomography (CT) in 72% (n=18) of the patients. Among these patients, 50% (n=9) had bronchiectasis in one lobe ,38.8% (n=7) had bronchiectasis in two lobes, 10.5% had bronchiectasis in three lobes (n=2), Before the PCD diagnosis, lobectomies were performed on two patients. The most prevalent microorganisms in sputum culture were *Haemophilus influenzae* (36%) (n=9), *Staphylococcus aureus* (12%; n=3), methicillin resistance *Staphylococcus aureus* (4%; n=1), *Streptococcus pneumonia* (4%; n=1), and *Pseudomonas aeruginosa* (4%; n=1) (**Table 2**).

Nineteen children older than six years were given pulmonary function testing. Decrease of forced expiratory volume in the first second (FEV1) (mean (±SD): 65.7% (19.8) predicted) and forced vital capacity (FVC) (mean (±SD): 65.6% (19.5) predicted) were observed in these children. Additionally, forced expiratory flow at 25 percent and 75 percent of the pulmonary volume (FEF25-75) was (mean (±SD): 73.7% (19.5) predicted).

In our study, 24 patients were aged two years and over, and their BMI was calculated. The mean BMI Z-score was 1.03 (±0.82). The mean height Z-score and weight Z-score of the patients were, respectively, -0.74 (±0.92) and -1.02 (1.1). There was a no correlation between BMI z-score and FEV1, FVC and FEF25-75 of patients (respectively: r;-0.42, p;0.864; r;-0.123, p;0.616; r;-0.16, p;0.513).

The following pathogenic variations were found through genetic analysis:16% DNAH5 within four patients, 16% with CCDC40 in four patients,12% with DNAAF2 in three patients,8% with DNAH11 in two patients, 8% with TTC25 in two patients,8% with DNAAF4 in two patients,8% with CCNO in twopatients4% with DYNC2H1 in one patient, 4% with DNAI1 in one patient, 4% with ARMC4 in one patient,4% with RSPH4A in one patient, 4% with HYDIN in one patient,4% with CCDC65 in one individual from each PCD gene (**Table 1** and **Table 3**).

Table 1
Primary ciliary dyskinesia patients' demographics and clinical traits as determined by genetic testing.

	DNAH5 (n=4)	CCDC40 (n=4)	DNAAF2 (n=3)	DNAH11 (n=2)	TTC25 (n=2)	DNAAF4 (n=2)	CCNO (n=2)	DYNC2H1 (n=1)	DNAI1 (n=1)	ARMC4 (n=1)	HYDIN (n=1)	RSPH4A (n=1)	CCDC65 (n=1)
Age of diagnosis, median (min-max) age	9 (2-17)	6.5 (1-11)	12 (6-14)	9.5 (8-11)	15.5 (16-15)	15.5 (16-15)	4.5 (1-8)	4	3	6	9	8	14
Female/Male n	3/1	2/2	2/1	0/2	1/1	2/0	2/0	1/0	1/0	0/1	1/0	1/0	0/1
Refugee	1	0	1	0	1	1	2	0	0	1	0	1	0
Consanguineous (%)	3 (75%)	4 (100%)	2 (66.6%)	1 (50%)	1 (50%)	1 (50%)	2 (100%)	0	1 (100%)	0	1 (100%)	0	1 (100%)
PICADAR score (min-max)	7 (6-9)	9 (8-9)	6 (5-8)	3.5 (2-5)	2,5 (2-3)	4,5 (3-6)	6 (5-7)	6	7	10	6	5	3
Situs inversus, n (%)	4 (100%)	4 (100%)	2 (66.6%)	0	0	1 (%50)	1 (%50)	0	1 (100%)	1 (100%)	1 (100%)	0	0
Recurrent sinusitis n (%)	2 (50%)	1 (25%)	2 (66.6%)	1 (%50)	1 (%50)	1 (%50)	2 (100%)	0	1 (100%)	1 (100%)	0	1 (100%)	1 (100%)
Recurrent otitis, n (%)	0	1 (25%)	1 (33,3%)	1 (%50)	0	0	0	0	1 (100%)	1 (100%)	0	1 (100%)	0
Neonatal respiratory distress,n (%)	2 (50%)	3 (75%)	2 (66.6%)	1 (%50)	0	1 (%50)	0	1 (100%)	0	1 (100%)	0	1 (100%)	0
Term/preterm n	4/0	4/0	3/0	2/0	2/0	2/0	2/0	0/1	1/0	1/0	1/0	1/0	1/0
Hearing impairment n (%)	0	1 (25%)	1 (33,3%)	0	0	0	0	0	0	1 (100%)	0	1 (100%)	0
Congenital heart defect, n(%)	0	0	1 (33,3%)	0	0	0	1 (%50)	1 (100%)	0	0	0	0	0
Clubbing, n (%)	0	0	0	0	0	0	1 (%50)	0	0	0	0	0	0
Bronchiolitis obliterans n (%)	0	0	0	0	0	0	1 (%50)	0	0	0	0	0	0
History of lobectomy, n (%)	0	0	1 (33,3%)	1 (%50)	0	0	0	0	0	0	0	0	0
Bronchiectasis n (%)	3 (75%)	3 (75%)	2 (66.6%)	2 (100%)	2 (100%)	2 (100%)	1 (50%)	0	0	1 (100%)	0	1 (100%)	1 (1005)

Table 2
Relationship between genetic findings and growth, pulmonary function tests, and the presence or absence of bronchiectasis.

	DNAH5 (n=4)	CCDC40 (n=4)	DNAAF2 (n=3)	DNAH11 (n=2)	TTC25 (n=2)	DNAAF4 (n=2)	CCNO (n=2)	DYNC2H1 (n=1)	DNAI1 (n=1)	ARMC4 (n=1)	HYDIN (n=1)	RSPH4A (n=1)	CCDC65 (n=1)
Height z score median (min-max)	-1.37 (-0.14) -(-2.32)	-1.0 (-0.68) -(-1.66)	-0.39 (-0.57)- (-0.22)	-0.06 (-0.56) -(0.43)	-0.815 (-1.24) -(-0.39)	0.51 (0.48 -0.54)	-0.78 (-2.1) -(0.58)	-1.29	-1.77	0.46	0.48	-0.77	-1.44
Weightz score median (min-max)	-2.1 (-3.05) -(-0.81)	-1.7 (-3.34) -(0.03)	-0.27 (-0.24)- (-0.31)	-0.57 (-1.3)- (0.21)	-0.2 (-1.29) -(0.82)	-0.59 (-0.97) -(-0.21)	-1.35 (-1.87.) -(0.83)	-0.36	-.242	-1.23	1.43	-3.22	-1.33
BMI z score	-1.8 (-3)-(-0.85)	-0.35 (-0.48)- (-0.13)	-1.05 (-1.99)- (-0.11)	-0.78 (-1.73)- (0.17)	0.12 (-0.8) -(1.04)	-1.03 (-0.57) -(-1.5)	-0.87 (-2.8.) -(1.06)	0.92	-1.7	-2.6	1.6	-4.51	-0.77
FEV1% median (min-max)	68.5 (61-76)	70 (60-80)	78.5 (69-88)	78.5 (69-88)	82.5 (82-83)	72.5 (50-95)	19	-	-	68	80	56	57
FVC% median (min-max)	77 (57-77)	56 (53-89)	77.5 (66-89)	77.5 (66-89)	86 (84-88)	69.5 (45-94)	23	-	-	71	82	55	50
FEEF25-75% median (min-max)	68.5 (68-69)	86.5 (78-95)	82.5 (70-95)	82.5 (70-95)	88.5 (82-95)	78.5 (57-100)	16	-	-	79	98	73	81
Microbiology of Mucus													
H. influenza, n	2	1	-	1	1	1	1	-	-	1	-	-	1
S. Aures n	-	-	1	-	1	-	-	1	-	-	-	-	-
P. aeruginosa, n	-	-	-	-	-	-	-	1	-	-	-	-	-
S. pneumonia n	-	-	-	1	-	-	-	-	-	-	-	-	-
No Growth n	2	3	-	-	-	-	-	-	-	-	-	-	-
Bronchiectasis n (%)	3 (75%)	3 (75%)	2 (66.6%)	2(100%)	2 (100%)	2 (100%)	1 (50%)	0	0	1 (100%)	0	1 (100%)	1 (100%)

Table 3
Classification comprehensive of identified variations in primary ciliary dyskinesia genes.

Patinets no	Sex	Gene	Transcript ID	Variant: Coding (HGVS nomenclature c.)	Variant: Protein (HGVS nomenclature p.)	Type	Zygosity	Reference Genome
1	F	CCD40	NM_001243342	c.940-1G>C	p.(?)	Splice-site	Homozygous	GRCh37/hg19
2	M	DNAH5	NM_001369	c.5579del	p.(Asn1860Ilefs*11)	frameshift	Homozygous	GRCh37/hg19
3	M	CCD40	NM_001243342	c.940-1G>C	p.(?)	splice_acceptor_-1	Homozygous	GRCh37/hg19
4	F	DYNC2H1	NM_001080463 NM_001080463	c.10366G>A c.2789T>C	p.(Gly3456Ser) p.(Leu930Pro)	Missense missense	Compound heterozygous	GRCh37/hg19 GRCh37/hg19
5	M	DNAH11 HYDIN	NM-001277115 NM_001270974	c.13008G>A c.7603C>T	p.(Trp4336*) p.(Arg2535Cys)	Nonse missense	Homozygous Homozygous	GRCh37/hg19 GRCh37/hg19
6	F	DNAAF4	NM_130810	c.583del	p.(Ile195*)	nonsense	Homozygous	GRCh37/hg19
7	F	CCD40	NM-017950	c.940-1G>C	p.(?)	splice_acceptor_-1	Homozygous	GRCh37/hg19
8	M	ARMC4	NM_018076	c.2528dupT	p.(Leu843Phefs*52)	frameshift	Homozygous	GRCh37/hg19
9	F	DNAH5 DNAH1	NM-001369 NM_015512	c.5747G>A c.1784A>G	p.(Trp1916*) p.(Lys595Arg)	Nonsense missense	Homozygous Homozygous	GRCh37/hg19 GRCh37/hg19
10	M	CCD40	NM-17950	c.2931_2944 dup	p.(asp982Glysf*50)	frameshift	Homozygous	GRCh37/hg19
11	M	TTC25	NM-031421	c.716G>A	p.(Trp239*)	nonsense	Homozygous	GRCh37/hg19
12	M	CCDC65	NM-033124	c.718C>T	p.(Arg240*)	nonsense	Homozygous	GRCh37/hg19
13	F	DNAAF2	NM_018139	c.1199_1214dup	p.(Gly406Argfs*90)	frameshift	Homozygous	GRCh37/hg19
14	F	RSPH4A	NM_001010892	c1351c>A	p.(Gln451Lys)	missense	Homozygous	GRCh37/hg19
15	F	TTC25	NM_031421	c.1079C>A	p.(Ser360*)	nonsense	Homozygous	GRCh37/hg19
16	F	DNAH5	NM_001369	c.6423_6424del	p.(Cys2141*)	nonsense	Homozygous	GRCh37/hg19
17	F	CCNO	NM_021147	c.842C>T	p.(Ser281Phe)	missense	Homozygous	GRCh37/hg19
18	F	DNAAF2	NM-001083908	c.1595A>G	p.(Glu532Gly)	missense	Homozygous	GRCh37/hg19
19	F	DNAAF4	NM_130810	c.583del	p.(Ile195*)	nonsense	Homozygous	GRCh37/hg19
20	M	DNAH11	NM-001277115	c.13008G>A	p.(Trp4336*)	Nonse missense	Homozygous	GRCh37/hg19
21	F	HYDIN	NM-001277115	c.13008G>A	p.(Trp4336*)	Nonse missense	Homozygous	GRCh37/hg19
22	M	DNAAF2	NM-001083908	c.1214_1215ins16	p.gly406Argfs*89	frameshift	Homozygous	GRCh37/hg19
23	F	DNAI1	NM_015512	c.138del	p.Ala47Profs	nonsense	Homozygous	GRCh37/hg19
24	F	DNAH5	NM_001369	c.5579del	p.(Asn1860Ilefs*11)	frameshift	Homozygous	GRCh37/hg19
25	F	CCNO	NM_021147	c.842C>T	p.(Ser281Phe)	missense	Homozygous	GRCh37/hg19

Discussion

In this study, the relationship between the phenotype and genotype of patients with PCD in the southeast Anatolian area of our country was investigated for the first time. More genetic diversity was found in our study than in two earlier studies published in our country.[9,10] Recent migration in this region might have contributed to a rise in genetic diversity. And also, PCD patients with PIBO were reported for the first time with CCNO genetics.

In our study, the mean age of patients at diagnosis was 9.2 years. In published research in our country, the age at diagnosis was found to be 8.3 years, while in another study, it was found to be nine years.[10,11] Our study's mean age was similar to those of previous studies. Studies from different countries show that PCD patients are diagnosed at different ages.[12-14] This difference may be related to regional and international variations in diagnostic capabilities. Patients will be diagnosed earlier as a result of the improvement of diagnostic techniques and their increased accessibility. Now that genetic testing is available in our location, we can diagnose patients earlier.

The percentage of consanguineous marriages among parents in our study was 64%. In two different studies conducted in our country, the rate of consanguineous marriage was 64% and 80.4%, respectively.[10,11] Studies conducted in other countries found between 13-19%.[15,16] This ratio is observed to be significantly higher in our country. People should be educated to minimize consanguineous marriage. PCD incidence may be decreased.

The clinical signs of PCD in children are quite heterogeneous. Some patients may show signs in early infancy, while others may show symptoms later in life. This varies based on the pathogenic effects of the patient's genes. PCD is characterized by a daily productive wet cough, recurrent lung infections, chronic rhinosinusitis, and chronic middle ear inflammation.[17] In contrast to other causes of respiratory distress in term newborns (e.g., transient tachypnea of the newborn—TTN), which often manifest during the first few hours of life, the majority of PCD patients are healthy immediately after delivery but develop respiratory distress at 12–24 hour of life.[17] 50% of PCD patients have situs abnormalities, and 10-12% have congenital heart diseases.[18] The clinical results of our group paralleled those of other studies. At the first examination, most patients (%96) had a history of persistent wet cough and recurrent lung infections. 60% of the patients had situs anomalies. Meanwhile, no laterality defects were observed in patients with DNAH11, TTC25, DYNC2H1, CCDC65, and RSPH4A mutations, as in previous reports.[10,19] 12% of patients also had congenital heart disease. Compared to other studies, we found a lower rate of respiratory symptoms (44%) in newborns.[20] This may be due to our patients' different genetic pathogenic variant outcomes.

Our research shows that patients with a high PICADAR score were diagnosed much earlier. However, there was no correlation between situs inversus and age at diagnosis. Similar to our study, Asfuroglu et al.[21] discovered a significant correlation between the PICADAR score and the age of diagnosis. In contrast to this investigation, there was no association between

situs inversus and the age of diagnosis. A patient with a high PICADAR score will have more complaints and make more frequent clinic visits. Therefore, early diagnosis is possible. However, if the patient has situs inversus and is asymptomatic, the patient's visits to the doctor and referrals to pediatric pulmonology for further diagnosis are decreased. Another reason there was no significant difference between situs inversus and the age at diagnosis in our study may be because there was no pediatric pulmonologist in the region before, and no method could be used for diagnosis in this region. In the last two years, the use of genetic diagnosis methods has increased the diagnosis of patients.

In our study, 72% of the patients had bronchiectasis at the time of diagnosis. 50% had bronchiectasis in one lobe, 38.8% had bronchiectasis in two lobes, and 10,5% had bronchiectasis in three lobes. Markus et al.[22] showed that 56% of pediatric patients had bronchiectasis. In another study, Bronchiectasis was detected in 80.4% of our patients.[9] Different research reports varying rates. This may be due to the patients in the study having different genes. Before the PCD diagnosis, lobectomies were performed on two patients because of bronchiectasis. In most situations, lung surgery is not suggested for PCD; lobectomy may be considered in cases with localized illness resistant to conservative treatment.[23] However, these two patients did not have refractory lung disease. Before deciding on a lobectomy, the cause of bronchiectasis should be investigated beforehand in patients with bronchiectasis.

The most common pathogens are *H. influenzae*, *S. aureus*, *Moraxella catarrhalis*, and *S. pneumonia*.[24] The most prevalent germ discovered in 36% of our patients were *H. influenzae*, which is consistent with findings from earlier investigations. One of our patients (DYCN2H1) had pseudomonas growth even though she was younger. It was linked to having a history of being born early and in the hospital for a long time in the intensive care unit.

Our study found no correlation between growth and nutrition and lung function in patients with PCD. Maglione et al.[24] did a long-term study and found no statistically significant link between the first FEV1 and FVC Z scores and the first BMI Z scores. They also found that the BMI and spirometry were stable during follow-up. The results of our study were similar to those of Maglionin et al.[24] Because PCD pulmonary disease is milder than diseases such as CF, it may not be associated with nutrients and growth.

There is no diagnostic gold standard for PCD. However, there are particular protocols to establish the diagnosis, ideally at a younger age.[4,25] Approximately 65-70% of PCD patients may be diagnosed using next-generation sequencing technologies, contributing to early diagnosis and management. Genetic testing cannot diagnose around 30% of patients, and a negative genetic test does not rule out PCD.[26] Genetic testing was used as a diagnostic method for the patients in our study. Even though six individuals in our research had PICADAR scores >5, situs inversus, and respiratory symptoms, a genetic diagnosis was impossible. We found that in our study, 80% of the patients could be diagnosed genetically, but only 20% could not be conclusively identified. For the diagnosis of PCD, further diagnostic procedures are required. DNAH5, CCDC40, and DNAAF2 were the most prevalent mutations in our study's area. This result was comparable to the genetic frequency discovered in the studies by Emiralioglu et al.[10] and Hornef et al.[27] As distinct from Emiralioglu et al.'s[10] study, we observed DNAAF4, DYNC2H1, CCDC65, and DNAAF2 alleles in our region.

In our study, one patient had PIBO, a previously undiagnosed sign of PCD that was recognized based on clinical symptoms, chest high-resolution CT scans, and lung function. The high-resolution CT scan revealed mosaic perfusion and air trapping (**Figure 1**, **Figure 2**), and the lung function test showed that the tiny airways were significantly obstructed. She had a poor clinical course. She needs oxygen and has a clubfoot. She was underweight, had retarded growth, and had poor lung function. Previously reported in a PCD patient with a DNHA1 mutation.[19] Our patients' mutation is different. This is the first reported case of PCD with PIBO in Turkey. The genetic result of this patient is CCNO. We know that in previous studies, CCNO more commonly presented severe respiratory symptoms.[19,28]

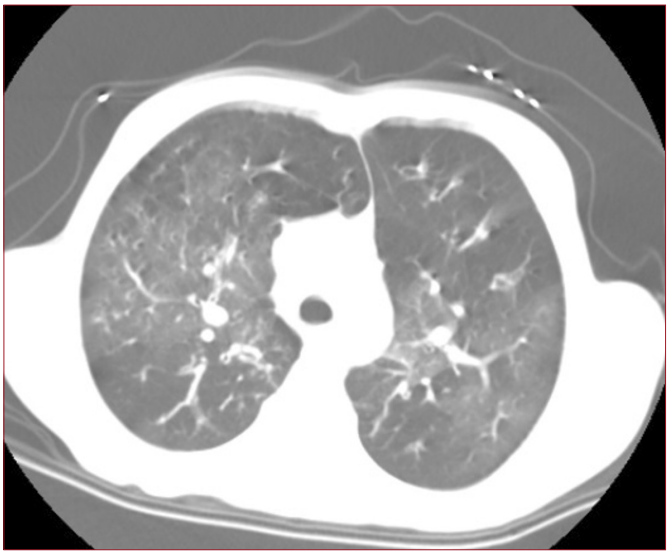

Figure 1. A axial view of HRCT of the lungs shows a mosaic attenuation pattern and air trapping.

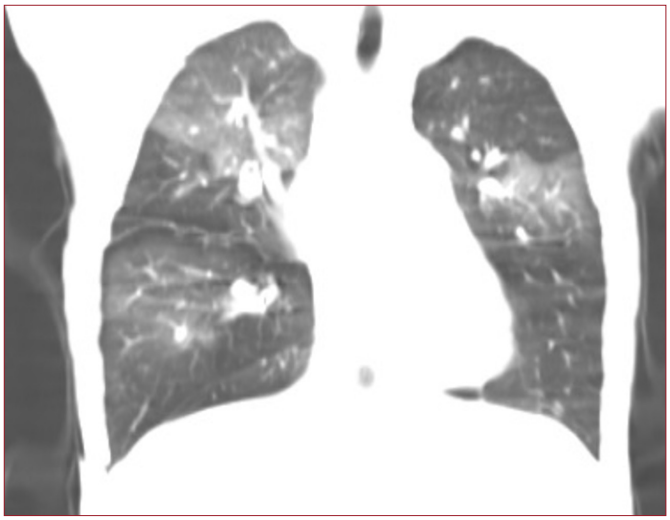

Figure 2. A coronal view of HRCT of the lungs shows a mosaic attenuation pattern and air trapping.

Conclusion

The data presented here are inconsistent with previous findings in Turkey describing the clinical and genetic spectrum of PCD. In contrast to prior research published in Turkey, we found DNAAF4, DYNC2H1, CCDC65, and DNAAF2 alleles in our location. This study reported PCD patients with PIBO for the first time with CCNO. Genotype and phenotype studies will help us determine the prognosis of patients in the future. These findings should increase our knowledge of PCD pathogenic pathways, hence enhancing early illness diagnosis, disease treatment, and prognosis.

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Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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