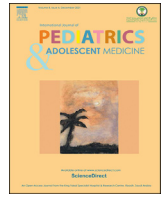


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Clinical and molecular characteristics of primary ciliary dyskinesia: A tertiary care centre experience

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ABSTRACT

Background: Primary ciliary dyskinesia (PCD) is a ciliopathy with diverse clinical and genetic findings caused by abnormal motile cilia structure and function. In this study, we describe the clinical characteristics of confirmed PCD cases in our population and report the radiological, genetic, and laboratory findings.

Methods: This was a retrospective, observational, single-centre study. We enrolled 18 patients who were diagnosed with confirmed PCD between 2015 and 2019. We then analyzed their data, including clinical findings and workup.

Results: In our cohort, 56% of patients had molecularly confirmed PCD, and RSPH9 was the most common gene identified. Transmission electron microscopy (TEM) showed an ultrastructural defect in 64% of samples, all of which matched the genetic background of the patient. Situs inversus (SI) was observed in 50% of patients, and congenital heart disease was observed in 33%. The median body mass index (BMI) was 15.87 kg/m², with a median z score of -1.48. The median FEV1 value was 67.6% (z score - 2.43). Radiologically, bronchiectasis was noted in 81% of patients at a variable degree of severity. Lung bases were involved in 91% of patients. We were unable to correlate the genotype-phenotype findings.

Conclusion: We describe the clinical and molecular characteristics of patients with confirmed PCD in a tertiary centre in Saudi Arabia and report 9 new pathogenic or likely pathogenic variants in one of the PCD-associated genes.

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Abbreviations: PCD, Primary ciliary dyskinesia; TEM, Transmission electron microscopy; BMI, body mass index; HSWA, high-speed video microscopy analysis; nNO, nasal nitric oxide; WES, whole-exome sequencing; NGS, next-generation sequencing; ODA, outer dynein arm; IDA, inner dynein arm.

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1. Background

Primary ciliary dyskinesia (PCD) is a ciliopathy that affects the structure and function of motile cilia. The prevalence is estimated to be 1:15,000–30,000 [1], and as expected, it is high in areas with a high rate of consanguinity [2]. The clinical presentation of PCD includes neonatal respiratory distress, recurrent respiratory infections, sinusitis and bronchiectasis due to impaired mucociliary clearance in the upper and lower airways. Male and female subfertility also results from defective cilia in sperm flagella and fallopian tubes, respectively. Approximately 50% of affected children have situs inversus (SI). Other clinical manifestations include hydrocephalus and complex heart disease due to defective ependymal cilia and nodal cilia, respectively [3].

Transmission electron microscopy (TEM) is used as the mainstay

to diagnose PCD. Approximately 30% of PCD cases have normal or nondiagnostic TEM findings [4]. Other diagnostic modalities include high-speed video microscopy analysis (HSVA), nasal nitric oxide (nNO) and abnormal ciliary protein patterns on immunofluorescent staining. With recent advances in molecular diagnostic techniques, including whole exome sequencing (WES), several novel PCD-associated genes are being increasingly reported. More than 40 different genes are already known to be associated with PCD. Nevertheless, 20%–30% of individuals with PCD do not have identifiable pathogenic variants in any of the associated genes [5,6].

PCD is underdiagnosed, and diagnosis is often delayed due to inadequate awareness, the complexity of diagnostic testing and lack of a consensus on a gold standard diagnostic test. This is unfortunate as a delayed diagnosis results in increased long-term pulmonary morbidity. The aim of this study was to identify the molecular and clinical characteristics of paediatric patients with PCD in Saudi Arabia.

2. Methods

We conducted a retrospective, observational, single-centre study. We enrolled all patients diagnosed with confirmed PCD between 2015 and 2019 who were followed up in our PCD programme at Children Specialized Hospital (CSH), King Fahad Medical City (KFMC), a tertiary centre in Saudi Arabia. Ethical approval was obtained from the institutional review board (IRB Log No. 19–524). Patient data were collected from our electronic health information medical system.

A total of 32 patients were followed up in our PCD programme. These patients were categorised as confirmed, likely or unlikely PCD in accordance with recent guidelines by the European Respiratory Society (ERS) and American Thoracic Society (ATS) [7]. Patients who had pathogenic/likely pathogenic biallelic variants in one of the PCD-associated genes were categorised as confirmed PCD (18 patients). Patients with clinical and radiological findings consistent with PCD but negative genetic tests were categorised as likely PCD after excluding other causes of chronic suppurative lung disease (14 patients). These 14 patients were excluded from our study. We calculated the PICADAR score, a recommended screening tool to predict the likelihood of PCD [8].

Spirometry results (FEV1 and FEF25–75), body mass index (BMI), respiratory secretion cultures (sputum and nasopharyngeal aspirate) and radiological findings were taken into account to describe the phenotype. Spirometry values are expressed in litres as the predicted percentages of an age-matched healthy population (Global Lung Initiative references). ATS standards were adhered to when spirometry manoeuvres were performed. The z score for BMI was calculated, and GLI equations were used to calculate spirometry indices.

TEM samples were obtained by ENT surgeons who are part of the PCD programme. Nasal brush biopsy was the standard method used to obtain samples. If a sample was inadequate, a repeat sample was taken through nasal punch biopsy. Bronchial brush biopsy was obtained if bronchoscopy was performed for any other reason. Samples were maintained in glutaraldehyde solution until fixation. All samples were interpreted by a single histopathologist to ensure consistency of the results.

In most patients, next-generation sequencing panels for PCD were performed in commercial laboratories. WES was performed using Agilent SureSelect version 5 kits on an Illumina HiSeq 4000 system to an average depth of coverage of 150x with automated adapter trimming of the fast sequences (BGI Europe). DNA sequence quality metrics were assessed using FastQC version 0.11.7 at King Fahad Medical City. Alignment, quality filtering and variant identification were undertaken using commercially

available algorithms (DNASTar and Qiagen Clinical Insight-Interpret software). Human reference assemblies were aligned against GRCh37.

3. Results

Eighteen patients (56%) were categorised as confirmed PCD based on positive genetic tests showing homozygous pathogenic or likely pathogenic variants in one of the PCD-associated genes (Tables 1 and 2). Additionally, two patients (patients 9 and 19) were homozygous for variants of unknown significance (VUSs) (*DNAH5*: c.972+5G > A (software prediction tool predicted Splicing impact) and *DNAL1* c.529G > C (p.Asp177His) (*In silico* prediction tools predicted a damaging effect)). In the remainder of patients, test results were negative in three individuals, while others were heterozygous for one or more variants, but none were compound heterozygous for variants in the same gene. TEM samples were obtained from 14 patients. Samples were obtained nasally in 79% of patients and 21% through a bronchial brush sample. Nine patients (64%) had positive findings, while the remaining had either inadequate findings or technical challenges. All results matched the genetic background of the patients (Table 1).

In patients with confirmed PCD, the mean age at diagnosis was 4.7 years (2 weeks–11.7 years), whereas the mean age at the last follow-up was 9.2 years (4 months–14 years). Unexplained neonatal respiratory distress was reported in 83% of patients (n = 15). All patients with confirmed PCD had chronic cough and chronic rhinitis. Moreover, 50% had SI and 33% had congenital heart disease in the form of septal defects. The average number of hospitalisations was 4.7 per patient. The average PICADAR score was 8.9, with 94% of patients scoring ≥ 5 and 39% scoring ≥ 10 . The median BMI was 15.87 kg/m², and the median z score was –1.48.

In our study, 12/18 patients with confirmed PCD could undergo spirometry reliably. The median values for FEV1 and FEF25–75 were 67.6% (z score –2.43) and 36.5% (z score –3.16), respectively. The z scores of FEV1 (r = 0.72) and FEF25–75 (r = 0.67) had a significant moderate positive correlation with the z score of BMI. The most common isolated organism was *Haemophilus influenzae* (35%), followed by methicillin-resistant *Staphylococcus aureus* (26%) and *Streptococcus pneumoniae* (22%). Other less frequently isolated organisms included methicillin-sensitive *Staphylococcus aureus* and *Pseudomonas* species.

CT scans of the chest were performed in 16 patients with confirmed PCD at a mean age of 8.2 years. Bronchiectasis, including early bronchiectatic changes (peribronchial thickening and signet ring) (2/13), cylindrical bronchiectasis (7/13) and cystic bronchiectasis (4/13), was found in 13 (81%) patients at variable severity. The lung bases were likely to be affected (92% of patients), with the right middle and left lower lobes being the most commonly involved areas. A tree-in-bud appearance was observed in 77% of patients (Fig. 1).

4. Discussion

PCD is a clinically and genetically heterogeneous disease that makes a confirmed diagnosis challenging [5]. An ultrastructural ciliary assessment through TEM was previously the gold standard for diagnosis. However, this changed recently as it has some drawbacks as a diagnostic test; some disease-causing mutations could result in a normal axonal structure, and there are difficulties to identify certain structural defects [4,9,10]. Extended gene panel testing is one of the best diagnostic modalities; however, it has limitations, including the interpretation of VUSs and the possibility of missing novel PCD genes that are not included in the panel [11]. Other diagnostic and screening tools are not without limitations.

Table 1
Summary of the clinical and investigation findings in patients with confirmed PCD. IDA: inner dynein arm and ODA: outer dynein arm.

Genetic defect	Mean Age of diagnosis (years)	PICADAR score	Situs inversus (n)	Cardiac defect (n)	BMI	Mean Spirometry		CT chest		TEM
						FEV1 (%)	Z score	Timing (years)	Findings	
<i>RSPH9</i> (n = 3)	9.5	6.3	0/3	1/3 (ASD)	15.9	64.9 (3/3)	-3.35	10	Bronchiectasis (cystic) (n = 3)	Absent central microtubules and supernumerary
<i>CCNO</i> (n = 2)	5.2	5.5	0/2	0/2	12.1	56.3 (1/2)	-3.43	6	Bronchiectasis (cylindrical) (n = 2)	No cilia (n = 2)
<i>ZMYND10</i> (n = 3)	7.6	9.7	2/3	1/3 (ASD)	17.7	74 (3/3)	-1.49	9.7	Early bronchiectatic changes (Signet rings) (n = 2) Bronchiectasis (cylindrical) (n = 1)	IDA and ODA
<i>DNAAF3</i> (n = 2)	0.2	11	2/2	0/2	15.5	47 (1/2)	-4.54	13	Bronchiectasis (cylindrical) (n = 1)	IDA and ODA
<i>DNAH5</i> (n = 1)	0.2	13	1/1	1/1 (ASD)	16	71.6 (1/1)	-2.05	5	Normal (n = 1)	NA
<i>DNAAF4</i> (n = 2)	0.2	11	2/2	1/2 (PDA)	14.5	83.4 (1/2)	-1.11	7	Normal (n = 1)	IDA and ODA
<i>DNAI1</i> (n = 1)	0.1	14	1/1	1/1 (ASD)	16	NA	NA	4	Normal (n = 1)	NA
<i>CCDC151</i> (n = 2)	7.1	8	1/2	0/2	18.4	79 (1/2)	-0.46	8	Bronchiectasis (cylindrical) (n = 2)	ODA
<i>DNAAF1</i> (n = 1)	6.4	8	0/1	0/1	14.3	59.6 (1/1)	-3.05	10	Bronchiectasis (cystic) (n = 1)	IDA
<i>CCDC39</i> (n = 1)	2.6	6	0/1	1/1 (PDA)	12	NA	NA	5	Bronchiectasis (cylindrical) (n = 1)	IDA/Abnormal ciliary orientation
Total (n = 18)	4.74	8.89	9	6	15.47	67.6	-2.4	8.19		9

Table 2
List of patients homozygous for variants in known PCD genes. Patients 4 and 32 are siblings and patients 6 and 15 are cousins. LP: likely pathogenic, P: pathogenic and VUS: Variants of unknown significance.

Patient no.	Gene	DNA nucleotide change	Protein amino acid change	ACMG classification	Note
1	<i>RSPH9</i>	c.804_806delGAA	p.Lys268del	P	—
2	<i>DNAAF1</i>	Exon 5 deletion	NA	LP	Deletion including exon 5 was reported (Davis et al., 2019)
3	<i>CCDC39</i>	c.1061del	p.Glu354Glyfs*2	LP	Previously unpublished
4	<i>CCDC151</i>	c.925G > T	p.Glu309*	P	—
5	<i>CCNO</i>	c.425del	p.Pro142Argfs*15	LP	—
6	<i>ZMYND10</i>	c.155_158del	p.Val52Alafs*23	P	Previously unpublished
7	<i>RSPH9</i>	c.804_806delGAA	p.Lys268del	P	—
9	<i>DNAH5</i>	c.9720+5G > A	NA	VUS	Previously unpublished, reported in ClinVar (Accession: VCV000454819)
11	<i>DNAAF3</i>	c.1105C > T	p.Gln369*	LP	Previously unpublished
14	<i>RSPH9</i>	c.804_806delGAA	p.Lys268del	P	—
15	<i>ZMYND10</i>	c.155_158del	p.Val52Alafs*23	P	Previously unpublished
16	<i>DNAAF4</i>	c.271+1G > T	NA	LP	Previously unpublished
17	<i>DNAH5</i>	c.2278_2279del	p.Gln760Gulfs*11	LP	Previously unpublished
19	<i>DNAL1</i>	c.529G > C	p.Asp177His	VUS	Previously unpublished
22	<i>CCNO</i>	c.833dup	p.Tyr278*	LP	Previously unpublished
24	<i>DNAAF4</i>	c.1111C > T	p.Arg371*	LP	Previously unpublished
27	<i>DNAL1</i>	c.1311+2T > A	NA	LP	Previously unpublished
29	<i>ZMYND10</i>	c.1091C > G	p.Ser364*	LP	Previously unpublished
30	<i>DNAAF3</i>	c.469C > T	p.Arg157*	P	—
32	<i>CCDC151</i>	c.925G > T	p.Glu309*	P	—

nNO requires a chemiluminescence analyser with a velum closure to yield accurate results and is technically a challenge. HSVM, which is used to measure ciliary beat frequency and pattern, requires a high degree of expertise and training [12,13].

In our practice, we utilised genetic tests as confirmatory diagnostic tests. TEM was used as a complementary test because it was recently introduced into our practice and because of the limited experience of our histopathologist. Unfortunately, we did not have access to HSVM or nNO, which is one of the limitations of our study. If confirmatory tests were positive, patients were categorised as confirmed PCD. Otherwise, they were categorised as likely PCD [7]. In the latter patients, we excluded other diagnoses by performing a CT chest scan, a sweat chloride test and an immunological work-up.

Eighteen (56%) patients had confirmed PCD. These patients had homozygous pathogenic or likely pathogenic variants in one of the PCD-associated genes (Table 2). Two patients in the likely PCD group (patients 9 and 19) were homozygous for previously unpublished VUSs. Both patients had recurrent sinopulmonary infection and bronchiectasis; one had SI and the other had two siblings with similar manifestations in addition to unexplained neonatal respiratory distress and SI. Further genetic testing and TEM are being conducted to help upgrade these variants.

The most common variant identified in our study was c.804_806delGAA (p.Lys268del) in the *RSPH9* gene (observed in three different families). In a recent large cohort study conducted in Saudi Arabia, this variant was the most common, which accounted

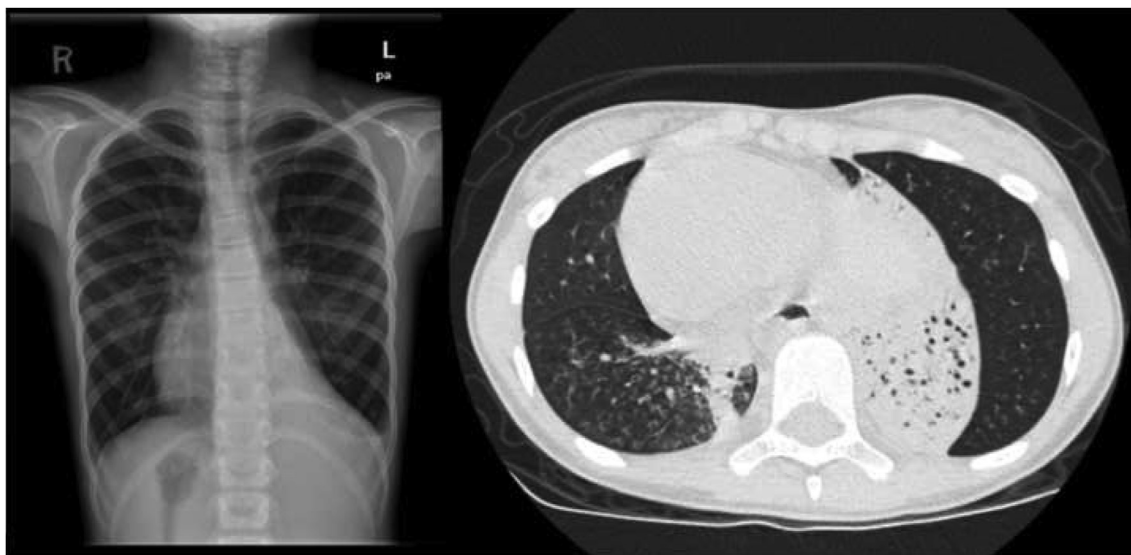


Fig. 1. Chest X-ray shows dextrocardia and hyperinflation with bronchial thickening. There is an atelectatic lung segment on the left lower lobe (arrow head). CT scan of the chest confirms these findings. There is an atelectatic bronchiectatic segment in the left lower lobe (arrow). A tree-in-bud appearance can be appreciated on the right lower lobe (star).

for 34% of families with molecularly confirmed PC, even though it depended on WES and our results were mainly through gene panels [14]. It is worth to mention that studies conducted in other Arab countries with different ancestries (Egypt and Tunisia) reported *CCDC39* as the most commonly involved gene in their population [15,16]. This is in contrast to the American Thoracic Society report of *DNAH5* and *DNAI1* being the most common genes associated with PCD [17]. All other pathogenic or likely pathogenic variants in our cohort were encountered in individual families. Nine of the 15 pathogenic or likely pathogenic variants that we report are novel (Table 2). Our cohort showed a high rate of parental consanguinity (83%), consistent with that reported in the cohort from Turkey but much higher than that reported in PCD cohorts from Belgium (19.6%) [18,19].

The identification of ciliary ultrastructure defects under TEM is one of the best diagnostic tools, with a detection rate of 74%, which is comparable to our rate of 69% [4,11]. We acquired samples through nasal brush biopsy. If a sample was inadequate, a repeat sample was taken through nasal punch biopsy. We performed bronchial brush biopsy only in patients who require bronchoscopy [20]. Ciliary ultrastructural defects are caused by alterations in different cytoplasmic proteins, and each is linked to a specific PCD genetic mutation [3,9]. In our study, combined outer dynein arm (ODA) and inner dynein arm (IDA) defects were the most common. These defects were identified in patients with mutations in the *ZMYND10*, *DNAAF1*, *DNAAF3* and *DNAAF4* genes [21–24]. ODA defects alone were associated with *CCDC151*, IDA defects in combination with ciliary axonal disorientation were associated with *CCDC39*, and the absence of cilia was associated with *CCNO* [9,25,26]. Patients with *RSPH9* mutations did not have the central microtubule complex; however, we could not appreciate the usual finding of radial spoke absence, which is difficult to distinguish because of the blurry, dense background [9,27].

Given the small number of patients, we could not ascertain the genotype-phenotype correlation. However, similar to reports in the literature, organ laterality was identified in 50% of patients, all of whom had SI [11,28,29]. Patients with situs solitus (SS) had a delayed mean age at diagnosis (6.8 vs 2.6 years) as compared to patients with SI (Table 3). The SS group had more severe lung disease even though the SI group was older at the final follow-up

(9.8 vs 8.5 years). This finding was also evident by the average number of admissions (6.56 vs 2.89), mean BMI (14.3 vs 16.7), FEV1 (61.6 vs 74%) and advanced bronchiectasis on CT scans in the SS group. This observation is also supported by Keuhni et al., who advocated for more clinical awareness and not to delay the investigation of patients with a clinical suspicion of PCD because early detection and appropriate management may decrease recurrent infections, the loss of lung function and the progression of bronchiectasis [7,12,30].

The loss of lung function starts in paediatrics and accelerates in older patients [31]. This loss occurs at an annual decline of 0.56%–0.8% in the predicted FEV1 [32,33]. There are discrepancies in reports of the factors that determine the severity of lung function regression, with the most frequently described discrepancies being the genotype-phenotype association, delayed age at diagnosis and nutritional status [18,31,34]. Lung function is closely associated with the nutritional status and both go through a period of steady decline augmented by recurrent exacerbations. Our data validate the link between BMI and lung function, with the nutritionally affected group having the worst lung function and FEV1 showing the strongest correlation with BMI.

A high-resolution chest CT scan might be helpful in making a PCD diagnosis by allowing for the detection of SI with bronchiectasis, and CT scan findings are useful for determining disease severity, which is correlated with FEV1 [35]. Santamaria et al. reported the presence of bronchiectasis in 71% of paediatric patients [36]. However, in our study, 81% of patients had bronchiectasis, with an average age of 8.2 years. Bronchiectasis is almost always central or diffuse and involves the middle and lower lobes. Other common features include a tree-in-bud appearance, atelectasis and mucus plugging [35,37,38]. These findings are similar to those found in our population.

5. Conclusion

In summary, we describe the clinical and molecular characteristics of patients with confirmed PCD in a tertiary centre in Saudi Arabia. We report nine new pathogenic or likely pathogenic variants. We found that *RSPH9* is the most common PCD-causing gene in our region. We recognise that there are some limitations to our

Table 3
Comparison between PCD patient with situs solitus and those with situs inversus.

	n	Age of diagnosis (years)	Average age of cases	PICADAR score	Recurrent admissions	Mean BMI	Spirometry		Radiological evaluation	Average age of study (years)
							FEV1 (%)	Z score		
Situs solitus	9	6.9	9.8	6.6	6.56	14.3	61.6	−3.1	Cylindrical bronchiectasis (N = 5)	6.4
Situs inversus	9	2.6	8.5	11.2	2.89	16.7	74	−1.8	Cystic bronchiectasis (n = 4)	10
									Normal (n = 3)	5
									Early bronchiectatic changes (n = 2)	10.5
									Cylindrical bronchiectasis (N = 2)	11

study, i.e. the lack of other diagnostic tests and the small sample size. However, we believe that it is essential to report new PCD genetic mutations to contribute to the PCD genetic pool.

Declaration of competing interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpam.2021.03.002>.

Funding

Not applicable.

Ethics approval and consent to participate

The Institutional Review Board (IRB) in King Fahad Medical City approved this study (IRB Log No. 19–524). The need to receive an informed consent from all patients was waived due to the retrospective nature of the study.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to confidentiality agreements but are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Visual abstract

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