Clinical manifestations and genotype of primary ciliary dyskinesia diagnosed in Korea: a nationwide, multicenter, retrospective study

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Abstract

Background: Primary ciliary dyskinesia (PCD) is a genetically heterogeneous disorder that leads to secondary ciliary dysfunction. PCD is a rare disease worldwide, and its data are limited in Korea. This study systematically evaluated the clinical symptoms, diagnostic characteristics, and treatment of pediatric PCD in Korea.

Methods: The medical records of paediatric patients diagnosed with PCD from January 2000 to August 2021 were retrospectively evaluated in this Korean nationwide, multicenter study.

Results: Overall, 42 patients were diagnosed with PCD in 15 medical institutions. The median age at diagnosis was 9.9±6.0 years (range: 0.5 months–25.5 years). Most patients (40/42) were born at full term, 18 (42.9%) had neonatal respiratory symptoms, and 15 (35.7%) had a history of admission to the neonatal intensive care unit. The most common complaint (59.5%) was chronic nasal symptoms. Among them, 18 patients had a PICADAR score over 5 points, 32 patients were diagnosed through transmission electron microscopy (TEM) and 14 patients through genetic studies. TEM mostly identified outer dynein arm defects (alone or combined with inner dynein arm defects, n = 16). The genes with the highest mutation rates were DNAH5 (three cases) and DNAAF1 (three cases). Chest computed tomography revealed bronchiectasis in 34 out of 42 patients.

Conclusions: Although PCD is a rare disease, early diagnosis can improve prognosis. However, a single gold standard diagnostic method has not yet been established, and its prevalence may be low owing to misdiagnosis.

Background

Primary ciliary dyskinesia (PCD) is a genetic disorder characterized by predominantly autosomal recessive inheritance and biallelic mutations,[1] with an estimated prevalence of 1 in 10000–40000 people worldwide.[2] PCD is characterized by impaired muco-ciliary clearance accompanied by ciliary dysfunction and gives rise to heterogeneous clinical manifestations such as neonatal respiratory distress, recurrent and chronic oto-sino-pulmonary disease, laterality defects, and infertility.[3] Respiratory tract motile cilia dysfunction is generally observed at an early age.[4] Patients show frequent productive cough and recurrent upper or lower airway disorders during childhood. In PCD patients, lung function starts to decline during childhood and continues to deteriorate with age.[5] In addition, ear diseases are predominant in PCD patients; therefore, inadequate diagnosis and treatment of patients during childhood can result in hearing impairment, speech delay, and learning disabilities in adulthood. Early diagnosis and proper management of pediatric PCD will help to avoid damage to the respiratory and auditory systems and improve the quality of life at later stages.

However, there is currently no single gold standard diagnostic test for PCD.[6] The American Thoracic Society and European Respiratory Society guidelines recommend that the diagnosis of PCD requires a combination of technically demanding investigations, including the nasal nitric oxide (nNO) test, high-speed video microscopy analysis (HSVA) of ciliary beat frequency and pattern, transmission electron microscopy (TEM), ciliary protein immunofluorescence staining, and genetic testing.[1,7] The analysis of TEM findings for the pathology of PCD should be performed by experienced pathologists, and the test should be conducted in the absence of respiratory infection. The quality of ciliary ultrastructure and/or function tests has recently improved. Approximately 30% of patients with PCD show normal ciliary structure and function.[8–10] Recently, methods for diagnosing PCD through genetic testing have been developed for newborns, in whom mucosal biopsy may be difficult, or for patients whose histological results are unclear. More than 40 genes have been identified to be involved in PCD development, although 20–30% of patients with a definite PCD diagnosis do not show a culprit genetic background.[11,12] Several studies have described a direct correlation between genetic anomalies and disease phenotypes.[13] However, the genetic spectrum associated with PCD differs among patients and across nationalities.

Currently, there are limited data and treatment practices for PCD in Korea, due to a lack of awareness and diagnostic approaches. The prevalence of PCD in Korea has not been reported, and most of the available evidence is documented in case reports.[14,15] This reflects the lack of awareness and proper medical practice for PCD among Korean clinicians. In the present study, we

analyzed the clinical characteristics, diagnostic approach, pulmonary function, and therapeutic options of patients with PCD in Korea in a nationwide multicenter study.

Methods

Study population

The medical records of pediatric and adolescent patients diagnosed with PCD between January 2000 and August 2021 at secondary and tertiary hospitals across Korea were reviewed retrospectively. PCD was diagnosed as a PrImary CiliAry DyskinesiA Rule (PICADAR) score of > 5 points based on clinical characteristics or positive results on transmission electron microscopy (TEM) or genetic testing.[3,11,12] Demographic, clinical manifestations, pulmonary function testing, chest computed tomography (CT), and echocardiography data, as well as those concerning any therapeutic options, were collected and documented. This study was conducted in accordance with the declaration of Helsinki and was approved by the institutional review board (IRB No. CNUH-2017-12-029). The authors affirm that written informed consent was obtained from all individual study participants.

PrImary CiliAry DyskinesiA Rule

Patients diagnosed with PCD had at least two out of the four important clinical symptoms (unexplained neonatal respiratory distress, laterality abnormality, chronic productive cough, or chronic nasal symptoms). The European Respiratory Society recommends a PICADAR score > 5 points as a simple diagnostic clinical prediction rule.[3] PICADAR involves patients with persistent wet cough from early childhood and has seven predictive factors: full-term gestational age, neonatal chest symptoms (wet cough, tachypnoea, need for oxygen), neonatal intensive care admittance, chronic rhinitis, ear symptoms, situs inversus, and congenital cardiac defect.

Transmission electron microscopy examination

All samples were obtained from nasal mucosal or bronchial biopsies. The main defects in cilia structure were investigated using TEM. PCD diagnosis requires the identification of an ultrastructural defect in the outer dynein arms (ODA), both ODA and inner dynein arms (IDA), IDA with microtubular disarrangement, microtubule defect with absence of a central pair (9 + 0), or off-central pair associated with transposition of the peripheral doublet to the center (8 + 1).[16]

Genetic analysis

Genomic DNA (gDNA) was extracted from the patients' whole blood samples. Genetic evaluation was performed using whole-exome sequencing. The gDNA extraction, library production and hybridization, and sequencing were performed by Macrogen (Macrogen Inc., Seoul, Korea) using an Agilent SureSelect Target Enrichment protocol for Illumina paired-end sequencing library (Version C2, December 2018) together with 1 ug input gDNA. In all cases, the SureSelect Human All Exon V6 probe set was used. Sequencing was performed using the HiSeq™ 2500 platform (Illumina, San Diego, CA). The raw sequence data were mapped to the hg19 reference genome. Variant analysis pipelines, annotation, and filtering processes were performed using Burrows-Wheeler Alignment Tool (bwa-0.7.12), Picard (v1.130), GAKT (v3.4.0), and SnpEff (v4.1g). For the analysis of whole-exome sequencing data, genes known to be associated with PCD were analyzed more intensively. The clinical significance of variants was analyzed according to the 2015 ACMG/AMP guidelines.[17]

Pulmonary function and imaging studies

Baseline spirometry was performed according to the American Thoracic Society guidelines.[18] Spirometric measurements were expressed as the predicted percentage. Chest CT was performed to evaluate the lung parenchyma. The images were assessed for severity, distribution of bronchiectasis, peribronchial wall thickening, atelectasis, and other findings.

Statistical analysis

Results are expressed as numbers and percentages for categorical data and as range for quantitative data with a non-normal distribution. Quantitative data were compared using the Kruskal–Wallis nonparametric test. A p-value of < 0.05 was considered significant.

Results

Baseline characteristics

In total, 42 patients were diagnosed with PCD across 15 medical institutions, of whom 22 (52.4%) were males (Table 1). The age at diagnosis was 9.9 ± 6.0 years (range: 0.5 months–25.5 years). Most patients (n = 40, 95.2%) were born at full term, 18 patients (42.9%) had respiratory symptoms (tachypnoea, cough, pneumonia) during the neonatal period, and 15 patients (35.7%) had a history of admission to the neonatal intensive care unit. The most common complaint was chronic nasal symptoms (n = 25, 59.5%). Nineteen patients (45.2%) had chronic ear symptoms such as glue ear and serous otitis media, and seven patients (16.7%) showed hearing defect (hearing loss, ear perforation). Seven patients (16.7%) had congenital heart abnormalities and eight patients (19.0%) had situs abnormalities.

Table 1
Demographics and clinical characteristics of patients with primary ciliary dyskinesia diagnosed in Korea

	N = 42
Age at diagnosis (years)	9.9 ± 6.0
Male (n, %)	22 (52.4)
Full term birth	40 (95.2)
Chest symptoms in the neonate	18 (42.9)
Admission neonatal intensive care unit	15 (35.7)
Situs abnormality	8 (19.0)
Congenital heart disease	7 (16.7)
Chronic nasal symptoms	25 (59.5)
Chronic ear symptoms	19 (45.2)
hearing symptoms	7 (16.7)
PICADAR score	
≤ 5	24 (57.1)
6–9	12 (28.6)
≥ 10	6 (14.3)
Data are presented as n (%) or mean ± SD, range. PICADAR: PrImary CiliARy DyskinesiA Rule	

Table 2
Diagnostic tests for patients with primary ciliary dyskinesia diagnosed in Korea

N = 42	
TEM findings (positive finding)	32/36
ODA defect	2
ODA and other defect	14
IDA defect	6
Tubulous disorganization with IDA defect	2
Central pair defect	8
Genetic study (positive finding)	14/18
TEM: transmission electron microscopy, ODA: outer dynein arm defect, IDA: inner dynein arm defect	

Diagnosis

Six patients (14.3%) had a PICADAR score of 10 points or higher and 12 patients (28.6%) scored between 6 and 9 points (Table 1). In total, 36 patients underwent nasal or bronchial mucosal biopsy for TEM analysis; four patients had either normal or inconclusive results. Thirty-two patients had a confirmed PCD diagnosis based on the pathological findings. The TEM results showed that ODA defects (alone or combined other defects = 16) were the most common. The absence of IDA (n = 6) was isolated or associated with microtubular disarrangement (n = 2). Microtubule defect with absence of central pair or single abnormalities were found in eight patients.

Genetic studies were performed in 18 patients. The genes with the highest incidence of mutations were DNAH5 (three cases), followed by DNAAF1 (three cases), CCDC39 (one case), DNAH11 (one case), CFAP300 (one case), RPGR (one case), DNAH8 (one case), NME5 (one case), HYDIN (one case), and DRC1 (one case). Four patients did not show any mutations (Table 3). Of the 18 patients who underwent genetic studies, 14 patients also underwent mucosal biopsy. The genetic study and TEM results are shown in Table 3. DNAH11 mutations were confirmed in those that had normal TEM results. Among them, four patients underwent mucosal biopsy, but the results were normal or inconclusive, and they were diagnosed through genetic testing. Among patients with situs abnormalities, five underwent biopsy, two had ODA and IDA abnormalities, one had a central pair defect, and two had inconclusive results. Genetic testing was performed in three of these patients; DNAH5 mutation was found in two patients and DNAAF1 mutation was found in one patient.

Table 3
Genetic and TEM information for patients with primary ciliary dyskinesia diagnosed in Korea

Patient No.	TEM	Gene	Variant	Previously reported	ACMG classification	Zygosity
1	Not done	DNAH5	c.5647C > T, (p.Arg1883Ter), c.10810dupA, (p.Ile3604AsnfsTer2)		Pathogenic; Likely pathogenic	Compound heterozygote
2	Normal	DNAAF1	c.376del, (p.Glu126LysfsTer35)		Pathogenic	homozygote
3	Microtubular disarrangement	CCDC39	c.1228C > T, (p.Gln410*),		Likely pathogenic	homozygote
4	Normal	DNAH11	c.2169 + 2T > C, c.3853-2A > G		Likely pathogenic; Likely pathogenic	Compound heterozygote
5	Not done	DNAH5	c.3066_3069dup, (p.Ala1024Hisfs*24), c.9365del, (p.Leu3122*)		Pathogenic; Pathogenic	Compound heterozygote
6	Tubular transposition defect	CFAP300	c.[309C > A];[361C > T] (p.[Cys103Ter]; [Arg121Ter]	c.309C > A: novel c.361C > T:[27]	Pathogenic; Pathogenic	Compound heterozygote
7	Not determined	DNAAF1	c.376del, (p.Glu126LysfsTer35), c.1198_1199delinsG, (p.Pro400ValfsTer80)		Pathogenic; Pathogenic	Compound heterozygote
8	Central pair defect	RPGR	c.154G > A (p. Gly52Arg)	Novel in PCD	Likely pathogenic	Hemizygote
9	Not determined	DNAH8	c.2182A > G (p.Met728Val)	Novel	Uncertain significance	Heterozygote
10	Not done	NME5	c.572G > A, (p.Trp191Ter), c.479_480del (p.Tyr160PhefsTer11)		Pathogenic; Likely pathogenic	Compound heterozygote
11	Not done	DNAH5	c.[1089delC](;) [9365delT] (p.[Leu364TyrfsTer3];[Leu3122Ter]	1089delC: novel c.9365delT:[28]	Likely pathogenic/Pathogenic	Compound heterozygote
12	IDA defect	Not detected				
13	Central pair defect	Not detected				
14	Microtubular disarrangement with IDA defect	Not detected				
15	ODA and IDA	HYDIN	c12121A > G (p.K4041E), c.5536G > A (p.E1846K)		Uncertain significance	Heterozygote
16	Not determined	Not detected				

TEM: transmission electron microscopy, ODA: outer dynein arm defect, IDA: inner dynein arm defect

Patient No.	TEM	Gene	Variant	Previously reported	ACMG classification	Zygosity
17	ODA and IDA	DRC1	DRC exon 1–4 deletion	DRC exon 1–4 deletion[29,30]		homozygote
18	Central pair defect	DNAAF1	c.1462C > T(p.Arg488Ter)		Likely pathogenic	Heterozygote

TEM: transmission electron microscopy, ODA: outer dynein arm defect, IDA: inner dynein arm defect

Imaging studies and pulmonary function testing

All patients underwent chest CT. As a result, bronchiectasis and atelectasis were found in 34 and 17 patients, respectively. The most common areas affected with bronchiectasis were the right middle lobe (n = 20), right lower lobe (n = 20), and left lower lobe (n = 18).

Initial lung function tests were performed at the age of 12.5 ± 4.5 years (range: 4.5–24.7 years). Lung function test findings were compared with the TEM results. Pulmonary function was preserved in patients with isolated ODA defect (forced vital capacity [FVC], % predicted: 90.5 ± 14.8, forced expiratory volume in the first second [FEV_1], %predicted: 94.0 ± 0, FEF_{25-75}, %predicted: 109.0 ± 15.6). However, patients with both ODA and IDA defect demonstrated decreased lung function (FVC, %predicted: 71.1 ± 23.8, FEV_1, %predicted: 66.3 ± 18.6, FEF_{25-75}, %predicted: 55.5 ± 47.5). Patients with IDA defect associated with microtubular disarrangement also showed decreased lung function (FVC, %predicted: 81.0 ± 15.6, FEV_1, %predicted: 74.0 ± 13.1, FEF_{25-75}, %predicted: 65.7 ± 14.5). Pulmonary function for patients with central pair defects or IDA alone was also quantified (FVC, %predicted: 87.9 ± 12.4, FEV_1, %predicted: 81.7 ± 14.8, FEF_{25-75}, %predicted: 75.5 ± 26.5). However, the difference was not statistically significant ($P > 1.0$).

Therapeutic options

Each health center had its own treatment practice. N-acetylcysteine (n = 11, 26.2%) was the most used inhalation therapy agent, followed by 7% hypertonic saline nebulizer (n = 8, 19.0%) and 3% hypertonic saline nebulizer (n = 8, 19.0%). Short-acting beta agonists, corticosteroid inhalation, long active beta agonist, and macrolide were used in 26.2% (n = 11), 26.2% (n = 11), 7.1% (n = 3), and 4.1% (n = 1), respectively.

Discussion

To our knowledge, this is the first study of aspects related to the diagnosis and treatment of PCD in children and adolescents using Korean multicenter clinical data. The age of diagnosis for PCD ranged from 0.5 months to 25.5 years, with an average of 9.9 years. The most used diagnostic techniques were TEM and biopsy, and the most common abnormality in PCD was ODA defects (isolated or combined with IDA). It was also observed that majority of the patients are still being followed up due to inconsistent treatments or protocols.

The European Respiratory Society has recommended the use of the PICADAR score, which includes various predictive factors.[3] The sensitivity and specificity of PICADAR score were reported to be 0.90 and 0.75, respectively, for a cut-off score of 5 points.[3] However, only 42.9% of the patients met these criteria in this study. PICADAR scoring helps identify clinical features, but it is not an absolute diagnostic tool for PCD.

PCD diagnosis is known to be delayed, with a median age at diagnosis of 5.5 years in Europe and 2–22 years in the United States.[19,20] The average age at diagnosis of PCD in our study was 9.9 years, suggesting that the diagnosis of PCD was delayed. This may be related to the fact that the most common complaint identified in our study was chronic nasal symptoms, which are very common even in healthy children and adolescents. Therefore, it is important to educate neonatologists, pediatricians, otolaryngologists, and primary care physicians about the clinical features of PCD. Primary symptoms of PCD include neonatal

respiratory distress, even in term babies, chronic persistent lower respiratory tract symptoms, chronic persistent upper respiratory symptoms, and/or lateral defects. If two or more of these clinical manifestations are present, the patients should be strongly suspected for PCD.

Abnormal pulmonary function begins at an early age, and as a result, many children show abnormal airflow function.[21,22] There is disagreement about the correlation between TEM results and lung function findings in patients with PCD.[13,23] One study reported that PCD patients with specific phenotypes such as IDA defects, central pair defects, or microtubular disorganization showed severely impaired pulmonary function.[13] In the present study, most patients showed decreased lung function, except those with isolated ODA defects, but statistical significance could not be achieved given the small number of patients analyzed. A longitudinal study reported that lung function can be preserved with aggressive treatment.[24] National registry management and regular follow-up of lung function should thus be employed to monitor disease progression.[16]

The most widely used PCD diagnostic method is the detection of ciliary abnormalities in clinically suspected patients using TEM. It is known that approximately 70% of cases can be diagnosed by histological examination.[9,10] However, this examination is dependent on adequate sample collection by skilled and experienced clinicians. In fact, in several centers across Korea, biopsy and pathology confirmation could not be performed due to the lack of experienced clinicians or neonatal patients. Moreover, 30% of patients did not exhibit ciliary defects and showed normal axonemal ultrastructure.[20] Mutations in DNAH11 are known to have normal cilia and beat frequencies; our study results are in line with these findings.[25]

To overcome the limitations of pathological testing, genetic tests and nNO are being studied. The introduction of genetic testing has greatly improved diagnosis. The youngest patient in our study was a full-term child with situs inversus and respiratory difficulties diagnosed on the 3rd day after birth, in whom a mutation was found in a genetic test.[15]

PCD is a genetically heterogeneous disease that does not have a clear racial or sexual preference. Mutations in any protein involved in ciliary assembly, structure, or function can cause this condition. Gene discovery has relied on a combination of experimental models and targeted screening of candidate genes encoding proteins of the ciliome. However, whole-exome and massive parallel sequencing has led to the identification of new genes through international collaboration in Europe and North America.[26–30] In the present study, one patient was diagnosed with PCD by TEM, but RPGR mutation was identified later through a genetic study. Since this mutation is known to be associated with retinitis pigmentosa, the patient underwent additional ophthalmologic examinations. Almost all genes associated with PCD are autosomal recessive, except for X-linked syndromic genes (RPGR and OFD1).[16] Furthermore, genetic counselling in patients should also be planned following disease confirmation.

As it is difficult to diagnose PCD through a single test, the diagnostic methods should be selected according to the clinical characteristics of the patient. Ciliary biopsy with TEM, PCD genetic panels, functional ciliary movement with HSVA, nNO, and immunofluorescence testing are the different diagnostic tools available. Nasal fractional exhaled nitric oxide is not invasive and can be easily tested, but it is not commonly standardized, and the functional ciliary movement analysis with HSVA is only possible in centers that are highly experienced in this technology.

Conclusions

The incidence of PCD could be estimated as 1 in 250000 live births for this study. Although we may not have included all cases, considering the prevalence reports in other countries, PCD is thought to be underdiagnosed in Korea. To prevent infection, patients with PCD should undergo daily airway clearance and standard vaccination.[31] In addition, the patients should visit a pulmonology clinic 2–4 times a year for pulmonary function monitoring. In this study, the clinical characteristics of PCD patients in Korea were highlighted, and this is expected to contribute to establishing appropriate follow-up and prognosis.

Abbreviations

PCD, primary ciliary dyskinesia; HSVA, high-speed video microscopy analysis; nNO, nasal nitric oxide; TEM, transmission electron microscopy; IDA/ODA, inner/outer dynein arms; PICADAR, PrImary CiliAry DyskinesiA Rule

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the declaration of Helsinki and was approved by the institutional review board (IRB No. CNUH-2017-12-029). The authors affirm that written informed consent was obtained from all individual study participants.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The data are not publicly available due to privacy or ethical restrictions.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MK: Formal Analysis, Investigation, Writing—Original Draft, Visualization. ML: Conceptualization, Resources. SH, JY, JC, DIS, HYK, HK, SJ, EL, SL, KJ, JYS, JK, HLC, YYJ, JK, JHS, JHK and JYA: Methodology, Resources, Formal Analysis, Writing—Review & Editing. KBS, KS, SYK: Resources, Investigation. SYK and EHC: Conceptualization, Resources, Funding, Writing—Review & Editing, Supervision. All authors read and approved the final manuscript.

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