The Relationship between Genotype and Phenotype in Primary Ciliary Dyskinesia Patients

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Abstract

Objectives: Primary ciliary dyskinesia (PCD) is a chronic genetic disease that affects the respiratory tract, characterized by different clinical and laboratory features. It has a very difficult diagnosis, and high morbidity. In recent years, with the advances in genetics, the rate of diagnosis has increased considerably. In this study, it was aimed to evaluate the relationship between PCD patients’ clinical, radiological and laboratory features and genetic analysis.

Methods: The study included 14 children who were diagnosed with PCD between 2015-2019 and underwent exome analysis. Diagnostic ages, body mass indexes (BMI)- Z score, clinical and radiological findings, pulmonary function tests, sputum culture reproduction and gene analysis were evaluated and compared.

Results: Six of the patients (43%) were girls and 8 (57%) were boys, and the median age at the time of diagnosis was 9 (min-max: 3-16) years. Genetic analysis revealed pathogenic mutations in DNAH5 (n=4, 29%), DNAH11 (n=2, 14%), RSPH4A (n=2, 14%), CCDC40 (n=2, 14%), DNAH9 (n=1, 7%), HYDIN (n=1, 7%), DNAH1 (n=1, 7%), and ARMC4 (n=1, 7%). Although not statistically significant, it was found that the diagnosis age was lower and the BMI Z-score was lower in CCDC40 mutations. Growth parameters were normal in DNAH5, DNAH11, RSPH4A and ARMC4 pathogenic variants. No significant correlation was found between genetic analysis and clinical features, culture reproduction and pulmonary function tests of the patients.

Conclusion: It is thought that more detailed information about the possible clinical features and prognosis of the disease can be obtained by genetic examinations of PCD. However, clinical trials with higher patient numbers are still needed.

Keywords: Bronchiectasis, genetic analysis, primary ciliary dyskinesia, situs inversus totalis.

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Primary ciliary dyskinesia (PCD) is a heterogeneous autosomal recessive disease characterised by abnormal cilia structure and function. Its reported frequency is between 1:2000 and 1:40,000. Common clinical findings of PCD include neonatal respiratory distress, recurrent lower respiratory tract infections, bronchiectasis, persistent otitis, and rhinosinusitis. Fertility problems, organ location disorders, and congenital heart diseases may also be present. The main causes of mortality and morbidity in these patients are bronchiectasis and chronic respiratory failure resulting from recurrent lower respiratory tract infections. Progression of the lung disease can be prevented by early diagnosis and treatment. Although many diagnostic methods such as nasal nitric oxide measurement, electron microscopy, high speed video microscopy, and immunofluorescence and genetic analyses...
have been developed for diagnosing PCD, there is no gold standard diagnostic method. Studies have reported that 70% of cases can be diagnosed by genetic analysis. Today, more than 40 mutations that cause PCD have been identified; thus, genetic examination has become an important method for diagnosing the disease, and new causative mutations have been identified by whole exome sequencing. This study examined the relationships of the genetic findings with the clinical, radiological, and laboratory features of patients with PCD.

**Methods**

The study enrolled 14 patients followed at the 07/05/2020-60012 between 2015 and 2019. This study was approved by the Istanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine ethics committee (08.05.2020/60012).

The patients' sex, body mass index (BMI), Z-score, age at diagnosis, pulmonary function test results, echocardiographic findings, sputum microbiology, genetic analysis, and other findings were recorded during follow-up. Patients with diseases causing recurrent lower respiratory tract infections, such as immune deficiency and cystic fibrosis, were excluded. Pulmonary function was tested in accordance with international standards, and the volume of air exhaled in the first second during forced expiration (FEV₁) and the forced vital capacity (FVC) were recorded. Radiological images were evaluated by the same radiologist. Patients with a Primary Ciliary Dyskinesia Rule score (PICADAR) ≥6 were considered to have PCD. PICADAR is a score to predict the likelihood of having PCD. It can be used in any patients with persistent wet cough and has seven predictive parameters: full-term gestation, neonatal chest symptoms, neonatal intensive care admittance, chronic rhinitis, ear symptoms, situs inversus and congenital cardiac defect. Sensitivity and specificity of the tool were 0.90 and 0.75 for a cut-off score of ≥6 for PCD diagnosis.

Exome analysis was performed in all patients, and the variants were evaluated using databases such as Online Mendelian Inheritance in Man (OMIM), ORPHANET, ExAC, 1000Genomes, and ESP; analysis programs such as Mutation Tester, PolyPhen2, PROVEAN, SIFT, GERP, and CADD; phred; and segregation patterns. We could not perform nasal nitric oxide measurements, electron microscopy, or high videomicroscopic examinations.

**Statistical Methods**

The results were analysed using SPSS for Windows ver. 25.0. Categorical variables are reported as numbers and percentages and numerical variables as medians (range). The chi-square test was used to compare categorical data. To compare numerical data between independent groups, the t-test (Student's t-test) was used if the assumption of normality was met, and the Mann–Whitney U-test was used otherwise. The Mann–Whitney U-test was used to compare two independent groups, and Pearson's chi-square and Fisher's exact tests were used to compare categorical variables. P<0.05 were considered significant in all tests.

**Results**

The patients included 6 (43%) women and 8 (57%) men. The median age at the time of diagnosis was 9 (range 3-16) years, whereas the median age at symptom onset was 1.2 months (range 1 month to 9 years). There were 9 (64%) consanguineous marriages involved (Table 1).

The genetic analysis detected pathogenic mutations in DNAH5 in 4 (29%) patients, DNAH11, RSPH4A, and CCDC40 in 2 (14%) patients each, and DNAH9, HYDIN, DNAH1, and ARMC4 in 1 (7%) patient each. Although the patients with CCDC40 mutations were younger at diagnosis, there was no significant (p=0.33) difference in the age at diagnosis among the mutation groups.

The patients' findings included chronic rhinitis and recurrent lower respiratory tract infections in 14 (100%) patients, recurrent sinusitis in 12 (85.7%), neonatal respiratory distress in 11 (78.6%), recurrent otitis in 9 (64.3%), clubbing in 6 (42.9%), congenital heart disease (atrial septal defect, ventricular septal defect, patent ductus arteriosus, or pulmonary valve insufficiency) in 6 (42.9%), situs inversus totalis in 5 (35.7%), and hearing loss in 4 (28.6%). Only a patient with PCD had been performed ventilation tube placement to the both ears.

The median BMI Z-score of the patients was -0.22 (range -2.90 to 1.62). Although not significant, the BMI Z-score was lower in the patients with CCDC40 mutations and normal in patients with the DNAH5, DNAH11, RSPH4A, and ARMC4 mutations (p=0.40) (Table 2).

On pulmonary function testing, the median FEV₁/FVC, was 86% (70-146%), FVC was 90% (78-159%), and FEV₁/FVC was 95% (76-109%). Pulmonary function could not be tested in one young patient. No relationships were detected between the BMI Z-score and pulmonary function indices (p=0.67). On chest computed tomography (CT), bronchiectasis was detected in 3, 2, 1, and 0 lobes in 6, 5, 2, and 1 patients, respectively. The most common microorganisms in sputum culture were Haemophilus influenzae (9, 64.3%), Streptococcus pneumonia (3, 21.4%), and Pseudomonas aeruginosa (2, 14.3%). There were no significant differences between mutation type and radiological findings (p=0.53) or sputum culture growth (p=0.68) (Table 2). There were no patients with cystic renal disease, hydrocephalus, reti-
nitis pigmentosa in our patients. Also no patients needed non invasive ventilation or oxygen therapy and lobectomy due to PCD.

Discussion

Due to difficulties in diagnosis, PCD is often diagnosed at a late stage. Recently, genetic analysis has been used to diagnose the disease, and new mutations are being identified, although data on the relationships of mutation type with the clinical features and prognosis are insufficient. We found that the mutation type was not a determinant of the clinical features of PCD. With some mutations, the disease progresses more intensely and is diagnosed earlier. Neonatal respiratory distress, recurrent upper and lower respiratory tract infections, situs anomalies, and congenital heart diseases are common findings in PCD. Up to 80% of patients have recurrent neonatal lower and upper respiratory tract infections, 50% have situs anomalies, and 10-12% have congenital heart diseases. Another study conducted in Turkey detected recurrent lung infections in all patients, neonatal respiratory distress in 89.1%, chronic rhinitis in 95.7%, chronic sinusitis in 78.3%, situs inversus to

Table 1. Comparison of the genetic analysis results and demographic characteristics of patients with primary ciliary dyskinesia

|                          | DNAH9 (n=1) | CCDC40 (n=2) | DNAH5 (n=4) | DNAH1 (n=1) | DNAH11 (n=2) | RSPH4A (n=2) | HYDIN (n=1) | ARMC4 (n=1) | All patients (n=14) | p   |
|--------------------------|-------------|--------------|-------------|-------------|--------------|--------------|-------------|--------------|---------------------|-----|
| Age of diagnosis, median (min-max) age | 9 (4-7) | 5.5 (9-16) | 11.5 (9-16) | 16 | 9.5 (9-10) | 7 (3-11) | 13 | 8 | 9 (3-16) | 0.33 |
| Female/Male               | 0/1 | 1/1 | 1/3 | 1/0 | 0/2 | 1/1 | 1/0 | 1/0 | 7/8 | 0.08 |
| Consanguine marriage, n (%) | 1 (100) | 1 (50) | 3 (75) | 0 (0) | 2 (100) | 2 (100) | 0 (0) | 0 (0) | 9 (64) | 0.46 |
| Respiratory distress in neonatal period, n (%) | 0 (0) | 2 (100) | 3 (75) | 1 (100) | 1 (50) | 2 (100) | 1 (100) | 1 (100) | 11 (78.6) | 0.31 |
| Chronic rhinitis, n (%)   | 1 (100) | 2 (100) | 4 (100) | 1 (100) | 2 (100) | 2 (100) | 1 (100) | 1 (100) | 14 (100) | 0.31 |
| Recurrent sinusitis, n (%) | 1 (100) | 1 (50) | 4 (100) | 1 (100) | 2 (100) | 1 (50) | 1 (100) | 1 (100) | 12 (85.7) | 0.85 |
| Recurrent otitis, n (%)   | 1 (100) | 0 (0) | 3 (75) | 1 (100) | 2 (100) | 1 (50) | 0 (0) | 0 (0) | 9 (64.3) | 0.74 |
| Recurrent LRTI n (%)      | 1 (100) | 2 (100) | 4 (100) | 1 (100) | 2 (100) | 2 (100) | 1 (100) | 1 (100) | 14 (100) | 0.31 |
| Hearing loss n (%)        | 1 (100) | 0 (0) | 0 (0) | 1 (100) | 1 (50) | 0 (0) | 0 (0) | 1 (100) | 4 (28.6) | 0.72 |
| Situs inversus totalis, n (%) | 1 (100) | 1 (50) | 2 (50) | 0 (0) | 1 (50) | 0 (0) | 0 (0) | 0 (0) | 5 (35.7) | 0.07 |
| Clubbing, n (%)           | 0 (0) | 0 (0) | 2 (50) | 1 (100) | 2 (100) | 1 (50) | 0 (0) | 0 (0) | 6 (42.9) | 0.56 |
| Congenital heart disease, n (%) | 0 (0) | 2 (100) | 2 (50) | 1 (100) | 1 (50) | 0 (0) | 0 (0) | 0 (0) | 6 (42.9) | 0.13 |

BMI: Body mass index; FEV₁: Forced expiratory volume in the first second of expiration; FVC: Forced vital capacity; H. influenzae: Haemophilus influenzae; P. aeruginosa: Pseudomonas aeruginosa; S. pneumonia: Streptococcus pneumonia.

Table 2. Comparison of genetic analysis and laboratory test results

|                          | DNAH9 (n=1) | CCDC40 (n=2) | DNAH5 (n=4) | DNAH1 (n=1) | DNAH11 (n=2) | RSPH4A (n=2) | HYDIN (n=1) | ARMC4 (n=1) | All patients (n=14) | p   |
|--------------------------|-------------|--------------|-------------|-------------|--------------|--------------|-------------|--------------|---------------------|-----|
| BMI-Z score median (min-max) | -1 | -2.13 | 0.13 | -1.9 | 0.19 | 0.415 | -1.54 | 0.80 | -0.22 | 0.40 |
| (min-max)                | (-2.56-1.71) | (-2.90-1.62) | (-0.34-0.72) | (0.41-0.42) | (-2.90-1.62) | 90 | 95 (92-98) | 111.5 | 78 | 84.5 (84-85) | 0.14 |
| FVC (%), median (min-max) | 90 | 95 (92-98) | 111.5 | 78 | 84.5 (84-85) | 89 | 81 | 89 | 90 (78-159) | 0.23 |
| FEV₁/FVC (%), median (min-max) | 96 | 99.5 (98-101) | 93 | 88 | 82 (96-88) | 89 | 109 | 104 | 95 (76-109) | 0.35 |
| FEV₁ (%), median (min-max) | 93 | 95 (94-96) | 105.5 | 70 | 82 (76-88) | 79 | 72 | 86 | 86 (70-146) | 0.35 |
| Sputum culture growth     | H. influenza, n (%) | 0 (0) | 2 (100) | 3 (75) | 1 (100) | 1 (50) | 1 (50) | 0 (0) | 1 (100) | 9 (64.3) | 0.69 |
| P. aeruginosa, n (%)      | 1 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (100) | 0 (0) | 0 (0) | 2 (14.2) | 0.64 |
| S. pneumonia, n (%)       | 0 (0) | 1 (50) | 1 (25) | 0 (0) | 1 (50) | 0 (0) | 0 (0) | 0 (0) | 3 (21.4) | 0.73 |
talis in 30.4%, recurrent otitis in 30.4%, and hearing impairment in 13% of patients.\cite{10} We obtained similar results regarding the rates of recurrent upper and lower respiratory tract infections, neonatal respiratory distress, and situs inversus totalis, whereas the rates of hearing impairment and congenital heart disease were higher. This difference may be due to the variety of pathogenic mutations. Hearing impairment was detected in patients with DNAH1, DNAH9, DNAH11, and ARMC4 mutations, while congenital heart diseases were detected in patients with DNAH1, DNAH5, DNAH11, and CCDC40 mutations.

The median age at diagnosis was 9 (3-16) years in our study. In a multicentre study conducted by Prescott et al.,\cite{11} the median age at diagnosis was 5.3 years, whereas it was 8±4.2 years in the study by Emiralioğlu et al.\cite{10} The age at diagnosis in our study is similar to that reported by Emiralioğlu et al. and higher than that reported by Prescott et al. This difference may be because patients are referred late to our centre, which is a tertiary hospital, and because the disease is difficult to diagnose. In our study, the rate of consanguineous marriages among the parents was 64%, compared with 80.4% in a similar study conducted in Turkey.\cite{10} The pulmonary function test results and BMI Z-scores in chronic lung diseases are closely related. Patients with a low BMI Z-score had reduced respiratory function, and those with a higher BMI Z-score had better respiratory function.\cite{12} Halbeisen et al.\cite{13} found positive correlations between the mean FEV₁ or FVC and BMI. Goutaki et al.\cite{14} reported better pulmonary functions in patients with good nutritional status. We found no relationship between BMI and respiratory function indices.

Bronchiectasis is common in PCD patients as a result of recurrent lung infections. We detected bronchiectasis in 3, 2, 1, and 0 lobes in 6, 5, 2, and 1 patients, respectively. Another study conducted in Turkey detected bronchiectasis in 80.4% of the patients, with involvement of two lobes in 52.2% and three lobes in 15.2%.\cite{10}

The microorganisms most commonly detected in sputum culture in PCD patients are S. pneumonia, H. influenzae, and Moraxella catarrhalis.\cite{12} In our study, the most common microorganism in sputum culture was H. influenzae (64.2%), similar to previous results.\cite{10,12}

Although many methods have been developed for diagnosing PCD, European Respiratory Society (ERS) and American Thoracic Society (ATS) guidelines concluded that there is no gold standart for diagnosis PCD. Both guidelines agreed that diagnosis of PCD can be confirmed with hallmark cilia ultrastructural defects or a positive genetic test in a person with clinical symptoms. There are some differences in both guidelines regarding the practical use of PCD diagnostic tests. The guidelines differ greatly in their evaluation of ciliary function by HSVMA; it is central to the ERS and not recommended by the ATS guideline.\cite{1,15}

Recently, different mutations have been detected by genetic analyses.\cite{1,4} Hornef et al.\cite{16} reported that the most common mutation in PCD occurs in DNAH5, found in 15-24% of patients. In a study of 46 Turkish patients, DNAH5 was the most common mutation (26.1%).\cite{10} Similar to other studies, DNAH5 was the most common mutation (29%) detected in our study. Very few studies have examined the relationships between mutations and clinical findings. Patients with axonemal organisation disorders of the inner dynein arm, such as those involving mutations in CCDC39 and CCDC40, and absence of cilia, such as those involving CCNO [Cyclin O] mutations, had more severe disease; consequently, these patients had an earlier age at diagnosis and lower BMI and respiratory functions.\cite{10,17} In a study of 118 PCD patients, those with CCDC39 and CCDC40 mutations had a worse clinical picture and earlier age at diagnosis, but much better BMI, especially in patients with external dynein arm mutations, such as in DNAH5.\cite{17} In our study, although not significant, patients with CCDC40 mutations had an earlier age at diagnosis and lower BMI, whereas patients with DNAH5 mutations had a higher BMI. In addition, growth and development were normal in the pathogenic variants DNAH11, RSPH4A, and ARMC4.

Neither our study nor that by Emiralioğlu et al.\cite{10} found any relationships between the type of genetic mutation and sputum culture results or radiological findings. Although, this was likely due to the small numbers of cases.

There are two main limitations in our study. The number of patients is small and we could not perform diagnostic methods such as nasal nitric oxide measurement, electron microscopy, and high speed videomicroscopic examination for PCD diagnosis.

**Conclusion**

Genetic analysis is a diagnostic method guiding the clinical findings and prognosis of rare heterogeneous diseases such as PCD. Patients will be diagnosed earlier, significantly reducing the morbidity and mortality of the disease. Nevertheless, studies with more patients are needed to aid clinicians.

**Disclosures**

**Ethics Committee Approval:** This study was approved by the Istanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine Ethics Committee (08.05.2020/60012).

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**Conflict of Interest:** None declared.
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