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【 CASE REPORT 】

FOXJ1 Variants Causing Primary Ciliary Dyskinesia with Hydrocephalus: A Case Report from Japan

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Abstract:
Primary ciliary dyskinesia (PCD) is a genetic disease characterized by motile cilia dysfunction, mostly inherited in an autosomal recessive or X-linked manner. We herein report a 29-year-old woman with PCD caused by a heterozygous frameshift mutation due to a single nucleotide deletion in exon 3 of *FOXJ1*. Heterozygous *de novo* mutations in *FOXJ1* have been reported as an autosomal-dominant cause of PCD. The patient had situs inversus, congenital heart disease, infertility, and hydrocephalus. However, the nasal nitric oxide level was normal. Long-term macrolide therapy was remarkably effective. This is the first case report of PCD caused by a *FOXJ1* variant in Japan.

Key words: primary ciliary dyskinesia, bronchiectasis, *FOXJ1*, hydrocephalus

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Introduction

Primary ciliary dyskinesia (PCD) is a genetically heterogeneous disease, with an incidence of approximately 1 in 10,000 individuals (1). PCD leads to functional disorders of motile cilia in various organs, especially the airway epithelial cells. Clinically, PCD is associated with sinusitis, otitis media, bronchiectasis, situs inversus, and infertility (2).

Almost all PCD cases are inherited in an autosomal recessive or an X-linked manner. Approximately 50 genes have been reported to be causative of PCD (3). In Europe and North America, more than 50% of PCD cases are attributed to the 4 most frequently occurring genes: *DNAH5*, *DNAH11*, *CCDC39*, and *CCDC40* (4); In Japan, the most common variant is a large deletion spanning exons 1-4 of *DRC1* (5-7). However, genomic DNA analyses sometimes fail to identify the causative variants of PCD. In addition, it is estimated that 20-30% of PCD cases are caused by unknown genetic mutations.

In 2019, Wallmeier et al. reported autosomal-dominant PCD cases caused by heterozygous *de novo* mutations in *FOXJ1*, which is consistent with haploinsufficiency (8). Obstructive hydrocephalus and congenital heart defects are characteristic features of PCD caused by a *FOXJ1* mutation (8-10).

We herein report a case of PCD caused by a *FOXJ1* variant that presented with situs inversus, hydrocephalus, and congenital heart disease. This is the first report of PCD caused by a *FOXJ1* pathogenic variant in Japan.

Case Report

A 29-year-old woman was referred to Fukujuji Hospital because of persistent wet cough. At two years old, she had been diagnosed with endocardial cushion defect and undergone the Fontan procedure. She had no other notable medical history except for infertility treatment. There were no

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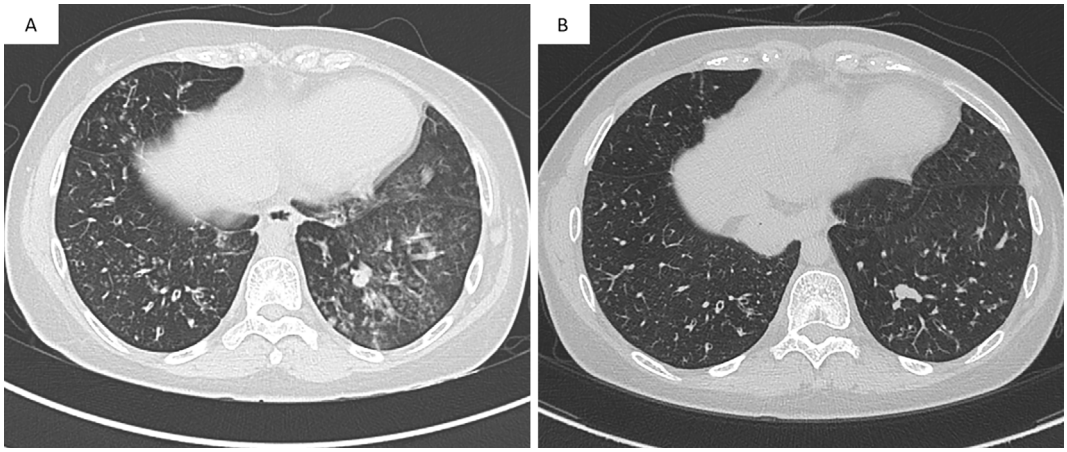

Figure 1. **Chest computed tomography images. Before (A) and after (B) the initiation of long-term macrolide therapy.**

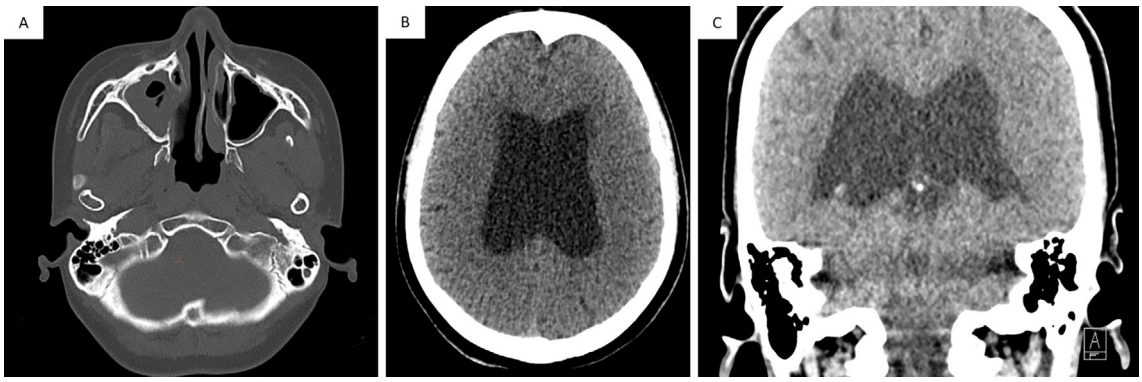

Figure 2. **Sinus computed tomography images showing fluid retention in the right maxillary sinus (A) and enlargement of the lateral ventricles. (B) Axial view, (C) coronal view.**

episodes of consanguineous marriage.

According to our interview, the patient had no respiratory symptoms during childhood. She had been suffering from persistent cough and sputum since she was 20 years old. At 22 years old, she had been diagnosed with diffuse panbronchiolitis (DPB), and long-term macrolide therapy had been initiated at a nearby hospital. After treatment, the patient's respiratory symptoms had improved, so she had stopped visiting the hospital. At 28 years old, her symptoms recurred, and she visited the hospital again. She was diagnosed with bronchiectasis exacerbation and prescribed antibiotics. She was referred to our hospital on suspicion of PCD because computed tomography (CT) revealed situs inversus.

She had no yellow nails or thoracic cage deformities. Chest CT showed cylindrical bronchiectasis and granular shadows in both the lower and middle lobes of the lungs (Fig. 1A). The modified Reiff score was 3. Sinus CT revealed fluid retention in the right maxillary sinus. No hypoplasia or aplasia was observed in the sinuses (Fig. 2A). We detected enlargement of the lateral ventricles on sinus CT and diagnosed the patient with hydrocephalus (Fig. 2B, C). There were no subjective symptoms of hydrocephalus or abnormal neurological findings. If she had a wet cough in childhood, her PrImary CiliAry DyskinesiA Rule (PICADAR) score was 9 points (11). The sputum culture test did not detect any bacterial species such as *Pseudomonas aeruginosa* or *Haemophilus influenzae*. The percent-predicted forced expiratory volume in 1 s (ppFEV1) was 50.3.

To diagnose PCD, we conducted nasal nitric oxide (nNO) measurement, electron microscopy (EM) of the ciliary ultrastructure, and a genetic analysis as described previously (12). The level of nNO was normal (123 nL/min). EM could not be performed because of the low number of ciliated cells obtained from a nasal biopsy. Pathogenic mutations were not found in the genetic analysis of 41 PCD genes. However, polymerase chain reaction (PCR) amplification of the *FOXJ1* gene using the primers 5′-GTTACTGTT CCGTCCCCTTCCTTC-3′ and 5′-CTTAGAGAGGATCTCC TTGCCCCA-3′ and subsequent Sanger sequencing of the PCR product using the primers 5′-CAGCGCCAGGTGCCA TTTCATCTC-3′, 5′-GGGATATGAATCCAGTGCAGCGTG-3′ and 5′-CTCTCATGGTGCAGGGTCCTTTAG-3′ identified a heterozygous frameshift mutation caused by a single nucleotide deletion at c.709delC (p.Arg237Glyfs*5) in exon 3 of the *FOXJ1* gene (NM_001454.4) (Fig. 3).

The patient was re-administered macrolides, and the symptoms improved again. After treatment, the patient

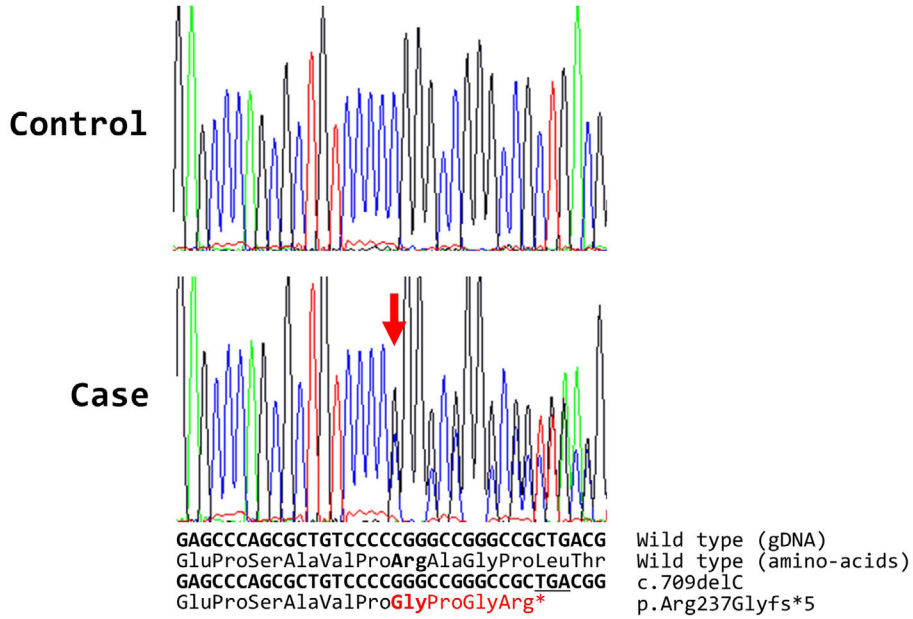

Figure 3. Electropherograms of Sanger sequencing of the *FOXJ1* gene. A heterozygous deletion of one C-nucleotide from a five C-nucleotide stretch was identified by Sanger sequencing of the PCR product. The genomic position of the deletion is chr17: 76137910-76137914 (GRCh38.p14) del G, and this mutation is registered in dbSNP as rs1555648767, but it was not found in 38KJPN (https: //jmorp.megabank.tohoku.ac.jp) or gnomAD (https://gnomad.broadinstitute.org/variant/17-76137909-CG-C?dataset=gnomad_r3) (accessed on June 20, 2023).

showed no exacerbation of bronchiectasis. The radiological findings also improved (Fig. 1B). Pulmonary function tests were not repeated after the long-term macrolide therapy.

Discussion

In this study, we encountered a case of PCD caused by a *FOXJ1* pathogenic variant of hydrocephalus. Similar to previous reports (Table), no abnormal findings were detected in the nNO measurements (8, 9). The patient started exhibiting respiratory symptoms in adulthood and was diagnosed with PCD at 29 years old. Long-term macrolide therapy was remarkably effective.

FOXJ1 is located on chromosome 17q25.1. The gene encodes a transcript of 2,641 base pairs, which produces a predicted protein with 421 amino acids (13). The *FOXJ1* protein plays a critical role in the regulation of genes involved in cilia formation and function. *FOXJ1* variants lead to a decrease in the number of cilia and misplacement of basal bodies in ciliated cells (8). *FOXJ1* is involved in ependymal cell maturation. The motor cilia of the ependymal cells lining the medial ventricles play a crucial role in maintaining the patency of narrow areas of cerebrospinal fluid (CSF) passage during brain formation. Therefore, patients with PCD may present with aqueductal stenosis and obstructive hydrocephalus. However, the mechanisms that cause obstructive hydrocephalus have not been fully elucidated.

Although hydrocephalus is not common in PCD patients, all reported cases of PCD caused by *FOXJ1* variants are complicated by hydrocephalus (8, 9) (Table). Hydrocephalus is an important hallmark of PCD, caused by *FOXJ1* variants. Indeed, in 7 of the 10 reported cases, hydrocephalus was detected in neonates and infants. When hydrocephalus is detected at a young age, investigation of a defect referred to as the reduced generation of multiple motile cilia, such as *FOXJ1* pathogenic variants, should be considered (3). Although no neurological symptoms were observed in the present case, they are likely to appear in the future and should be closely monitored.

Infertility was observed in this woman. In previous studies, up to 80% of men and 61% of women with PCD were infertile (14). Most men with PCD are infertile because of their poor sperm motility. Female infertility is thought to be caused by abnormalities of the oviducts and uterine cilia. In previous reports, women with PCD caused by *FOXJ1* variants presented with infertility (8) (Table). Further studies on female infertility in PCD patients are needed.

In an official American Thoracic Society clinical practice guideline for the diagnosis of PCD, nNO measurement is recommended as a screening test for adult and pediatric patients five years old or older (2). When the cut-off was set at 77 nL/min, the sensitivity and specificity of nNO were 92% and 86%, respectively. The sensitivity was even lower in patients with normal ultrastructures (85%) (15). However, nNO levels were normal in the present case and in previous reports (Table). Various other genetic mutations, such as *DNAH9*, *CCDC103*, and *RSPH1*, have been reported to cause PCD with normal NO levels (16-18). Why nNO is not reduced in PCD patients with these genetic mutations is unclear. Although PCD patients with *FOXJ1* variants exhibit

Table. Summary of Pathogenetic Variants, Patient Characteristics, and Abnormal Findings from Previously Published and Current Reports.

Case no.	*FOXJ1* variants	Gender	Age* (years)	Nasal nitric oxide (nL/min)	Situs inversus	Infertility	Congenital heart disease	Obstructive hydrocephalus	Respiratory distress syndrome	Bronchiectasis	Sinusitis
FRC130 (present case)	Exon3; c.709delC; p.Arg237Glyfs*5	Female	29	123	Yes	Yes	ECD	Yes	No	Yes	Yes
OP1743 II1 (8)	Exon3; c.901G>T; p.Glu301*	Male	0	n/a	No	Unknown	-	Yes	Yes	Yes	Yes
OP1933 II1 (8)	Exon3; c.826C>T; p.Gln276*	Male	0	141	No	Unknown	VSD	Yes	Yes	Yes	Yes
OP2950 II1 (8)	Exon3; c.868_871dup; p.Thr291Lysfs*12	Female	54	122	Yes	Yes	-	Yes	Yes	Yes	Yes
RBH II1 (8)	Exon3; c.967delG; p.Glu323Serfs*10	Female	0	215	No	Yes	-	Yes	No	Yes	Yes
US-1 II1 (8)	Exon3; c.826C>T; p.Gln176*	Male	0	n/a	Yes	Unknown	-	Yes	Yes	Yes	Yes
US-2 II1 (8)	Exon3; c.939delC; p.Ile314Serfs*19	Male	0	328	Yes	Yes	-	Yes	Unknown	Yes	Yes
UNC-1459 (9)	Exon3; c.945delC; p.Phe315Leufs*18	Female	0	n/a	Yes	Unknown	ASD	Yes	Yes	No	Yes
UNC-0852 (9)	Exon3; c.929_932delACTG; p.Asp310Glyfs*22	Male	0	234	No	Unknown	-	Yes	Yes	Yes	Yes
Chinese (22)	Exon3; c734_735ins20; p.Glu246Alafs*3	Female	4	54	Yes	Unknown	-	Yes	No	No	Yes

ESD: endocardial cushion defect, VSD: ventricular septal defect, ASD: atrial septal defect

*Age at the diagnosis of hydrocephalus

normal nNO levels, PCD can be suspected based on other specific findings, such as hydrocephalus, situs inversus, and congenital heart disease.

The European Respiratory Society (ERS) and British Thoracic Society (BTS) guidelines recommend long-term macrolide therapy for non-cystic fibrosis bronchiectasis with a history of three or more exacerbations in one year. The efficacy of long-term macrolide therapy in patients with PCD remains controversial (19). In the BEATCILIA study, patients prescribed azithromycin had a significantly lower exacerbation rate than those prescribed placebo [rate ratio 0.45 (95% confidence interval 0.26-0.78); p=0.004] (20). Although long-term macrolide therapy was effective in the present case, there have been some reports of a poor response to long-term macrolide therapy in PCD (21). The efficacy of long-term macrolide therapy may depend on the severity and genetic subtypes of PCD. In a previous report (9), a 51-year-old man (UNC-0852) with an FEV1 of 0.84 L had undergone lung transplantation, but the history of macrolide use was unknown. Other reports of PCD with *FOXJ1* variants also did not provide details regarding the effects of macrolides. Further studies are needed to determine the differences in the efficacy of macrolides depending on the type of genetic mutation in PCD.

This report lacks some information that would be helpful. First, immunofluorescence and high-speed video microscopy analyses were not performed in our hospital. Second, we could not perform EM because of the low number of ciliated cells obtained in this case. Third, a genetic analysis was not performed for the patient's father and mother. However, neither the patient's father nor mother showed symptoms suggestive of PCD.

We present a case of PCD caused by a heterozygous frameshift mutation resulting from a single nucleotide deletion at c.709delC (p.Arg237Glyfs*5) in exon 3 of the *FOXJ1* gene. This is the first case report of PCD caused by *FOXJ1* variants in Japan.

The authors state that they have no Conflict of Interest (COI).

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