Identification of a Novel OFD1 Variant in a Patient with Primary Ciliary Dyskinesia

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Background: OFD1 encodes a protein with 1012 amino acids, which is a component of basal bodies and centrioles, essential for cilia biogenesis. OFD1 was reported to be associated with X-chromosome linked dysmorphology syndrome in early studies and recent studies reported a few cases with primary ciliary dyskinesia (PCD) caused by OFD1 deficiency.

Case Presentation: We report a 31-year-old man who suffered from recurrent respiratory infections with typical manifestations of primary ciliary dyskinesia. In addition to respiratory manifestations, the patient also had situs inversus, obesity, gastroesophageal reflux, and hearing impairment. Clubbing fingers and mild stellomicrodactyly were also observed.

Examination Result: We performed whole-exome sequencing to identify a novel variant c.2795delA:p.(Lys932Argfs*3) inOFD1. The hemizygous variant was predicted to be likely pathogenic by bioinformatic analysis software and ACMG guideline. High-speed video microscopy (HSVVM), transmission electron microscopy (TEM), and immunofluorescence were performed to analyze the respiratory cilia. A high beating frequency and a stiff beating pattern were observed under HSVVM, while there were no significant abnormalities in TEM and immunofluorescence. The sperm flagella examinations were also generally normal.

Conclusion: Our study identified a novel frameshift variant inOFD1 causing PCD, enriched the genetic spectrum of OFD1 variants, and verified that OFD1 mutation can lead to only a PCD characteristic phenotype, while other OFD1-associated syndromic symptoms such as dysmorphic features and renal symptoms were not present.

Keywords: OFD1, primary ciliary dyskinesia, cilia, situs inversus

Primary ciliary dyskinesia (PCD, MIM 244400) is an inherited genetic motile ciliopathy with a global prevalence estimated at least 1 in 7554 individuals.1 So far, more than 50 genes that affect ciliary structure and function have been reported to be associated with PCD.2 PCD is characterized by recurrent respiratory infections. Since cilia in the respiratory tract, embryonic node, fallopian tube, and flagella shared similar components, patients with PCD may have symptoms involving multiple systems such as bronchiectasis, sinusitis, situs inversus, and infertility.3,4

OFD1, located on chromosome Xp22.2, encodes a protein with 1012 amino acids, which has been known to be associated with dysmorphology syndrome and oral-facial-digital syndrome type 1 (OFDS1). This male-lethal syndrome inherited in an X-linked dominant pattern was first described in 1954.5 Despite controversy exists, two X-linked recessive diseases, Simpson-Golabi-Behmel syndrome type 2 and Joubert syndrome type 10, were also reported to be related to OFD1.6,7 Dysmorphic features, skeletal abnormalities, and renal symptoms are characteristic of these syndromes and the clinical manifestations of OFD1 hemizygous mutation are highly variable between different syndromes or the same syndrome. The OFD1 is essential for cilia biogenesis as a component of centriolar satellites, the particles surrounding centrosomes and basal bodies.8 Patients with a frameshift hemizygous variant of the OFD1 gene exhibiting recurrent...
respiratory infections were reported in 2006. The typical clinical manifestations of the respiratory system and high-speed video microscopy (HSVM) results of the family suggested that the variant of *OFD1* is associated with PCD.9

Herein, we present a case of a patient with a novel variant of *OFD1*, who has typical respiratory manifestations but no associated syndromic symptoms such as dysmorphic features and renal symptoms.

**Case Presentation**

The patient is a 31-year-old male who works as a motor mechanic. His mother died in a car accident and both his father and mother did not have respiratory symptoms (Figure 1A). He has been suffering from coughing and expectoration for 2 years. He was admitted to the hospital because of the aggravation of cough, expectoration and sudden hemoptysis (40 mL). His CT examination revealed bronchiectasis, sinusitis, and situs inversus (Figure 1B–C). Sputum culture showed *Staphylococcus aureus* and *Pseudomonas aeruginosa* infection. The symptoms improved after piperacillin-tazobactam treatment.

We also carried out a detailed examination of the patient. The patient’s height was 158 cm with a weight of 70 kg (body mass index: 28.04), indicating obesity. However, there was no obesity history in the patient’s family. The patient also had fatty liver and hypodensity lipoprotein cholesterolemia. He complained that he had gastroesophageal reflux and hearing impairment in recent years. Lung function test showed predicted forced vital capacity (FVC): 72%, predicted forced expiratory volume during the first second (FEV1): 51%, FEV1/FVC: 73%. His nasal nitric oxide concentration (nNO) was normal: 135.3 nL/min (normal >77nL/min).10 No obvious abnormality was found in the immune system (immunoglobulin and lymphocyte subsets). The patient had clubbing fingers and mild strebloomicrodactyly in the distal interphalangeal joint, but no obvious deformity was found in his head and face (Figure 1D–E).

![Figure 1](https://doi.org/10.2147/PGPM.S365740)

Figure 1 (A) Pedigree of the family with inherited *OFD1* variant. Black arrow, proband; solid symbol, affect family members; crossed-out symbols subject who has passed away. (B) CT scan showed bronchiectasis with infection and situs inversus. (C) CT scan showed sinusitis. (D) The clubbing fingers and mild strebloomicrodactyly of the patient. (E) The fingers of patient's brother. No significant deformity has been found.
Genetic Analysis and Detailed Examinations

Identification of the OFD1 Variant

To further clarify the patient's etiology, whole-exome sequencing was performed and a hemizygous variant of OFD1 was identified after the selection of rare variation (minor allele frequency <1%) related to PCD. The hemizygous variant c.2795delA:p.(Lys932Argfs*3) happened in Exon 21 which was predicted to be disease-causing by Mutationtaster (http://www.mutationtaster.com), which might be associated with the patient's disease. The variant was classified as likely pathogenic according to the ACMG guideline (PVS1 + PM2). Sanger sequencing confirmed that the variant was found in the patient while not in his brother (Figure 2A–B). The primer sequences were designed as follows: forward, 5'-CTGTACTTGAAGGATCGGGATT-3'; reverse, 5'-ATGGCACTGGCACCTTATCT-3'.

Detailed Examination of the Respiratory Cilia and the Sperm Flagella

We also collected respiratory epithelium from patients and performed motile cilia analysis including HSVM, transmission electron microscopy (TEM), and immunofluorescence. The nasal brush sample was suspended in Gibco Medium 199 (12350039 Gibco). Strips of ciliated epithelium were imaged using an upright Olympus BX53 microscope (Olympus, Tokyo, Japan) with a 40x objective lens. Videos were recorded using a scientific complementary metal oxide semiconductor (sCMOS) camera (Prime BSI, Teledyne Photometrics Inc, USA) at a rate of 500 frames per second at room temperature as described. The ciliary beat frequency was calculated using CiliarMove, a validated software. A mix of normal cilia beating and stiff beating was observed and the beat frequency of the patient with the OFD1 variant was higher than that of the healthy control (13.02 ± 3.61 Hz vs 9.52 ± 0.52 Hz) (Figure 3A and Supplementary video 1 and 2). The normal ultrastructure of the axoneme was confirmed in TEM. The normal 9+2 pattern and the presence of dynein arms were observed in all the analyzed cross sections (Figure 3B). Also, both inner and outer dynein arms (DNALI1 and DNAH5) seem normal under the immunofluorescence (Figure 3C). The patient also underwent the fertility examination. The patient had no genital abnormalities. Semen evaluations were performed and more than 200 spermatozoa were observed under the microscope after Papanicolaou staining for morphology analysis. The computer-aided sperm analysis (CASA) and morphological analysis were generally normal except the CASA showed abnormal progressive motility and morphological examination showed that the rate of short flagella deformity was slightly higher than normal (Table 1 and Figure 3D).

Figure 2 (A) The result of Sanger sequencing of our proband and his brother. The variant c.2795delA:p.(Lys932Argfs*3) was found by whole-exome sequencing and validated by Sanger sequencing in our proband. (B) Structure of the OFD1 gene and protein.
PCD is caused by the dysfunction of respiratory cilia, which can also involve motile cilia present on the surface of other organs in the human body. Motile cilia have a highly conserved complexed 9+2 axonemal structure among different species that generate functional ciliary motility. The dysmotility and dysfunction of cilia in the respiratory epithelium result in impaired mucociliary clearance, leading to recurrent sinusitis and bronchiectasis. The abnormal flagella in spermatozoa results in male infertility, and the immotility of cilia in the embryonic node causes laterality defects.

Clinical features of PCD include respiratory distress, pneumonia, and nasal obstruction during the neonatal period. In childhood, PCD patients typically have chronic coughs with sputum, rhinosinusitis, otitis media, pneumonia, and bronchiectasis. The clinical diagnosis of PCD requires typical clinical manifestations combined with pathological changes in TEM or biallelic pathogenic variants in a known gene associated with PCD. The European Respiratory Society recommended that a diversity of technically demanding methods (including nNO, HSVM, TEM and genetic testing) are necessary for the diagnosis of PCD.

Although the clinical symptoms of PCD have certain characteristics, it is difficult to diagnose PCD early and the diagnostic rate has been low due to the lack of understanding of the disease and the limitations of detection methods.

As a component of basal bodies and centrioles, OFD1 has been demonstrated to promote primary cilium biogenesis by autophagy. Cep90 (centrosomal protein of 90 kD), MNR (moonraker) and OFD1 work together as DISCO (distal centriole complex) to promote cilia formation by limiting the length of centriole. In earlier reports, mutations in OFD1 were thought to be associated with severe ciliopathy syndrome. These syndromes often involve the skeleton, nervous system, and kidney. However, the typical respiratory manifestations and situs inversus of PCD are rarely reported in OFD1 mutation. A family with OFD1 mutation that had complications of severe mental retardation, macrocephaly and...
recurrent respiratory tract infections was reported in 2006. Later, \textit{OFD1} mutation was considered to be a pathogenic gene of PCD and was added to the screening panel of whole-exome sequencing of PCD. We reviewed all previously reported cases of \textit{OFD1} mutations, and only 4 patients had only typical PCD symptoms and no other facial or finger abnormalities. All patients are male and three of them have situs inversus. The ultrastructure of the respiratory cilia was normal and the HSVA revealed a stiff beating pattern. Moreover, all mutations were located in exons 20 and 21 (Table 2).

\textbf{Table 1} The Result of Semen Analysis for the Patient

| Semen Parameters | Sperm Morphology |
|------------------|------------------|
| Semen Volume (mL) | Sperm Count (10⁶/mL) | Motility (%) | Progressive Motility (%) | Normal Flagella (%) | Absent Flagella (%) | Short Flagella (%) | Coiled Flagella (%) | Angulation (%) |
| Patient | 7.0 | 107.6 | 65.6 | 30.6 | 77.2 | 4.4 | 5.6 | 8.4 | 9.6 |
| Reference | >1.5 | >15.0 | >40.0 | >32.0 | >23.0 | <5.0 | <1.0 | <17.0 | <13.0 |

Notes: Semen parameters were evaluated according to the World Health Organization guidelines, and reference limits of morphologically abnormal spermatozoa observed in fertile individuals. More than 200 spermatozoa were observed after Papanicolaou staining for morphology analysis.

\textbf{Table 2} The Clinical Feature of This Case and Previous 4 Patients with \textit{OFD1} Mutations Who Had Only Typical PCD Symptoms and No Other Facial or Finger Abnormalities

| | This Case | Case 1 \(^{24}\) | Case 2 \(^{22}\) | Case 3 \(^{22}\) | Case 4 \(^{21}\) |
|---|---|---|---|---|---|
| Sex | Male | Male | Male | Male | Male |
| Age at diagnosis | 31 | 32 | 17 | 17 | 5 |
| \textit{OFD1} mutation | c.2795delA, p. Lys932ArgfsTer3 | c.2868delT, p. Pro957LeufsTer2 | c.2615_19delAAATT, p. Gln872fsTer26 | c.2797G>T, p.Glu933Ter | c.2852delC, p. Ser951LeufsTer8 |
| Sinusitis | Y | Y | Y | Y | N |
| Conductive hearing impairment/recurrent otitis media | Y | N | Y | Y | N |
| Situs inversus | Y | Y | Y | N | Y |
| Gastroesophageal reflux | Y | NA | Y | NA | NA |
| Mental development | Normal | Normal | Normal | Normal | Normal |
| Facial or digital abnormalities | Mild streblomicrodactyly | N | N | N | N |
| Obesity | Y | NA | Y | Y | N |
| nNO (nL/min) | 135.3 | 54.5 | 61.3 | 59.1 | NA |
| HSVM | Stiff beating | NA | Sparse cilia, stiff beating, reduced bending, immotile or nearly immotile cilia | Sparse cilia, stiff beating, reduced bending | Stiff beating, 5.5Hz |
| TEM | Normal 9 + 2 structure of cilia | Normal | NA | Sparse cilia, cytoplasmic accumulation of basal bodies | Microtubular disarrangement |
| Immunofluorescence | Normal | NA | Sparse or abnormally long cilia | Sparse or abnormally long cilia | NA |
| Pathogens | \textit{S. aureus, P. aeruginosa} | \textit{H. influenza} | \textit{H. influenza}, \textit{P. aeruginosa}, \textit{S. aureus} | \textit{H. influenzae, S. pneumoniae} | NA |

Abbreviations: Y, yes; N, no; NA, not available.
This suggests that the mutations near the C-terminal of OFD1 protein will lead to the classic PCD respiratory phenotype without other severe skeletons, nervous system, and renal symptoms. Compared with other patients with OFD1 mutation, these patients have normal intelligence, and only present with respiratory symptoms, gastroesophageal reflux, and obesity. Auditory problems have been reported in 8 of 135 OFDS I cases before, which can be attributed to abnormal development of the nervous system and craniofacial developmental malformations. However, in PCD patients with OFD1 variants, chronic sinusitis can also cause otitis media, resulting in hearing impairment.

In this study, the patient has typical bronchiectasis, sinusitis, and situs inversus. A hemizygous variant in PCD-related gene OFD1 was identified, which is the basis for the diagnosis of PCD in this patient, whereas most other PCD-related examinations such as nNO and TEM do not meet the diagnostic criteria. The nNO of the patient (135.3 nL/min) was normal and significantly different from the criteria for the diagnosis of PCD (normal >77nL/min). However, given the typical clinical symptoms of PCD, we continued to carry out other tests of PCD according to the guidelines, although the patient’s nNO value was inconsistent with PCD. The different nNO values could be explained by the interference of age, health status, and other environmental or genetic factors. The TEM is an important method for direct observation of ciliary ultrastructural abnormalities. In line with the previous studies, the normal 9 + 2 structure of cilia in our patient with OFD1 variant was seen under TEM. The stiff beating of cilia under HSVM in our patients illustrated the effect of OFD1 variant on ciliary function. These phenomena have also been reported in previous patients with OFD1 variants. Nevertheless, in previous reports, OFD1 can lead to the abnormal anchoring of cilia and reduction of cilia on the surface of epithelial cells, which cannot be seen in the TEM and immunofluorescence in our patients. So far, the relationship between clinical manifestations, ciliary ultrastructural defects, genotypes, and ciliary beating patterns in patients with PCD has not been clearly established because of the heterogeneity of the disease, contributing to the extreme difficulty to diagnose PCD.

Infertility is common in male PCD patients. The reason behind infertility is that the flagella of sperm may be involved, such as immotile spermatozoa and multiple morphological abnormalities of the sperm flagella (MMAF). In this study, the patient’s sperm examination was basically normal, with slight abnormalities in the sperm progressive motility and the rate of short flagella. Considering the current results of sperm examinations, it is rather difficult to determine whether the OFD1 variant will lead to infertility. Only one male with OFD1 variant who had offsprings has been reported before, but the author declared that it was uncertain whether the patient had used assisted reproductive technologies. The frameshift OFD1 variant may lead to abnormal ciliary function and affect sperm progressive motility and sperm morphology, which in turn, results in infertility. Besides, in previous reports, some patients with OFD1 variants showed genitourinary malformations. We will continue to track whether patients can reproduce in the future follow-up, and suggest that PCD patients who carry OFD1 variants should monitor the morphology and function of sperm in adolescence in addition to routine genital examination at birth. Fertility counseling and appropriate assisted reproductive technologies are necessary for PCD infertile patient care.

In the present study, we describe a male with PCD that carries a novel hemizygous variant in the OFD1. This patient presented chronic sinusitis, bronchiectasis, and situs inversus. Notably, he has only shown chronic cough in the last two years without obvious symptoms of the respiratory system before, which is inconsistent with the clinical manifestations of classic PCD patients in the past. The patient has obvious clubbing fingers and mild strebloomicrodactyly, and the sperm examination results seem to be normal. Our case expanded the mutation spectrum of OFD1 in PCD and verified that OFD1 mutation can lead to only a PCD characteristic phenotype, while other associated syndromic symptoms were not present.

Data Sharing Statement
The data that support the findings of this study are available from the corresponding author Hong Luo upon reasonable request.

Ethical Compliance
The Review Board of the Second Xiangya Hospital of Central South University in China approved the study protocol. The patient and his brother were given informed consent for publication and for the use of any associated images. All
experiments strictly followed the relevant guidelines and provisions. The clinical data of the subjects, including nasal nitric oxide (nNO) level, computed tomography (CT) and chest radiographs were collected for reviewing.

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**Author Contributions**
All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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**Disclosure**
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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