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CASE REPORT

# A Novel Pathogenic Splicing Mutation of *OFD1* is Responsible for a Boy with Joubert Syndrome Exhibiting Orofaciodigital Spectrum Anomalies, Polydactyly and Retinitis Pigmentosa

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**Abstract:** Joubert syndrome (JS) is an infrequent congenital neurodevelopmental ciliopathy, typically identified in children around the average age of 33 months. This disorder is characterized by developmental delay, cognitive impairment, and infantile hypotonia that may evolve into ataxia. Mutations in *OFD1* results in Joubert syndrome with a variety of phenotypes. Here, we identified a child who presented with Joubert syndrome exhibiting orofaciodigital spectrum anomalies, polydactyly, and retinitis pigmentosa. Whole exome sequencing and Sanger sequencing revealed a splicing mutation (NM_003611.2, c.2387+1G>A) in the *OFD1* gene of the patient and his mother. mRNA sequencing further confirmed this mutation. However, since the patient is homozygous and the mother is heterozygous, only the patient has the phenotype and the mother is normal. This mutation can lead to the loss of sixth coiled-coil domains of OFD1 protein, which further disrupt the ciliary signaling pathway and Hedgehog signaling pathway. This study presents a new case of JS and expands the mutant spectrum of *OFD1*, but also enhances our understanding of the mechanism by which *OFD1* is associated with ciliosis.

**Keywords:** Joubert syndrome, *OFD1*, splicing mutation, ciliosis

## Introduction

Joubert syndrome (JS) (MIM 213300) is an infrequent congenital neurodevelopmental ciliopathy, typically identified in children around the average age of 33 months.[1] This disorder is characterized by developmental delay, cognitive impairment, and infantile hypotonia that may evolve into ataxia.[2] Additional early symptoms of JS have included irregular respiration patterns during infancy, nystagmus, oculomotor apraxia, and premature retinal dystrophy. The prevalence of JS in the population reaches 1.7 per 100,000 for ages 0–19 years.[3,4] The diagnosis of JS is now based on the identification of a distinctive malformation of the midbrain-hindbrain junction, which results in a brain imaging finding known as the "molar tooth sign" (MTS).[5,6] There is a reported range of severity for the MTS, and mild MTS presentations can be challenging to evaluate.[7] On the other hand, other cerebellar and brainstem malformations are sometimes incorrectly interpreted as a mild MTS, leading to incorrect diagnoses.

Most of JS are autosomal recessive, while only JS caused by *OFD1* mutation is an X-linked recessive genetic disease.[8] *OFD1* comprising 23 exons and encoding a centriole and centriolar satellite protein, is located on the Xp22.2.[9] *OFD1* plays a crucial role in ciliogenesis by regulating the length of the centriole and the formation of distal appendages in the mother centriole.[10] The lack



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of OFD1 leads to abnormal elongation in the distal regions of centrioles, resulting in long centrioles that form structurally unstable microtubules with unusual post-translational modifications. In human, *OFD1* mutations lead to abnormal ciliary morphology and malformations in the face, oral cavity, and digits.[11] In zebrafish, the absence of OFD1 results in bent body axes, edema, and disrupted fluid flow in Kupffer's vesicles due to shortened and disrupted axonemes in cilia.[12]

In this study, we identified a child who presented with Joubert syndrome exhibiting orofaciodigital spectrum anomalies, polydactyly, and retinitis pigmentosa. Through whole exome sequencing and Sanger sequencing, we identified a splice mutation in the patient's *OFD1* gene. Our research not only expands the mutation spectrum of *OFD1*, but also provides new insights into the study of Joubert syndrome.

# Materials and Methods

## Subjects

The research received authorization from the Maternal and Child Health Care Hospital of Hunan Province (Approval no.:2023-S190). The participant in this study signed informed consent forms, and the proband's guardian consented to the collection of proband information. In this study, a boy with orofaciodigital spectrum anomalies, polydactyly and retinitis pigmentosa came to Hunan Provincial Maternal and Child Health Care Hospital for treatment in November 2023. A compilation of family health histories, additional diagnostic tests, and peripheral blood specimens has been gathered for further analysis. The peripheral blood was collected from the patient (III-1), as well as his father (II-1) and mother (II-2).

## Whole-Exome Sequencing and Sanger Sequencing

Genomic DNA was isolated from the lymphocytes in the patients' peripheral blood with the DNeasy Blood and Tissue Kit provided by Qiagen, located in Valencia, CA. The core component of the Whole Exome Sequencing (WES) was supplied by Berry Genomics Company Limited, based in Beijing, as outlined in our earlier work.[13] All the exomes were enriched using the Agilent Sure Select Human All Exon V6 kits and sequenced by Illumina HiSeq X Ten platform. Using a Mastercycler® X50 PCR machine (Eppendorf, Germany), we conducted Polymerase Chain Reaction (PCR) using designed primers (Forward 5'-3' CCCTACCATCACCCACT, Reverse 5'-3' ACTGCCTTGGCATGTTC). RNA was extracted from blood using the RNA Extraction Kit (YESEN, 19211ES60), and then reverse transcribed into cDNA using the Reverse Transcription Kit (ProMab Biotechnologies Inc, E30101). The amplified products were then sequenced on an ABI 3100 Genetic Analyzer (ABI, USA).

## Bioinformatics Analysis

The MetaDome website (https://stuart.radboudumc.nl/metadome/) was utilized to analyze whether the affected amino acid positions were located in intolerant regions. Structural changes were examined through protein modeling with the aid of the SWISS-MODEL tool (https://swissmodel.expasy.org/). The Mutation Taster website (https://www.mutationtaster.org/) was employed to assess whether the mutations were pathogenic.

# Results

## Case Presentation

Here, we enrolled a 4.5-year-old Chinese child diagnosed with Joubert syndrome. The patient exhibited congenital brain developmental abnormalities, externally manifested as a forehead protrusion (Figure 1A). Magnetic resonance imaging revealed elongation and deformation of the midbrain's superior cerebellar peduncles, forming the characteristic "molar tooth sign" on axial views (Figure 1B). Simultaneously, the patient had an enlarged fourth ventricle with abnormal morphology, resembling a "bat-wing" shape. Additionally, the patient exhibited retinitis pigmentosa (Figure 1C) and polydactyly, with six fingers on both the left hand and left foot (Figure 1D and E). An X-ray of the patient's hand revealed the development of phalanges on the sixth finger of the left hand (Figure 1F).

## Genetic Analysis

An investigation of the patient's family history revealed that no other family members of the patient exhibited a similar phenotype (Figure 2A). To investigate the genetic defect in this patient, whole exome sequencing and Sanger sequencing

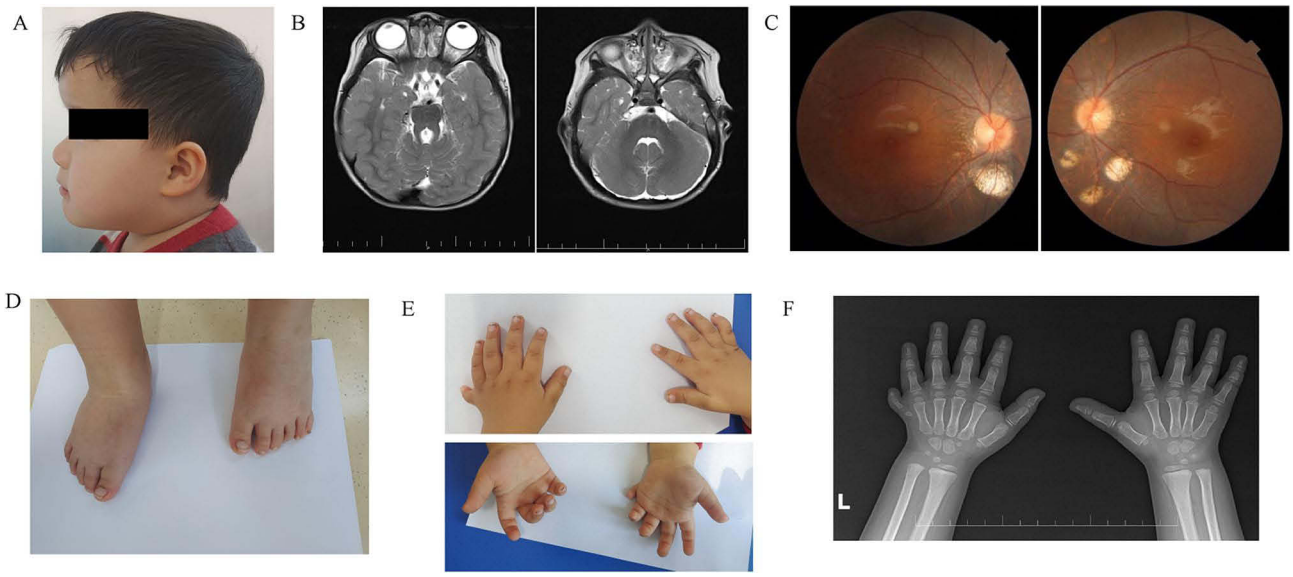


**Figure 1** Clinical and genetic data of proband with Joubert syndrome. (**A**) The proband's head appearance, with a forehead protrusion. (**B**) Magnetic resonance imaging of the proband's head. The junction of the midbrain and cerebellum shows a "molar tooth" change (left), and the upper part of the fourth ventricle is enlarged, forming a "bat wing" shape (right). (**C**) Eye examination of the proband, showing retinitis pigmentosa. (**D**) Morphological photo of the proband's foot, showing a sixth toe on the left foot. (**E**) Morphological photo of the proband's hand shows that the patient has six fingers on his left hand. (**F**) X-ray of the proband's hand, showing a sixth finger with phalanges on the left hand.

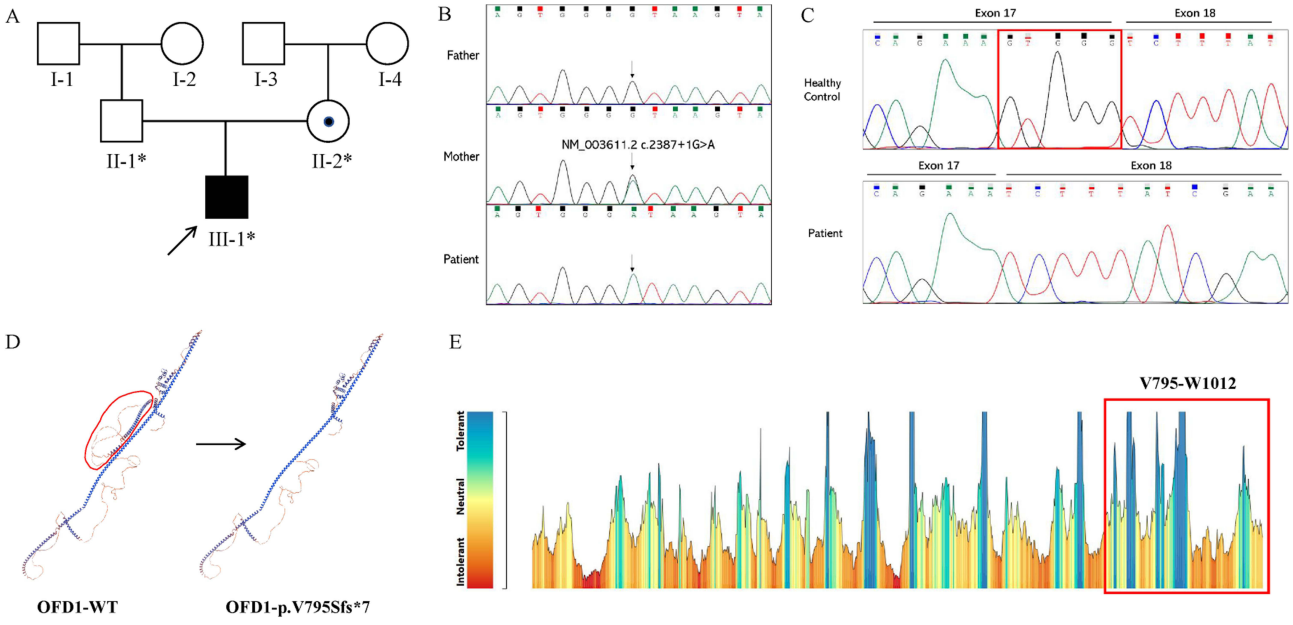


**Figure 2** Whole exome sequencing revealed a novel mutation in the *OFD1* gene. (**A**) The family pedigree is shown, with members identified by generation and number. Squares represent males, circles represent females, black symbols indicate individuals with Joubert Syndrome, the arrow points to the proband, asterisks mark participants in this study, and circles with a dot denote carriers of the sex-linked recessive gene. (**B**) Sanger sequencing chromatogram shows that the proband's homozygous mutation in the *OFD1* gene (NM_003611.2, c.2387+1G>A) originates from the heterozygous mutation carried by the mother. (**C**) Sanger sequencing of cDNA obtained from reverse transcription of RNA shows that the RNA sequence of the proband lacks the last 5 bases of exon 17, which are normally present in healthy members. (**D**) Comparative analysis of the protein structure before and after OFD1 mutation, showing that the mutated protein has lost a large segment of its structure. (**E**) Analysis of amino acid tolerance in the OFD1 protein reveals that the lost structure is enriched with a significant number of intolerant amino acids after the mutation.


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were utilized. The detailed steps were described as our previous study.[14] After data filtering, we identified a novel splicing mutation (NM_003611.2, c.2387+1G>A) of *OFD1* gene in the proband and Sanger sequencing further validated that the novel mutation was in the proband and his mother (Figure 2B). Sanger sequencing of cDNA confirmed that the splice mutation led to the deletion of five bases "GTGGG" at position c.2382_2383, which resulted in a premature stop codon at position 802 (Figure 2C). We utilized Swiss-model to analyze and predict the protein structures before and after mutation, revealing that the mutated protein lost a large segment of its coiled structural domain (Figure 2D). Subsequently, we employed the MetaDome website to assess the amino acid tolerance of the OFD1 protein, and the results indicated that the affected amino acid sequences contained a significant number of intolerant regions (Figure 2E). The Mutation Taster website predicted that the mutation is pathogenic. According to the ACMG guidelines, this variant was assessed as pathogenic (PVS1+PS3+PM1+PM2+PM3+PM4+PP1 +PP3).

## Discussion

The *OFD1* gene is expressed in a variety of tissues, including the brain, heart, lungs, kidneys, and spleen. The protein structure of OFD1 is primarily composed of one LisH domain and six coiled-coil domains.[15,16] Previous studies revealed that the OFD1 protein played a crucial role in cell division, the formation and maintenance of the cell cytoskeleton, and intracellular material transport.[9]

The zebrafish presented bent body axes, hydrocephalus, and edema after antisense morpholinos disrupting Ofd1 expression. Further studies demonstrated that Ofd1 deficiency led to short cilia which further disrupted axonemes and perturbed intravesicular fluid flow in Kupffer vesicle.[17,18] Meanwhile, Ferrante et al generated the Ofd1 knockout mice and observed that mutant male embryos exhibited defects in left-right axis specification, and ultrastructural analysis revealed an absence of cilia in the embryonic node.[19,20]

In 2001, Ferrante et al identified the *OFD1* mutation in the patients with oral-facial-digital syndrome.[21] Since then, more than 100 *OFD1* mutations were detected in patients with Simpson-Golabi-Behmel syndrome, Joubert syndrome and retinitis pigmentosa. Recently studies considered that OFD1 was a cilioprotein that involved in cilia formation and establishment of left–right asymmetry. This protein is associated with both primary and motile ciliopathies, presenting phenotypes that range from isolated retinitis pigmentosa to multiorgan involvement.

Some studies have revealed that within the first 16 exons of the *OFD1* gene, missense mutations are more likely to lead to Joubert Syndrome, while frameshift mutations are more likely to result in OFD1 syndrome. OFD1 syndrome is an X-linked dominant genetic disorder that is often fatal for males and frequently causes significant deformities in females, including abnormalities in the mouth and skeleton.[22] This phenomenon may be due to frameshift mutations occurring in earlier positions that often lead to extensive truncation of the OFD1 protein, thereby reducing its binding capacity to functionally related proteins, such as lebercilin. Mutations beyond the 16th exon do not affect the fifth coiled-coil domain and previous domains of the OFD1 protein, nor do they affect its localization around the centrosome, thus rarely leading to very severe phenotypes. Furthermore, some researchers have found that the same mutation of *OFD1* can lead to different phenotypes in different populations. For example, the c.2789–2793del mutation reported by Thauvin-Robinet presents as Joubert Syndrome in a French family,[23] while the same mutation, as reported by Yifei Xu, manifests as primary ciliary dyskinesia in a Japanese family.[24] The mechanisms behind these differences require further investigation.

OFD1 and lebercilin both play crucial roles in the ciliary signaling pathway and may further regulate the Hedgehog signaling pathway involved in the development of multiple organs. The conserved coiled-coil domains 5 and 6 of the OFD1 protein are responsible for the interaction between OFD1 and the ciliary protein lebercilin.[15] Figure 3 showed a brief overview of all pathogenic variants in OFD1 gene listed in HGMD and a schematic representation of the OFD1 protein structure.[25–27] The splice mutation in *OFD1* (c.2387+1G>A) that we reported is located exactly between the 5th and 6th coiled-coil domains of OFD1.

This mutation leads to a truncation of amino acids starting from position 795, resulting in the loss of the 6th coiled-coil domain. This further disrupts the ciliary signaling pathway and the Hedgehog signaling pathway, ultimately leading to orofaciodigital spectrum anomalies, polydactyly, and retinitis pigmentosa.[28] Our research further validated the pathogenicity of this splice mutation and confirmed the clinical diagnosis in affected patients.

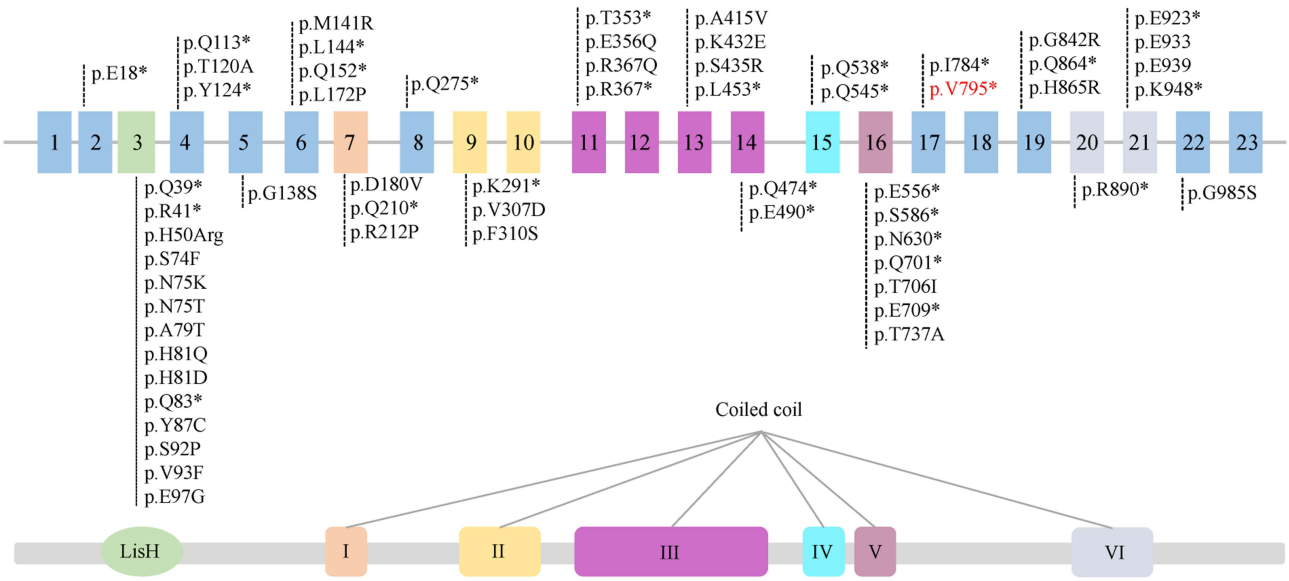


**Figure 3** A summary of *OFD1* gene mutations reported in the HGMD has been compiled, along with the protein domains of OFD1. The domains are represented by colored sections corresponding to the exon regions they map to, with the red parts indicating the mutations reported in this study.

## Conclusions

In summary, we detected a novel splice mutation (c.2387+1G>A) of *OFD1* in a child with orofaciodigital spectrum anomalies, polydactyly and retinitis pigmentosa. mRNA sequencing validated that the splice mutation resulted in a deletion mutation c.2382_2383delGTGGG of *OFD1*. Our research not only broadens the spectrum of *OFD1* gene mutations but also provides valuable information for genetic counseling of Joubert syndrome patients. Moreover, the mutation we identified is one of the few that occur after the 17th exon, leading to the loss of the sixth coiled-coil domain in the OFD1 protein. This work offers some reference for further exploration of the function of the sixth coiled-coil domain of OFD1.

## Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Ethical approval

We confirmed that we have read the journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. The publication of case details in this article does not require institutional approval.

## Informed Consent

Written informed consent has been provided by the patient's parent for the publication of case details and any accompanying images.

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We thank all subjects for participating in this study.

## 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 no competing interests in this work.

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