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Long-Surviving Adult Siblings With Joubert Syndrome Harboring a Novel Compound Heterozygous *CPLANE1* Variant

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Abstract

Background and Objectives

We describe 2 long-surviving siblings with a mild phenotype of Joubert syndrome (JBTS) harboring a novel compound heterozygous missense variant in the *CPLANE1* gene.

Methods

Targeted sequencing data of 2 middle-aged siblings (sister and brother) with JBTS were analyzed.

Results

The patients were older than 60 years and presented with an inborn facial anomaly and ataxia, accompanied by a molar tooth sign on brain MRI. The male patient showed mild intellectual disability, abnormal eye movements, and progressive gait disturbance. Targeted sequencing revealed a compound heterozygous missense variant of *CPLANE1* p.Arg1193Cys_Gln1223Pro; c.3577C>T_3668A>C. Multiple in silico assays predicted that the missense sites were pathogenic.

Discussion

The phenotype-genotype correlation of *CPLANE1* remains controversial, although many cases have been previously reported in children and young adults. Our study revealed a novel pathogenic variant of *CPLANE1* in patients, confirming the role of this gene in JBTS, thus providing an opportunity for neurologists to recognize JBTS as a differential diagnosis for chronic progressive ataxia in an aging society.

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Glossary

CADD = combined annotation-dependent depletion; **IFT** = intraflagellar transport; **JBTS** = Joubert syndrome; **MMSE** = Mini-Mental State Examination; **MTS** = molar tooth sign; **SHH** = sonic hedgehog; **SIFT** = Sorting Intolerant From Tolerant.

Joubert syndrome (JBTS), first described in 1969, is a rare lethal congenital disorder¹ characterized by hypotonia, abnormal breathing patterns, oculomotor apraxia, intellectual disability, and a specific brain malformation—the “molar tooth sign (MTS).”^{2,3} Associated variants have been reported in more than 35 genes coding for proteins of the primary cilia, which play an important role in the development of the skeleton, retina, neurons, kidney, and liver.³ We describe the cases of 2 adult siblings with JBTS presenting with a mild clinical phenotype and harboring an unreported gene variant.

Methods

Patients

We enrolled a pair of siblings who visited the Division of Neurology, Kobe University Graduate School of Medicine and were diagnosed with JBTS. Detailed clinical characteristics, blood tests, electrophysiologic examinations, and brain images were also assessed.

Genome Analysis

Genomic DNA was isolated from peripheral blood leukocytes of both patients using a DNA extraction kit (Qiagen, Germany). Targeted sequencing was performed using an Ion Torrent system (Illumina, San Diego, CA) for 24 genes (*AH11*, *ARL13B*, *CPLANE1*, *CC2D2A*, *CEP290*, *CEP41*, *CXORF5*, *EXOC8*, *INPP5E*, *IQCB1*, *KIF7*, *NPHP1*, *NPHP4*, *RPGRIP1L*, *SDCCAG8*, *TCTN1*, *TCTN3*, *TMEM138*, *TMEM216*, *TME M231*, *TMEM237*, *TMEM67*, *TTC21B*, and *ZNF423*).

The variants of *CPLANE1* (NM_023073.4) were confirmed by direct Sanger sequencing of the genomic DNA and subcloning. The PCR primers used for subcloning were *CPLANE1* forward (5'-CCTCAGGAAGATGGTGATGATCTTCTTTTAAAAGC-3') and reverse (5'-GGTGGAACTAACCTAGAGACCCTGTTCAGA-3'). PCR was performed using a PCR master mix (KOD One [KMM-101], TOYOBO, Osaka, Japan) and cloning vector (TOPO TA cloning kit for sequencing [45-0030], Invitrogen, MA). Variant pathogenicity was predicted using sorting intolerant from tolerant (SIFT), Polyphen-2, and Combined Annotation-Dependent Depletion (CADD v1.6).⁴⁻⁶

Ethical Approval

This study was approved by the ethics committee of the National Institute of Neuroscience, National Center of Neurology and Psychiatry (Approval No. A2014-036), and written consent was obtained from all patients.

Data Availability

The data in this study are available from the corresponding author (N.C.) on request.

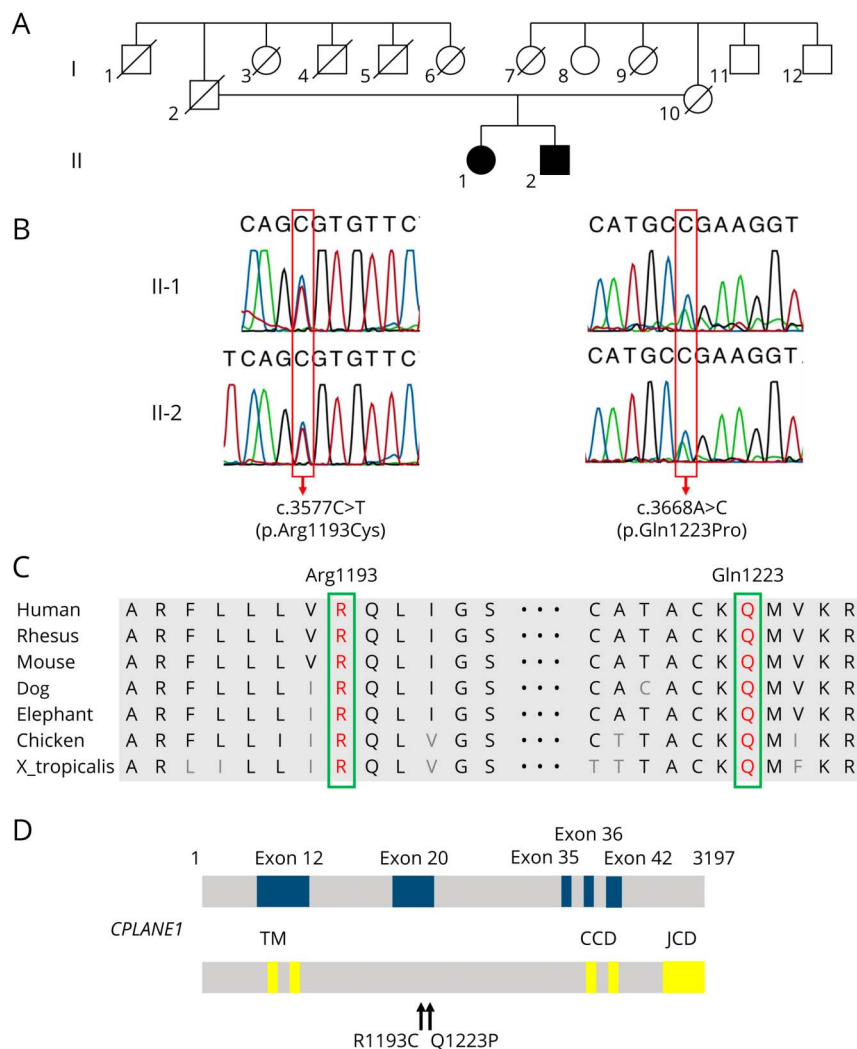
Results

Case Presentation

Patient 1 was a 64-year-old Japanese woman with unrelated parents (Figure 1A). She exhibited a flat nasal root (Figure 2A), but no oral or digital abnormalities. She had no apparent history of respiratory failure or dizziness at age 62 years. The patient's Mini-Mental State Examination (MMSE) score was 26. Neurologic examination revealed strabismus, saccadic pursuit of extraocular movements, slurred speech, and postural tremors. The patient showed decomposition and dysmetria in the nose-finger-nose test. Brain MRI revealed MTS with fourth ventricle dilatation, absent cerebellar vermis, profound interpeduncular fossa, and elongated superior cerebellar peduncles (Figure 2B).

Patient 2 was her 63-year-old brother. He was born in a breech position with strabismus, had a delayed first cry, dysarthria, was not good at running, and was diagnosed with cerebral palsy in his childhood. The patient had graduated from university. He had no history of excessive alcohol consumption. The patient presented with a complaint of slow progressive gait disturbance. He exhibited a broad and high forehead, hypertelorism, flat nasal root, thin upper lip, large chin, and broad toes (Figure 2C). He had no apparent oral findings. His MMSE score was 28, with a total IQ of 90 (verbal IQ = 102; performance IQ = 76) on the Wechsler Adult Intelligence Scale III. Neurologic examination revealed saccadic pursuit of extraocular movements and postural tremors of the upper limbs. His motor strength, sensory function, and deep tendon and plantar reflexes were normal. He had dysdiadochokinesis, left-dominant dysmetria, and intention tremors. He showed decomposition in the lower extremities and ataxic wide-based gait. He had no bladder-rectal disorders or orthostatic hypotension in the head-up tilt test. Blood test results showed high triglyceride and hemoglobin A_{1c} levels (6.7%). Hypercapnia or hypoxia was not observed in the arterial blood-gas test. Radiography revealed no evidence of digital malformation. CT revealed no cystic lesions in the kidneys or liver. EEG findings were normal, but polysomnography showed severe central sleep apnea syndrome with an apnea-hypopnea index of >40. Brain MRI revealed an MTS similar to that in patient 1 (Figure 2D). Magnetic resonance tractography also suggested a deficit in the crossing of the superior cerebellar peduncles. In addition, 123I-IMP single-photon emission CT revealed normal perfusion in the brainstem and cerebellum.

Figure 1 Family Pedigree, Genetic Findings, Conservation Analysis, and the Scheme of *CPLANE1*



(A) The family pedigree of the cases is shown. I-2: died of choking due to dysphagia at age 78 years; I-10: suffered from cerebral hemorrhage and died at age 84 years; II-1: patient 1; and II-2: patient 2. (B) Genetic findings in cases with the *CPLANE1* variant. Targeted sequencing analysis of genomic DNA revealed a compound heterozygous c.3577C>T_3668A>C variant. (C) Comparison of *CPLANE1* from different species generated by the UCSC Genome Browser. Arg1193 and Gln1223 were highly conserved. (D) The black arrows indicate the location of the variants. Arg1193 and Gln1223 are located in exon20. CCD = coiled coil domain; JCD = Joubert syndrome-associated conserved domain; TM = transmembrane domain.

Exon-Targeted Sequence Revealed a Novel Missense Variant in the *CPLANE1* Gene

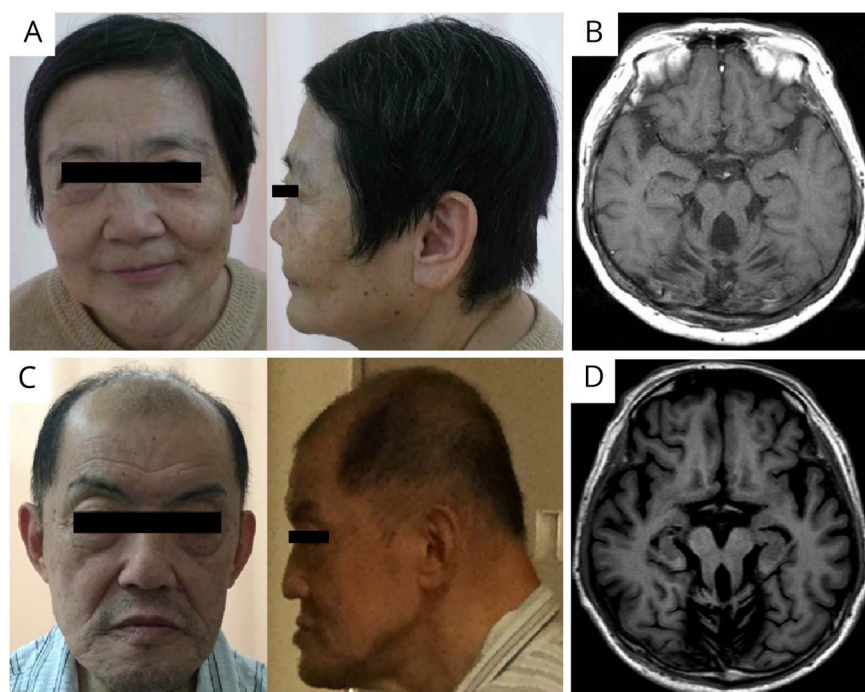
A previously unreported compound heterozygous missense variant in the *CPLANE1* gene was identified—p.Arg1193Cys_Gln1223Pro; c.3577C>T_3668A>C. Sanger sequencing confirmed the variant in both patients (Figure 1B). Each PCR product of the variant was subcloned and confirmed as trans (eFigure 1, links.lww.com/NXG/A548). Although the c.3577C>T variant has already been described in the literature,^{7,8} the c.3668A>C missense variant was present in neither the gnomAD nor the Human Gene Mutation Database and had moderate (PM5) or supporting pathogenicity (PP2) in the American College of Medical Genetics and Genomics guidelines.⁹ Both the SIFT and Polyphen-2 algorithm analyses predicted the variant to be damaging. Mutation Taster analysis also indicated a likely pathogenic gene variant. The combined annotation-dependent depletion (CADD)-phred scaled score was 27.4 for 3577C>T and 23.9 in 3668A>C. Arg1193 and Gln1223 were highly conserved among the species (Figure 1C; University of California, Santa Cruz (UCSC) Genome

Browser), and the location of *CPLANE1* is shown in Figure 1D.³

Discussion

CPLANE1, also known as *JBTS17* or *C5ORF42*, is responsible for ciliogenesis and the planar polarity effector.¹⁰ JBTS is a ciliopathy, and the related gene encodes primary cilia proteins with important roles in the development of many organs, with variants causing mid-hindbrain malformation. *CPLANE1* proteins are localized in the ciliary transition zone and aid in the recruitment of intraflagellar transport (IFT) proteins to the basal body of cilia. The IFT system links cargo to microtubule motors for bidirectional transport in the axonemes. In the absence of *CPLANE1*, IFT-A proteins fail to localize to the basal bodies and assemble. Fibroblasts from *CPLANE1*-mutated patients show fewer and shorter cilia and a diminished response to a sonic hedgehog (SHH) agonist.¹¹ Macrocephaly and facial widening are general signs of disturbed SHH signalling.

Figure 2 Craniofacial Features and Imaging of Middle Age JBTS Siblings



(A) Patient 1 had a broad and high forehead, flat nasal root, and large ears. (B) The T1-weighted MRI of patient 1 showed a MTS. MRI. (C) Patient 2 showed craniofacial features similar to those of patient 1: broad and high forehead, hypertelorism, flat nasal root, thin upper lip, and large chin. (D) T1-weighted MRI of patient 2 shows. JBTS = Joubert syndrome; MTS = molar-tooth sign.

To date, more than 125 *CPLANE1* variants have been definitively associated with JBTS,³ but patients aged older than 60 years have not been reported. *CPLANE1* was first reported as a causative gene of JBTS in a portion of families.¹² The presence of truncated variants in *CPLANE1* is associated with oral-facial-digital syndrome type VI and results in a severe phenotype and early death.¹³ The c.3577C>T variant has already been described to be associated with developmental delay and without respiratory, kidney, or liver abnormality.^{12,13} Our male patient had a relatively severe facial anomaly, developmental delay, and gait disturbances. However, the target genes causing the neurodevelopmental outcomes in our case remain unknown.

Although both the SIFT and Polyphen-2 assays predicted the variants to be damaging, the CADD scores were low, indicating mild phenotypes. This report highlights novel potential pathogenic variants of JBTS in long-term surviving adult patients. Thus, it widened the disease entity to include a mild phenotype with a low CADD score genotype.

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Disclosure

K. Matoba, N. Chihara, W. Satake, H. Tokuoka, Y. Otsuka, T. Ueda, K. Sekiguchi, M. Itoh, and R. Matsumoto report no disclosures relevant to the manuscript. Full disclosure form information provided by the authors is available with the full text of this article at [Neurology.org/NG](https://www.neurology.org/NG).

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Kento Matoba, MD	Division of Neurology, Kobe University Graduate School of Medicine, Kobe	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Norio Chihara, MD, PhD	Division of Neurology, Kobe University Graduate School of Medicine, Kobe	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
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Hideki Tokuoka, MD	Division of Neurology, Kobe University Graduate School of Medicine, Kobe	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data

Appendix (continued)

Name	Location	Contribution
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Takehiro Ueda, MD, PhD	Division of Neurology, Kobe University Graduate School of Medicine, Kobe	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Kenji Sekiguchi, MD, PhD	Division of Neurology, Kobe University Graduate School of Medicine, Kobe	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Masayuki Itoh, MD, PhD	Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data
Riki Matsumoto, MD, PhD	Division of Neurology, Kobe University Graduate School of Medicine, Kobe	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; additional contributions: supervised the study

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