Novel CC2D2A compound heterozygous mutations cause Joubert syndrome

DAIMIN XIAO¹, CHUNLI LV¹, ZHIMIN ZHANG², MINGSONG WU², XUANG ZHENG², LEI YANG², XUEYING LI², GUAN WU³ and JINDONG CHEN³

¹Clinical Laboratory, Zunyi Medical University Affiliated Hospital; ²Department of Genetics, Zunyi Medical University, Zunyi, Guizhou 563099, P.R. China; ³Department of Urology, University of Rochester Medical Center, Rochester, NY 14642, USA

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Abstract. Joubert syndrome (JS) is an autosomal recessive disorder, which is characterized by hypotonia, ataxia, psychomotor delay, and variable occurrences of oculomotor apraxia and neonatal breathing abnormalities. JS is clinically and genetically heterogeneous. The present study investigated a typical JS family. The ‘molar tooth sign’ was observed in the proband through magnetic resonance imaging. Other symptoms of JS include cerebellar vermis hypoplasia/dysplasia, oculomotor apraxia and intellectual disability. High-throughput sequencing revealed that JS was caused by coiled-coil and C2 domain containing 2A (CC2D2A) compound heterozygous mutations. One CC2D2A allele was affected with a missense mutation, c.2581G>A, which led to a p.Asp861Asn amino acid replacement. The other allele was affected with a c.2848C>T nonsense mutation, which resulted in a truncated CC2D2A protein (p.Arg950Ter). Both of these alterations are novel. Further investigation indicated that the proband’s father was the c.2581G>A carrier, whereas the proband’s mother was the c.2848C>T carrier. These results indicated that JS in the proband was caused by novel compound heterozygous mutations in CC2D2A, which were inherited from both parents. These findings may be used to establish prenatal molecular diagnostic criteria, which may be beneficial in future pregnancies.

Introduction

Joubert syndrome (JS) is a recessive or X-linked genetic disorder, which is characterized by hypotonia, apnea/hyperpnea in infancy, oculomotor apraxia, psychomotor delay/mental retardation, and cerebellar vermis hypoplasia and dysplasia. JS is accompanied by brainstem abnormalities, which result in radiological detection of the ‘molar tooth sign’ (1-6). JS can be classified into six phenotypic subtypes, based on other clinical abnormalities detected alongside JS, including occipital encephalocele, polymicrogyria, kidney lesions, polydactyly, hepatic fibrosis and ocular coloboma (6). JS has an incidence of 1 in 80,000-100,000 individuals in the United States (6). Several of the JS phenotypes are similar to those associated with Meckel syndrome (MKS) (7-9), which is a lethal recessive disorder that is characterized by renal cystic dysplasia; occipital encephalocele, or other central nervous system phenotypes; polydactyly and hepatic fibrosis. Therefore, some researchers consider JS and MKS to be different phenotypes of the same disease. JS is clinically and genetically heterogeneous, and overlaps with several other ciliopathies, including nephronophthisis, Senior-Loken syndrome and MKS (10). To date, >30 causative genes (11,12) have been identified in JS.

Coiled-coil and C2 domain containing 2A (CC2D2A) was initially described in JS and MKS in 2008, and further investigations have elucidated the role of CC2D2A in ciliary function (13-19). Previous studies have linked JS, and other syndromic disorders, to defective cilia (14,16,20-22). The present study identified a JS family with novel compound heterozygous mutations in CC2D2A.

Case report

Ethical permission for genetic analysis and collection of test data in the present study was granted by the Research Ethics Committee at Zunyi Medical University (Zunyi, China). Written informed consent was obtained from the participants or participants’ family. The proband was an 8-month-old male who was diagnosed with JS before being subjected to cerebral magnetic resonance imaging (MRI) using a 1.5 Tesla MRI scanner (Siemens AG, Munich, Germany). The patient matched the JS diagnostic criteria: Cranial MRI demonstrated brainstem ‘molar tooth sign’ (Fig. 1) (6,23). Further
examinations indicated that the proband exhibited hypotonia, psychomotor delay/intellectual disability, nystagmus and jerky eye movements. Other tests, including complete blood count, blood biochemistry, serum lactate, urine ammonia, plasma and urine amino acids, and metabolic screening of blood, were conducted in our laboratory.

Upon JS diagnosis, 10 µg DNA was extracted from a blood sample collected from the proband according to the protocol of the Invitrogen genomic DNA extraction kit (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), in order to determine what genetic alterations caused the disease. High-throughput exome sequencing was performed on the DNA samples obtained from the proband by Shenzhen BGI Diagnosis Technology Co., Ltd. (Shenzhen, China). The sequencing results revealed that the proband possessed *CC2D2A* compound heterozygous mutations (c.2581G>A/c.2848C>T), and demonstrated that two *CC2D2A* alleles were mutated (Fig. 2). The c.2581G>A mutation is located on exon 21, whereas the c.2848C>T alteration is situated on exon 23 of the gene. The c.2581G>A mutation results in an amino acid replacement (p.Asp861Asn), whereas the c.2848C>T mutation leads to an immediate stop code (p.Arg950Ter), which results in a truncated form of the *CC2D2A* protein.

To determine the source of the mutations, the proband’s parents and grandparents were recruited for genetic examination. Furthermore, the mother was pregnant (6 months) and also decided to screen the fetus. An MRI analysis indicated that the female fetus displayed molar tooth sign. Blood samples were collected for DNA extraction from the proband’s parents, grandparents, and the proband. The present study screened for mutations in the samples obtained from the proband’s available parents and grandparents using routine polymerase chain reaction (PCR) and DNA sequencing on exons 21 and 23. The PCR primers were designed and synthesized by Invitrogen (Thermo Fisher Scientific, Inc.). PCR was performed according to the manufacturer’s protocol [Thermo Scientific Dream Taq Green PCR Master Mix (2X); #K1081; Thermo Fisher Scientific, Inc.]. Briefly, 50 pg patient DNA was mixed with 1 µM PCR primers and 25 µl Taq Green PCR Master Mix; nuclease-free water was added to ensure the final volume was 50 µl. The mixture was initially denatured for 3 min at 95°C; PCR was then conducted for 35 cycles at 95°C for 30 sec, 58°C for 30 sec and 72°C for 1 min; finally, the PCR products underwent extension at 72°C for 5 min. DNA sequencing was carried out by Shenzhen BGI Diagnosis Technology Co., Ltd. The PCR primer sequences were as follows: Exon 21, forward...
In the present family, JS was caused by novel compound heterozygous mutations in CC2D2A inherited from the parents. These findings may be used to establish prenatal molecular diagnostic criteria, and may be beneficial in future pregnancies. In the present study, the proband's mother was pregnant and was subject to prenatal diagnosis based on the family history. The pregnancy was terminated at the parents' choice due to the confirmed compound heterozygous CC2D2A mutations and phenotypic defects.

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References


Discussion

The present study identified a JS family with novel compound heterozygous mutations in CC2D2A. Both mutations detected are not present in the 1000 Genomes database (1000genomes.org) or the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/ EVS/), thus indicating that they are novel CC2D2A mutations. Since the proband's parents are mutation carriers and not affected, both mutations are causative based on the recessive genetic two-hit theory.


