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Identification of 1q21.1 microduplication in a family

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Abstract

BACKGROUND

Copy number variation (CNV) has become widely recognized in recent years due to the extensive use of gene screening in developmental disorders and epilepsy research. 1q21.1 microduplication syndrome is a rare CNV disease that can manifest as multiple congenital developmental disorders, autism spectrum disorders, congenital malformations, and congenital heart defects with genetic heterogeneity.

CASE SUMMARY

We reported a pediatric patient with 1q21.1 microduplication syndrome, and carried out a literature review to determine the correlation between 1q21.1 microduplication and its phenotypes. We summarized the patient's medical history and clinical symptoms, and extracted genomic DNA from the patient, her parents, elder brother, and sister. The patient was an 8-mo-old girl who was hospitalized for recurrent convulsions over a 2-mo period. Whole exon sequencing and whole genome low-depth sequencing (CNV-seq) were then performed. Whole exon sequencing detected a 1.58-Mb duplication in the CHR1:145883867-147465312 region, which was located in the 1q21.1 region. Family analysis showed that the pathogenetic duplication fragment, which was also detected in her elder brother's DNA originated from the mother.

CONCLUSION

Whole exon sequencing combined with qPCR can provide an accurate molecular diagnosis in children with 1q21.1 microduplication syndrome, which is of great significance for genetic counseling and early intervention.

INTRODUCTION

Copy number variation (CNV) has become widely recognized in recent years due to the extensive use of gene screening in developmental disorders and epilepsy research^[1]. In this study, we performed molecular genetic analysis on a child admitted to the hospital in October 2019 with unexplained seizures and found approximately 1.58 Mb of duplication in the 1q21.1-1q21.2 (CHR1:145883867-147465312 *3) region. Previous literature reported that epilepsy was associated with CNV of region 1q21.1, but it is mainly seen in microdeletion syndrome and rarely in microduplication syndrome^[2,3]. The existing reports show that 1q21.1 microduplication syndrome leads to a phenotype that includes developmental delays, autism spectrum disorders, congenital malformations, and congenital heart defects (typically, tetralogy of Fallot) along with characteristic facial dysmorphic signs ^[4-6]. Here, we describe a 1q21.1 microduplication in a patient with a 1.58 Mb duplication. The patient experienced seizures, developmental delay, and congenital heart disease in the form of ventricular septal defect. In addition, we summarized and analyzed the clinical characteristics and CNV regions of children with 1q21.1 microduplication syndrome in order to further illustrate the correlation between the duplication region and phenotype.

CASE PRESENTATION

Chief complaints

The patient was an 8-mo-old girl who was hospitalized for recurrent convulsions over a 2-mo period.

History of present illness

She presented with repeated convulsions after admission to the hospital that manifested as upturned eyes, followed by involuntary blinking, cyanosis of the lips and hands and confusion. This was not considered a tonic seizure, and she had no limb shaking. The convulsions occurred approximately 10 times a day, each lasting about 30 s; some episodes were self-relieving. The patient did not present with fever before the convulsions and there was no history of birth asphyxia or trauma. The convulsions were relieved after treatment with sodium valproate. The patient also suffered from developmental retardation as she could not lift her head in the prone position until 6 mo old, and was unable to sit independently at admission (age: 8 mo). Her gross and fine motor skills were poor, but her language and speech skills seemed to be fine. The patient had clear consciousness. Her weight was in the normal range, and she did not show macrocephaly or short stature.

History of past illness

Ventricular septal defect (VSD) was found when she was 4 mo old, and she underwent open-heart repair of the VSD.

Personal and family history

She delivered naturally at term. There was no hypoxia or suffocation or intraventricular hemorrhage during birth. Her parents denied any family history of developmental disorders, mental disorders, or genetic disorders. Both parents were in good health and are unrelated. The mother claimed that she developed a cold in the first 3 mo of the pregnancy. The mother denied any possible exposure to toxic substances such as alcohol, drugs, or other harmful environmental factors during the pregnancy and perinatal period. The child has a 5-year-old sister in good health and a 2.5-year-old brother who has language developmental delay and a two-time history of febrile convulsions. The patient's brother had no hypoxia or asphyxia or intraventricular

hemorrhage at birth, has poor gross and fine motor skills, and he also appeared to have poor speech and language skills. He did not present with macrocephaly or short stature.

Physical examination

On physical examination, there was no remarkable dysmorphism in the patient's appearance. A 6-cm-long surgical scar was observed on the anterior chest. She had normal limb muscle strength and muscle tone, and her physiological reflexes were normal, with no evidence of any pathological signs.

Laboratory examinations

Routine blood, urine, and stool tests showed no abnormalities. Her thyroid hormone and growth hormone profiles were normal.

Imaging examinations

Electrocardiography showed sinus rhythm, left atrial burden, and T-wave changes in partial leads (V5). Chest radiography revealed increased pulmonary vascular markings, enlarged heart shadow, right upper mediastinal widening, and a thymus. Her video electro-encephalography (VEEG) detected sharp slow wave release in the mid-line CZ (central midline point) and PZ (parietal midline point) zone during the sleep course. Cranial magnetic resonance imaging (MRI) showed left lateral ventricle enlargement and dysgenesis of the corpus callosum.

Further diagnostic work-up

Genomic DNA extraction: Briefly, 2 mL peripheral blood was drawn from the patient, her parents, and siblings, and sent to Kaiumph Medical Diagnosis (Beijing, China) for molecular karyotyping. We used a blood genomic DNA extraction kit (QIAamp DNA Blood Mini Kit, Qiagen) to extract genomic DNA.

Chromosome microarray: we first extracted DNA from peripheral blood and then processed the DNA samples by CMA using whole-genome bacterial artificial

chromosome (BAC) microarrays, in accordance with the protocol. Data analysis was performed using algorithm fixed settings. The genomic coverage resolution of this microarray platform is up to 1 Mb. There were a minimum of 3 consecutive BAC clones used for microarray probes to determine copy number variation. We compared the detected CNVs with the known CNVs in the public database, and also classified detected CNVs as “Clinically Significant,” “Likely Benign,” or as “Variants Of Uncertain Clinical Significance (VOUS).” The result of the chromosome microarray test was negative.

Whole exome sequencing: Genomic DNA was quantified using Nanodrop 2000 (Thermal Fisher Scientific, USA). We used the NEXT flex Rapid DNA-Seq Kit to construct a genomic DNA library, used xGen Exome Research Panel v2 (IDT, USA) to capture the constructed libraries, and then used Novaseq 6000 (Illumina, USA) to sequence for 10–12 GB. We screened for variants based on minor allele frequencies in normal population and performed variant function and prediction by Mutation Tester, Polyphen2 and SIFT. The mutant pathogenicity was assessed in accordance with guidelines of the American College of Medical Genetics and Genomics. We carried out analysis of CNV of patient’s total exome detection results based on Cap CNV (capture copy number variation) analysis which was published in previous articles^[1]. The results of gene sequencing and analysis with seizure-related genes such as *AAAS*, *AARS*, *AASS*, and *ABAT*, and another 1254 genes failed to show any pathogenic variation at the exon region.

Cap CNV analysis: we calculated the depth of each exon using bedtools-2.16.2 coverage for the same batch of samples. the sequencing quantity ratio of the sample and the batch mean was calculated to obtain the corrected depth in order to correct the bias in the sequencing data quantity. The depth ratio is used to determine whether a potential CNV exists in a exonic area, obtained from the corrected sample depth over the batch mean depth. a potential deletion is judged by a depth ratio of less than 0.7, and a potential duplication is judged by a depth ratio of greater than 1.3. The criterion for

judging the low-quality area is that the rate of abnormal depth ratio in this area exceeds 20% in this batch, and then it is filtered.

The criteria for screening candidate CNV regions are as follows: (1) It is required that at least two consecutive exons have abnormal depth ratio for OMIM disease-related gene exon regions; and (2) It is required that at least ten consecutive exons have abnormal depth ratio for non OMIM disease-related gene exon regions. Then the regions of candidate CNV were annotated with Decipher and DGV.

The analysis of CNVs based on the depth of the exon captured sequence showed that there was a 1.58 Mb duplication of heterozygosity in the 1q21.1 region, which included the following 11 genes *GJA5*, *FMO5*, *CHD1L*, *PRKAB2*, *BCL9*, *BNBPF11*, *BNBPF12*, *BNBPF24*, *GJA8*, *GPR89B*, *GPR89C* (Figure 1, Table 1).

SYBR green fluorescent quantitative polymerase chain reaction: Primers for fluorescent quantitative polymerase chain reaction (PCR) were designed for *FMO5*, *CHD1L*, *GJA5* and *ABL1* genes in the microduplication region. *ABL1*, as a housekeeping gene, is used as an internal reference. The relative quantitative values (RQ values) of *FMO5* (forward, GAGCCCCATCCCACCTTCC; reverse, CCAACGCCATACCATTCAGG), *CHD1L* (forward, CTCCTCAAGACAGCTGGTG; reverse, GCCCACCAGATCCTGATTCC) and *GJA5* (forward, CAGAGCCCCGGACCTCTTT; reverse, TCCCCATCTCCCACATTCG) genes were calculated with *ABL1* (forward, CTAAAGGTGAAAGCTCCG; reverse, GACTGTTGACTGGCGTGAT) as the internal reference gene by an ABI QuantStudioTM 6 Flex fluorescence quantitative analyzer for statistical analysis. Fluorescence qPCR was performed to determine the expression of related genes in duplicate regions of the genomic DNA from the patient, her parents and normal controls. We purchased the PCR reaction reagents from Cowin Biosciences Company (Beijing, China), and the primer sequences were synthesized by Shanghai Sangon Biotech Company (Shanghai, China). We randomly selected exons from the *FMO5*, *CHD1L*, and *GJA5* genes in the 1.58 Mb heterozygous duplication region, which was detected by CNV analysis, according to the results of whole exome sequencing (WES) to

design the fluorescent quantitative PCR primers with the *ABL* as the internal reference gene. The results showed that the patient and her mother were carriers of a heterozygous duplication in *FMO5*, *CHD1L*, and *GJA5* genes (the patient's RQ was approximately 1.5, and the mother's RQ was between 1.4 and 1.8). The father, however, did not show microduplication of *FMO5*, *CHD1L* and *GJA5* genes (RQ: approximately 1), which indicated that the mutation originated from the mother (Figure 2).

Familial analysis of low-depth whole genome sequencing: Low-depth whole genome sequencing detected a duplication of 2.096 Mb of heterozygosity in chromosome 1q (CHR1:145828373-147924436). The region has not yet been reported in the Decipher database. Low-depth whole genome sequencing, which was performed on the DNA of the patient's mother and brother, also revealed a 2.096 Mb duplication of heterozygosity in the CHR1:145828373-147924436 region (as shown in Figure 3). These results indicated that the duplicate fragment originated from the mother and was also carried by the brother.

The patient's family tree: The patient, her brother and mother had a duplication of heterozygosity in the 1q21.1 region, her father and sister did not (Figure 4).

FINAL DIAGNOSIS

The patient was diagnosed with 1q21.1 microduplication syndrome.

TREATMENT

During the hospital stay, the patient had no epileptic seizures. The convulsions were relieved after treatment with sodium valproate.

OUTCOME AND FOLLOW-UP

Detailed history-taking and follow-up examinations showed that the patient and her brother are currently lagging behind in motor development and have language developmental delay and are likely on the autistic spectrum.

DISCUSSION

The 1q21.1 region is considered the most genetically unstable fragment due to multiple low-copy duplication, and one of the largest regions in the human genome that carries repeated duplication, thereby making it prone to CNV^[2,4]. CNVs in the region are associated with developmental disorders, feeding problems, intellectual disability (ID), developmental delays (DD), congenital heart defects, behavioral problems (including autistic disorder and attention deficit hyperactivity disorder), and various other congenital deformities. There are 20-40 genes in the 1q21.1 region. The CNV of 1q21.1 can present as two main types: the one containing only the distal end region of 1q21.1 is called type I (approximately 1.8 Mb), and the one extending proximally to encompass the thrombocytopenia absent radius syndrome region is called type 2 (approximately 2.7 Mb)^[4,5].

1q21.1 microduplication syndrome is a rare chromosome 1 mutation. It can be an autosomal dominant inheritance or a novel mutation. There is no significant difference in the sex ratio. Microduplication of 1q21.1 is observed in approximately 0.03% adults, and the frequency in live births is estimated to be 1/6309. It presents with variable and partially explicit phenotypes; the common clinical manifestations are multiple congenital developmental disorders, which include DD, autism spectrum disorders, congenital malformations, and congenital heart defects, wherein tetralogy of Fallot is the most common. It can also be seen in normal individuals^[4,6-8]. In 2017, Busè *et al*^[9] expanded the phenotype of the 1q21.1 syndrome and suggested that the 1q21.1 microduplication syndrome showed special facial features such as macrocephaly or relative macrocephaly, prominent forehead, and widening of the eye distance. The relationship between triangular head deformity and 1q21.1 microduplication syndrome was also mentioned^[9]. Some researchers believed that it is a predisposing locus instead of a clinically distinct syndrome, given its incomplete dominance and variable phenotype^[10]. With the development of genome microarray and other methods for detecting CNVs, the improved accuracy of CNVs detection, and the widespread use of

gene screening in the study of DD and epilepsy, an increasing number of cases of 1q21.1 microduplication have been reported.

1q21.1 microduplication has been linked to a range of neurodevelopmental disorders, including autism spectrum disorders, ID, and even epilepsy, although there is no known gene for neurological disorders in this region^[11]. The UCSC Genome Browser lists two genes located in the distal microduplication region, *CHD1L* and *PRKAB2*, which may be associated with epilepsy^[2]. The *CHD1L* gene is also a candidate gene for attention deficit hyperactivity disorder and autism spectrum disorders, and the repetition of *CHD1L* is implicated in delayed language development^[10]. The *CHD1L* gene, which is associated with a variety of cancers, encodes a helicase responsible for DNA repair. It comes from the same family as *CHD2*, which is related to epileptic encephalopathy and various generalized epilepsy syndromes^[2]. *PRKAB2* acts as a regulator of cellular responses to numerous stimuli, encodes the $\beta 2$ subunits of AMP-activated protein kinase, and appears to play an important role in brain function^[12]. With regard to the previously mentioned macrocephaly, it has been reported that it may be related to *DUF1220* in this region, because the deletion of *DUF1220* was found to be associated with microcephaly in 1q21.1 microdeletion syndrome. *HYDIN2*, in region 1q21.1, is a gene involved in the *DUF1220* protein domain, and a homolog of the *HYDIN* gene in region 16q22.2. *HYDIN*, expressed only in the brain, is associated with hydrocephalus, so it may affect head circumference^[13-15]. Regarding congenital heart defect, the *GJA5* gene is believed to play an important role in the heart phenotype in the 1q21.1 region. It encodes gap junction protein 40 (CX40), a cardiac gap junction protein expressed through the right ventricular outflow tract, which plays a key role in intercellular communication and cellular adhesion and makes *GJA5* gene a major candidate for congenital heart defect phenotype in this region^[5,16-18].

At present, the sample size of 1q21.1 microduplication syndrome is limited, and the reported phenotypes are different. Using "1q21.1 microduplication syndrome" as the key word, we searched the biomedical literature database (PubMed) for nearly 10 years of literature, and we counted the 1q21.1 duplication regions and the genes contained in

the region (Table 2). Therefore, we still need more research to clarify the connection between genotype and specific phenotype of 1q21.1 microduplication syndrome. In our case, the result of the patient's gene analysis detected microduplication which contained both the *GJA5* and *CHD1L* genes in the 1q21.1 region. This might explain her seizures and congenital heart disease. In the familial analysis, her mother and brother both carried microduplication of the *GJA5* and *CHD1L* genes. However, her brother, who had a heterozygous duplication of about 2.096 Mb in the CHR1:145826755-147924436 region, showed symptoms presenting with language DD and febrile convulsions, while her mother showed no abnormalities. VEEG and cranial MRI of the patient's mother did not show any abnormalities, and the language ability and reading ability of her mother were normal as assessed by the simple Wechsler Adult Intelligence Scale-Revised in China. In 2015, Judith and Verhagen *et al*^[17] reported a case of 1q21.1 microduplication in a family of 11 people over three generations. Their patient suffered from severe learning difficulties and was diagnosed with chronic depression and anxiety at the age of 18. All carriers in her family showed hypertelorism and other facial dysmorphism but no congenital heart defect. At the first pregnancy of the proband, ultrasound examination at 20 wk of gestation revealed excessive fetal growth and a complex congenital heart defect, the pregnancy was terminated on the advice of her obstetrician. With these two reports of the same genotype but different phenotypes, we could speculate that the same genetic changes may result in different phenotypes. The phenotypes of our patient and her relatives still require long-term follow-up, for any new potential phenotypes.

The development of the nervous system is a complex process involving nearly 70% gene expression, which may explain why different CNVs have common clinical features. However, there is no explanation as to why the same position and CNV would result in a different phenotype^[19]. Genes are epigenetically inherited and subject to environmental modifications; genetic alterations may make CNV carriers more susceptible to environmental influences, which can lead to different phenotypes, depending on the severity of the environmental impact^[20,21]. Furthermore, the variable

expressivity and incomplete penetrance suggest that the influence of CNV is modified by other genetic loci or environmental factors^[22]. Xu *et al*^[23] suggested that women have a higher tolerance for pathogenicity/potential CNVs and have an inclination to pass these CNVs on to their offspring of males. At present, all CNVs studies of the Chinese population are concentrated on describing the genotype-phenotype correlation and explaining the mechanism of pathogenesis. Therefore, understanding the clinical significance of CNVs or offering optimum treatment options to CNV carriers at prenatal consultation continues to remain challenging.

CONCLUSION

1q21.1 microduplication syndrome is a rare CNV disease. This finding has extended our knowledge of the clinical manifestations of 1q21.1 microduplication syndrome and enhanced our understanding of CNV. However, further research is required to clarify the connection between genotype and specific phenotype of 1q21.1 microduplication syndrome. WES combined with qPCR can provide an accurate molecular diagnosis for children carrying this genetic mutation, which is of great significance for genetic counseling and early intervention.

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