Compound variants of *FKTN*, *POMGNT1*, and *LAMB1* gene identified by prenatal whole-exome sequencing in three fetuses with congenital hydrocephalus

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Abstract

Congenital hydrocephalus (CH) is a severe birth defect, and genetics components is an important etiology. Whole-exome sequencing (WES) has been proven to be a feasible approach for prenatal diagnosis of CH. In this study, we carried out WES on three fetuses with cerebral ventriculomegaly. After bioinformation analysis and data filtering, three compound variants, c.919C>T(p.Arg307Ter)/c.1100del(p.-Phe369fs) in *FKTN*, c.1449_1450insACAACG/c.1490G>C(p.Arg497Pro) in *POMGNT1*, and c.2690+1G>A/ c.1447C>T(p.Arg483Cys) in *LAMB1* were detected in the three fetuses. All the six variants were classified as likely pathogenic or pathogenic in accordance with the American College of Medical Genetics and Genomics-Association for Molecular Pathology guidelines. This study provides support for the potential of WES for the accurate prenatal diagnosis of fetal hydrocephalus and further demonstrated the genetic heterogeneity in patients with CH. The novel variants (c.1449_1450insACAACG and c.1490G>C in *POMGNT1*, c.2690+1G>A in *LAMB1*) expanded the gene mutational spectrum of CH and contributes to genetics counseling and pregnancy management.

Key words: congenital hydrocephalus, genetic etiology, novel variants, whole-exome sequencing.

Introduction

Congenital hydrocephalus (CH) is a severe birth defect with an estimated prevalence of 1 per 1000 births, a high perinatal mortality rate, and poor prognosis with neurological and physical disabilitie.[1] The etiology of CH is heterogeneous and includes secondary causes, such as neural tube defects, intracranial hemorrhages, teratogens, intrauterine infections, tumors, and trauma. The pattern of the family aggregation of CH shows that genetic components play a role in the etiology of this defect.[2] Genetic studies including linkage studies, targeted gene sequencing, and high-throughput DNA sequencing have identified 13 genes causing CH. Although numerous genetic causes of CH have been identified, they only explain approximately 20% of CH cases,[3] other many potential CH-associated genes have yet to be identified.

Here, we carried out whole-exome sequencing (WES) in three fetuses with CH on antenatal ultrasound. We

found compound heterozygous variants in the *FKTN*, *POMGNT1*, and *LAMB1* genes in the three fetuses, respectively, which provided precise prenatal diagnosis to aid genetic counseling and understanding of recurrence risk for future pregnancies. Our results further proved that *FKTN*, *POMGNT1*, and *LAMB1* are associated with CH.

Case Report

The present study was approved by Guangxi Maternal and Child Health Hospital Medical Ethics Committee (approval no. (2022) 1-2). Three fetuses with CH diagnosed by prenatal ultrasound were recruited with informed consent from their parents. Their parents were asymptomatic and nonconsanguineous. Maternal age, gestational weeks, family history, and fetal clinical symptoms are summarized in Table 1.

At 22 weeks of gestation, the ultrasound revealed severe hydrocephalus (the width of the left lateral brain ventricle was 2.0 cm and that of the right was 2.2 cm, and the transverse cerebellar diameter was below the 10th percentile for gestational age, Figure 1a) in fetus 1. A standard ultrasound screening for fetus 2, carried out at 22nd week of gestation, confirmed hydrocephaly: the width of the left lateral brain ventricle was 1.55 cm, and that of the right was 2.52 cm (Figure 1b). No other fetal structural abnormalities were identified. Ultrasound of fetus 3 at 24 weeks of gestation showed bilateral dilatation of the cerebral posterior horns (1.67 cm [left], 1.29 cm [right]) (Figure 1c) and agenesis of the corpus callosum (Figure 1d).

Cordocentesis of the three women were performed and 2 mL fetal cord blood for each fetus were aspirated into ethylene diamine tetraacetic acid (EDTA) disodium salt tubes. Genomic DNA was extracted from fetal cord blood and their parents' peripheral blood samples by using Lab-Aid DNA kit (Xiamen Zeesan Biotech Co, Ltd.). Fetal genomic DNA was used for WES, which was performed according to Illumina's TruSeq Exome Enrichment Guide (Illumina, Inc.). Variant–phenotype prioritization was performed via TGex software, and the potential causal variants were validated in their family by Sanger sequencing. The primer sequences covering the candidate variants were designed by Primer 3 (https://bioinfo.ut.ee/primer3-0.4.0/). Polymerase chain reaction (PCR) was performed with a 25 μL reaction volume containing 12.5 μL of Premix Taq (Takara Biotechnology Co., Ltd.), 1 μL of each primer (concentration: 20 μM), 1 μL of DNA

TABLE 1 Prenatal phenotype and genotype for fetuses

Patient	Fetus 1	Fetus 2	Fetus 3
Maternal age (years)	30	34	25
Gestational age (weeks)	22	22	23
Left lateral ventricle width (cm)	2.00	1.55	1.67
Right lateral ventricle width (cm)	2.20	2.52	1.29
Other malformation	/	/	Agenesis of the corpus callosum
Outcome	Termination of pregnancy	Termination of pregnancy	Termination of pregnancy
Gene	*FKTN*	*POMGNT1*	*LAMB1*
Variant	c.919C>T(p.Arg307Ter) / c.1100del(p.Phe369fs)	c.1449_1450insACAACG (p.Arg485_Arg486ins GlnArg) / c.1490G>C (p.Arg497Pro)	c.2690+1G>A / c.1447C>T (Arg483Cys)
Parental origin	Paternal / Paternal	Maternal / Paternal	Paternal / Maternal
Family history	/	/	/
Disease	Muscular dystrophy-dystroglycanopathy	Muscle-eye-brain disease	Lissencephaly 5
Inheritance model	AR	AR	AR
Pathogenicity classification	P (PVS1 + PM2 + PP1) / P (PVS1 + PM2 + PM3)	LP (PM1 + PM2 + PM4) / LP (PM1 + PM2 + PM3 + PP3)	LP (PVS1 + PM2) / LP (PM1 + PM2 + PM3 + PP3)
Reference	PMID: 17878207 / PMID: 31742715	Novel / Novel	Novel / ClinVar ID:716744

Abbreviations: AR, autosomal recessive; P, pathogenic; LP, likely pathogenic.
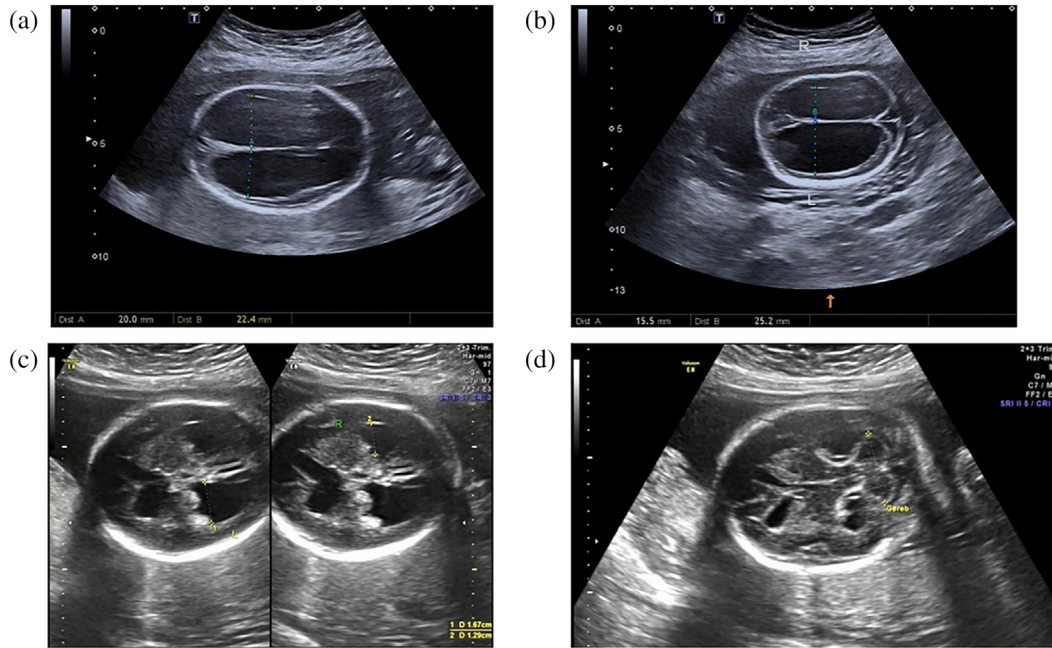
FIGURE 1 Ultrasounds results of three fetuses. Panels (a, b, and c) showing fetal cerebral ventriculomegaly of fetus 1, 2, and 3, respectively. (d) Revealing agenesis of the corpus callosum of fetus 3

(concentration: 50 ng/μL), and 9.5 μL of distilled water. PCR was performed with the following steps: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation 30 s at 95°C, annealing at Tm for 30 s for both primer pairs, and extension at 72°C for 60 s followed by a final 7 min extension cycle at 72°C. The PCR products were sequenced on an ABI3730XL Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.) for variant analysis. Compound heterozygous variants in the *FKTN* (NM_006731.2), *POMGNT1* (NM_017739.3), and *LAMB1* (NM_002291.2) genes were detected in the three fetuses (Table 1) and confirmed by Sanger sequencing (Figure 2a–c). Neither pathological CNVs nor other pathogenic variants related to CH were identified in the three fetuses.

Compound heterozygous pathogenic variants c.919C>T (p.Arg307Ter) and c.1100del (p.Phe369fs) in *FKTN* (NM_006731.2) were detected by WES in fetus 1, which were reported previously,[4,5] and both were evaluated pathogenic on the basis of American College of Medical Genetics and Genomics-Association for Molecular Pathology (ACMG/AMP) guidelines. WES identified compound heterozygous variants (c.1449_1450insACAACG and c.1490G>C(p.Arg497Pro)) in the *POMGNT1* (NM_017739.3) gene, which were novel variants absent from any public database, including the

Human Gene Mutation Database (HGMD; www.hgmd. cf.ac.uk/ac/), ClinVar (www.ncbi.nlm.nih.gov/clinvar/), National Center for Biotechnology Information Single Nucleotide Polymorphism Database (dbSNP; www.ncbi. nlm.nih.gov/SNP), Genome Aggregation Database (gnomAD; https://gnomad.broadinstitute.org), 1000 Genomes Project database (http://www.internationa lgenome.org/1000-genomes-browsers/), and Ensembl (http://grch37.ensembl.org/index.html). The variant c.1449_1450insACAACG was an in-frame insertion variant that led to the inclusion of two additional amino acid residues at position 485 (p.Arg485_Arg486ins-GlnArg). The missense variant c.1490G>C was predicted to be damaging (score: 0), probably damaging (score: 1), disease causing, and deleterious (score: 27.3) based on prediction tools SIFT, Polyphen, Mutation Taster and CADD, respectively. Conservation analysis revealed that the amino acid positions of Arg497 was relatively conserved among diverse species (Figure 2d). According to ACMG/AMP guidelines, c.1449_1450ins-ACAACG could be classified as likely pathogenic, and c.1490G>C was likely pathogenic as well. Fetus 3 was identified to have compound likely pathogenic heterozygous variants (c.2690+1G>A and c.1447C>T(p.-Arg483Cys)) in the *LAMB1* gene (NM_002291.2). The novel c.2690+1G>A was a splice variant, which may
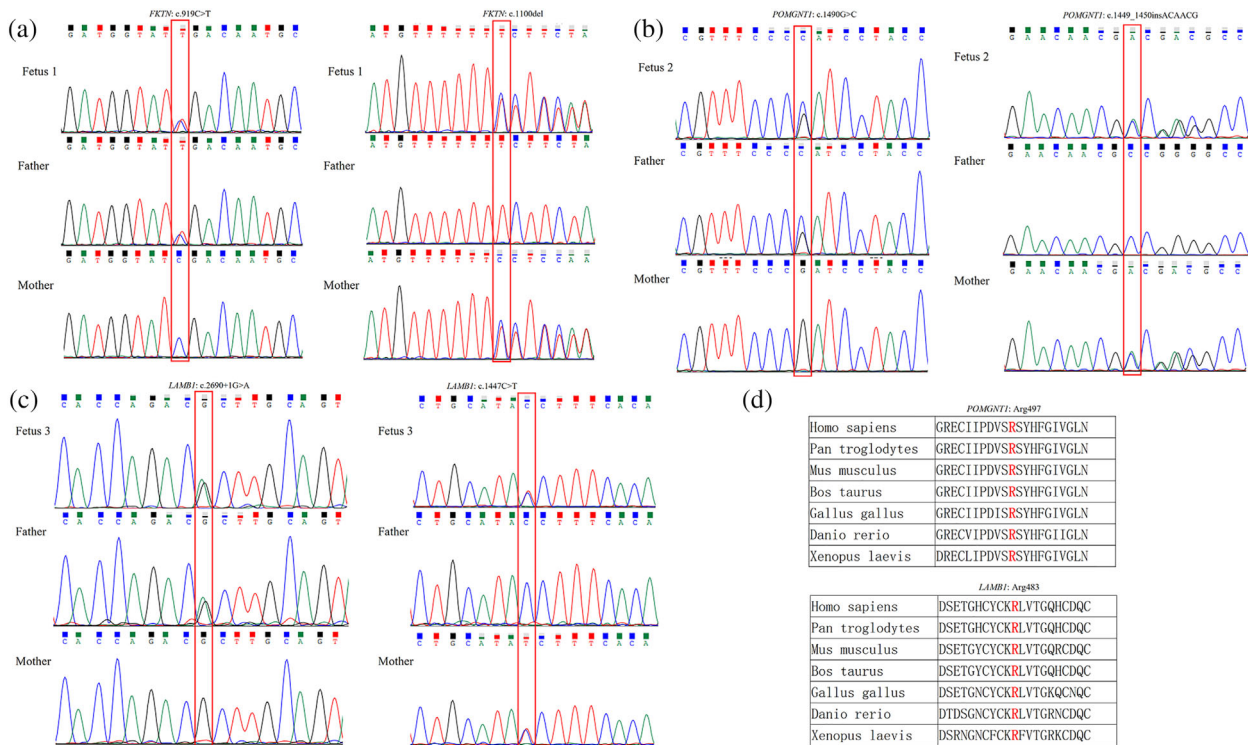
FIGURE 2 Sequencing chromatograms and multiple sequence alignment. (a) Compound heterozygous pathogenic variants c.919C>T (p.Arg307Ter) and c.1100del (p.Phe369fs) in *FKTN* of fetus 1. (b) Compound heterozygous variants (c.1449_1450insACAACG and c.1490G>C (p.Arg497Pro)) in *POMGNT1* of fetus 2. (c) Compound heterozygous variants (c.2690+1G>A and c.1447C>T(p.Arg483Cys)) in *LAMB1* of fetus 3. (d) Multiple sequence alignment of two missense. The multiple amino acids sequence alignment reveal that Arg497 in *POMGNT1* and Arg483 in *LAMB1* are highly conserved during evolution

result in the abnormal splicing of pre-mRNA, consequently affecting protein function. The variant Arg483Cys has been documented in gnomAD with extremely low frequency and was predicted to be damaging (score: 0), probably damaging (score: 1), disease causing and deleterious (score: 32) according to SIFT, Polyphen, Mutation Taster, and CADD, respectively. Multiple sequence alignment results suggested that Arg483 was an evolutionarily conserved amino acid among all species in the comparison (Figure 2d). Consequently, the two variants can be classified as likely pathogenic in accordance with ACMG/AMP guidelines.

Discussion

CH is a common central nervous system malformation that leads to serious pathological consequences for infants. Fetal cerebral ventriculomegaly is one of the most frequent phenotype observed in most patients with CH.[6] Ultrasound examination is an indispensable first-line screening method, which can help us identify CH within the fetus. However, considering the genetic heterogeneity and nonspecific phenotype of CH in the fetal period, ultrasound in isolation is not sufficient to distinguish and identify the etiology of CH in the prenatal age. With the advancement in genetic testing techniques, WES has become a fast and effective tool for identifying the causative variants of Mendelian disorders and has been proven to be a feasible approach for prenatal diagnosis.[7] In this study, we performed prenatal WES on three fetuses suspected with CH based on prenatal ultrasound, and they had compound heterozygous variants in three different genes, highlighting the importance of genetic test in accurate diagnosis of hydrocephalus for genetic counseling. All the parents decide to terminate pregnancies according to the genetic result even though fetus 1 and fetus 2 had no structural abnormalities other than hydrocephalus.

WES identified compound heterozygous pathogenic variants c.919C>T (p.Arg307Ter) and c.1100del (p.-Phe369fs) in the *FKTN* gene in fetus 1. The *FKTN* gene, which is 82 988 bp long and composed of 10 exons, encodes fukutin, a protein comprising 461 amino acids. Fukutin is located on the Golgi apparatus and expressed ubiquitously in human tissues. The function of fukutin has not been completely elucidated, but it has been proven to play an important role in the glycosylation of dystroglycan and it affects the development of the brain.[8] Variants in *FKTN* cause four different phenotypes: muscular dystrophy-dystroglycanopathy type A, 4 (OMIM no. 253800), muscular dystrophy-dystroglycanopathy type B, 4 (OMIM no. 613152), muscular dystrophy-dystroglycanopathy type C, 4 (OMIM no. 611588) and dilated cardiomyopathy-1X (OMIM no. 611615). In these clinical symptoms, brain malformations including hydrocephalus can provide important clues for prenatal diagnosis. However, variants in *FKTN* in the fetus have rarely been reported.[9,10] The present study described the first fetus with compound heterozygous variants in the *FKTN* gene from a Chinese families. These fetus highlighting the importance for *FKTN* gene testing in fetuses with hydrocephalus, encephalocele, cerebellar hypoplasia, and so on.

POMGNT1 encodes a protein that catalyzes the transfer of N-acetylglucosamine to O-mannoses of glycoproteins.[11] Variants in *POMGNT1* can lead to three different forms of congenital muscular dystrophies, namely, muscle-eye-brain (MEB) disease (OMIM no. 253280), congenital mental retardation (OMIM no. 613151), and a milder limb-girdle form with normal intellect (OMIM no. 613157). POMGNT1 protein can be divided into cytoplasmic tail (residues 1–37), transmembrane domain (residues 38–59), carbohydrate-binding stem domain (residues 92–299), and catalytic domain (residues 300–646).[12] The Arg497Pro and Arg485_Arg 486insGlnArg we identified in fetus 2 were both novel variants and located in the catalytic domain. Arg497 and Arg485 are highly conserved amino acids among many species, and the 3D protein structure is likely changed due to the missense variant and in-frame insertion. Previous studies have suggested that *POMGNT1* variants near the 5′ terminus may lead to more severe neurodevelopmental malformations, while variants near the 3′ terminus are associated with mild MEB phenotypes.[13] Our fetus was consistent with prior results, as the compound variants were near the 3′ terminus and only characterized by hydrocephaly.

LAMB1 encodes laminin subunit beta-1, which plays an important role in the outgrowth, differentiation, and migration of neuronal cells.[14] Variants in *LAMB1* have been shown to result in lissencephaly-5 (OMIM no. 615191), which is characterized by delayed psychomotor development, seizures, hydrocephalus, ocular anomalies, and other neurodevelopmental malformations. In this study, bilateral dilatation of the cerebral posterior horns and agenesis of the corpus callosum were observed in fetus 3 by prenatal ultrasound. Although hydrocephalus has been observed in previous cases, it is present later in childhood or in adolescence.[14,15] In addition, agenesis of the corpus callosum in our fetuses was first reported in the cases of neurological malformations resulting from *LAMB1* variants. Our fetus was the first case with neurological malformations resulting from the *LAMB1* variant in China, and the novel variant c.2690+1G>A extended the *LAMB1* gene variant spectrum.

In conclusion, we report the successful implementation of WES for the molecular diagnosis of three fetuses with prenatal CH, and they had compound heterozygous variants in three different genes, which confirmed that WES can provide accurate and fast diagnosis in fetuses with CH resulted from different genetic etiology. Three families were likely to occur in future pregnancies with 25% recurrence risk, therefore, all parents received genetic counseling for invasive prenatal testing and preimplantation genetic diagnosis in future pregnancies. In the present research, three of six variants identified in the fetuses were novel, enriching the variant spectrum of the *POMGNT1* and *LAMB1* genes in the Chinese population and improve understanding the etiology of CH. Furthermore, this study suggests that more, as yet undefined, genes may be causal etiology to fetal hydrocephalus and highlighting the importance of prenatal molecular test in the diagnosis of CH.

Author contributions

Acknowledgments

Funding information

Conflict of interest

The authors declare that they have no competing interests.

Data availability statement

The data that support the findings of the current study are available from the the corresponding author on reasonable request.

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