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Prenatal diagnosis of Walker–Warburg syndrome due to compound mutations in the *B3GALNT2* gene

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Abstract

Background: Congenital hydrocephalus is one of the symptoms of Walker–Warburg syndrome that is attributed to the disruptions of the genes, among which the *B3GALNT2* gene is rarely reported. A diagnosis of the Walker–Warburg syndrome depends on the clinical manifestations and the whole-exome sequencing after birth, which is unfavorable for an early diagnosis.

Methods: Walker–Warburg Syndrome was suspected in two families with severe fetal congenital hydrocephalus. Whole-exome sequencing and Sanger sequencing were performed on the affected fetuses.

Results: The compound heterozygous variants c.1A>G p.(Met1Val) and c.1151+1G>A, and c.1068dupT p.(D357*) and c.1052 T>A p.(L351*) in the *B3GALNT2* gene were identified, which were predicted to be pathogenic and likely pathogenic, respectively. Walker–Warburg syndrome was prenatally diagnosed on the basis of fetal imaging and whole-exome sequencing.

Conclusions: Our findings expand the spectrum of pathogenic mutations in Walker–Warburg syndrome and provide new insights into the prenatal diagnosis of the disease.

KEYWORDS
B3GALNT2, congenital hydrocephalus, prenatal diagnosis, Walker–Warburg syndrome, whole-exome sequencing

1 | INTRODUCTION

Congenital hydrocephalus (CH) is defined as the abnormal accumulation of the cerebrospinal fluid in the cerebral ventricles.[1,2] With an incidence of approximately 1 per 2000 births, CH is one of the leading causes of the morbidity and mortality in children[1,3] and is greatly attributed to disruptions of the genes.[4] Publications about disruptions of the *B3GALNT2* gene leading to CH have become available recently. To date, mutations in the *B3GALNT2* gene were identified in four patients with CH, all of which were diagnosed Walker–Warburg Syndrome (WWS).[5,6]

WWS is an autosomal recessive disease characterized by multi-system abnormalities involving the brain, eye and muscle.[7,8] Lissencephaly, cerebellar malformation, hydrocephalus, hypoplasia of

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midline structures, retinal malformation, cataract, microphthalmia, anophthalmia, coloboma and muscular dystrophy are common clinical manifestations.[7,9–13] Patients with WWS usually die in infancy.[4] The diagnosis of WWS mainly depends on the clinical manifestations after birth, which is unfavorable for an early detection and diagnosis.

In the present study, we describe several cases from two unrelated families with severe hydrocephalus. Prenatal whole-exome sequencing followed by Sanger sequencing identified compound heterozygous mutations in the *B3GALNT2* gene that were predicted to be pathogenic, and therefore WWS was prenatally diagnosed. The present study is the first to identify the mutations in the *B3GALNT2* gene leading to WWS by as early as week 20 of gestation, which undoubtedly provides new insights into the prenatal diagnosis of WWS.

2 | MATERIALS AND METHODS

2.1 | Case report

Patient 1 (II 1), male, 22 months old, was the first child of a non-consanguineous couple. Dilated bilateral ventricles, 22 mm in the left and 24 mm in the right lateral ventricles, were observed during week 24 of gestation by ultrasound scan (US). Fetal magnetic resonance imaging (MRI) revealed severe hydrocephalus (Figure 1C-D). After

birth, the child suffered from severe developmental delay and microphthalmia, and manifested low-set ears and a single palmar flexion crease. The enzyme profile at his 15th month was: serum creatine kinase (CK) = 4588 U/l and creatine kinase isoenzyme (CK-MB) = 112 U/l. Cranial MRI at the age of 16 months showed severe hydrocephalus, lissencephaly, callosal agenesis, and enlargement of the fourth and third ventricles (Figure 2). WWS was suspected. The couple came to our clinic for genetic counselling because severe hydrocephalus was found in the second conception (II 2) in week 20 of gestation.

Patient 2 (II a) was the first conception of a healthy non-consanguineous couple. A slight dilatation of the bilateral cerebral ventricles was noted in week 16 of gestation. The progressively dilated bilateral ventricles, 16 mm in the left and 19 mm in the right lateral ventricles, were observed in week 20 (Figure 3B). The pregnancy was terminated after genetic counseling during week 22 because Dandy–Walker malformation was suspected[14] and no genetic tests were performed on the fetus. The 34-year-old woman came to our hospital because her second fetus (II b) was found to have severe hydrocephalus during week 24 by US.

The study was approved by the Ethics Committee of Women's Hospital, School of Medicine Zhejiang University (IRB-20210230-R) and conformed to the Declaration of Helsinki. All participants provided their written informed consent.
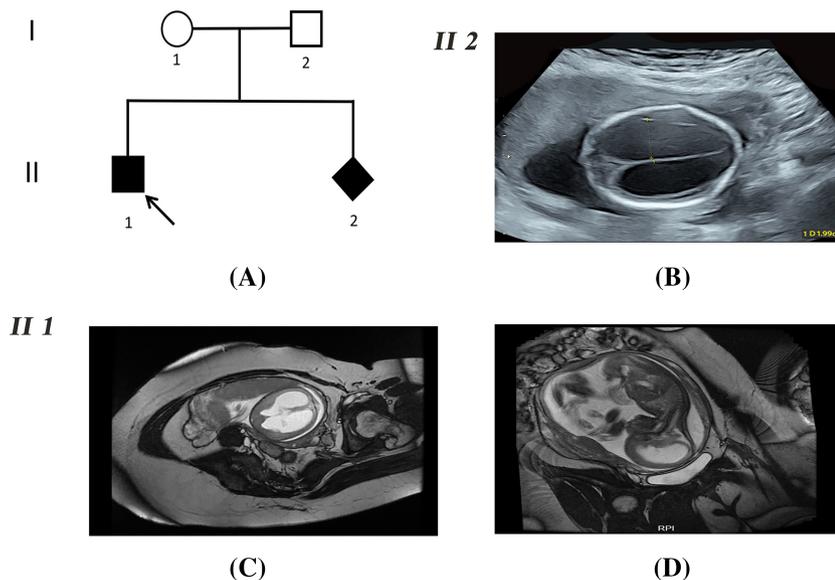
FIGURE 1 Pedigree and fetal ultrasound scan and MRI findings in the first family. (A) Pedigree of the first family. The arrow refers to the proband. The solid rhombus and square (male) represent the affected siblings. (B) The enlarged bilateral ventricles in the fetus (II 2). (C,D) the enlarged bilateral ventricles and severe hydrocephalus in the probnd (II 1) in coronal and vertical section, respectively
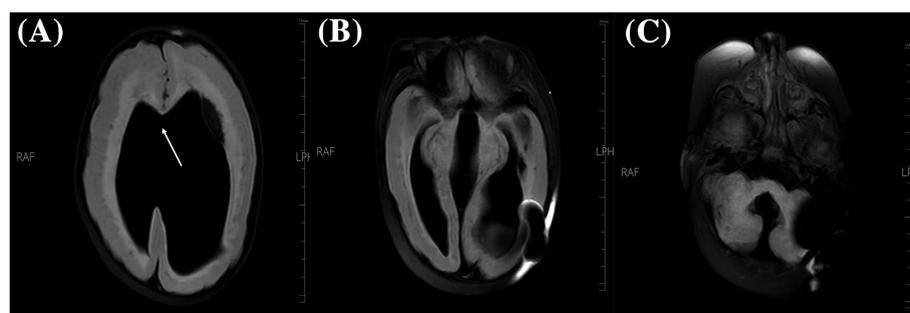
FIGURE 2 Magnetic resonance imaging of the patient 1 (II 1) at 16 months of age. (A) Dysplastic cerebral cortex; reduced white matter volume; severe hydrocephalus; and callosal agenesis (arrowhead). (B) Massively dilated third ventricle. (C) Massively dilated fourth ventricle

2.2 | Fetal sampling

Amniocentesis was performed at the fetus (II 2) in week 20 of gestation. Transabdominal umbilical blood puncture was performed at the fetus (II b) in week 24 of gestation. In total, 5 ml of blood and 20 ml of amniotic fluid were taken for karyotyping or DNA extraction under the sonographic guidance as a routine.

2.3 | Chromosome karyotype analysis

Samples of umbilical cord blood or amniotic fluid were cultured and prepared for G-banding karyotyping. Chromosome karyotype analysis was conducted on metaphase preparations using the GSL-120 Cyto-Vision platform (Leica). In total, 30 metaphases were counted and five metaphases were analyzed. The karyotypes were described following the International System for Human Cytogenetic Nomenclature.[15]

2.4 | DNA extraction

Genomic DNA was collected from peripheral blood of the couple (I 1, 1 2 and I a, I b) and the proband (II 1), amniotic fluid (II 2) and umbilical blood (II b) (Figures 1A and 3A). DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Germany) in accordance with the manufacturer's instructions. The DNA concentrations were detected using a NanoDrop 2000 (Thermo Fisher Scientific).

2.5 | Chromosomal microarray (CMA)

CMA was performed using CytoScan™ HD array (Affymetrix) in accordance with the manufacturer's instructions. Chromosome Analysis

Suite (ChAS) software (Thermo Fisher Scientific) was used to analyze the raw data. The copy number variants were interpreted according to American College of Medical Genetics' standards and guidelines.[16]

2.6 | Whole-exome sequencing (WES)

WES was performed on Illumina HiSeq2000 platform (Illumina) in accordance with the manufacturer's instructions. The mean sequence coverage of the exons was at least 90-fold and, for those more than 95% region-of-interest, the coverage was at least 20-fold. Variants were filtered by population databases, which included genome Aggregation Database (gnomAD: http://gnomad.broadinstitute.org) and Exome Aggregation Consortium (ExAC: http://exac.broadinstitute.org). Next, the variants were compared by multiple databases, including the Human Gene Mutation Database (HGMD: http://www.hgmd.cf.ac.uk/ac/index.php), Leiden Open Variation Database (LOVD: http://www.dmd.nl) and ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar) and only those with allele frequency ≤ 1% were retained. To predict the pathogenicity of the mutations, SIFT (http://sift.jcvi.org) and Mutation Taster (http://www.MutationTaster.org) were mainly used. The variants were interpreted under the guideline of the the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) and only those relevant to the patients' manifestations were recorded.

2.7 | Sanger sequencing

Sanger sequencing was carried out to validate the variants. The forward primer (5′-GCCCCTCAGTCTCCGCACTT-3′), reverse primer (5′-TTAACGCTCCAAGGATGAAA-3′) and forward primer
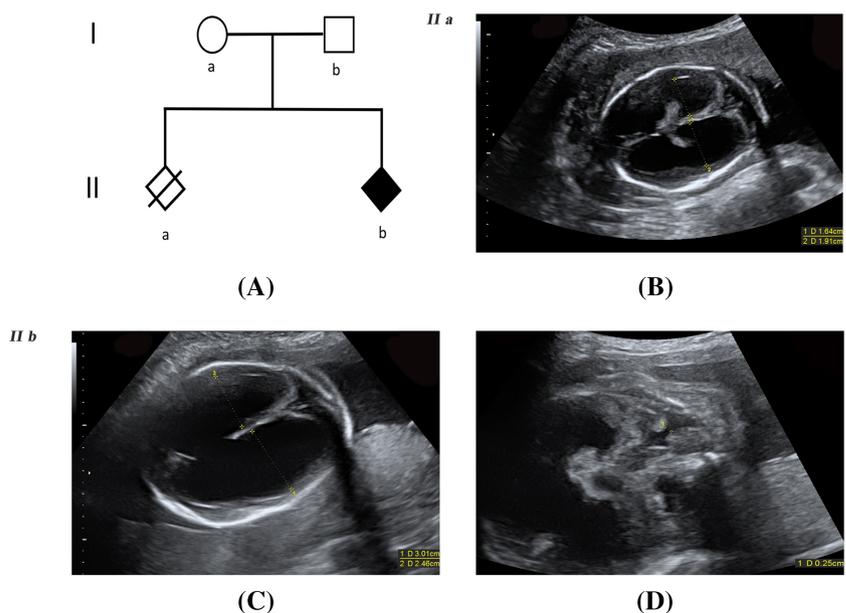
FIGURE 3 Pedigree and fetal ultrasound scans findings in the second family. (A) Pedigree of the second family. The solid rhombus represents the affected fetus. (B) The enlarged bilateral ventricles in the fetus (II a). (C) The enlarged bilateral ventricles and hypoplasia of midline structure in the fetus (II b). (D) A separation width of 2.5 mm in the inferior of the cerebellar vermis (II b)

(5′-AGGACTGTGGAAACAACGAG-3′), reverse primer (5′-AGCCAGCATGACTGACCTAG-3′) were used to amplify the PCR products in the *B3GALNT2* gene in the first family. The forward primer (5′-TGTTAGTGACGCCACTGCATTG-3′) and reverse primer (5′-TCAGGTACACGAATGCCAATGG-3′) were applied in the second family. The products were sequenced with the ABI 3730 DNA analyzer (Applied Biosystems).

3 | RESULTS

3.1 | Fetal ultrasound scans

In the first pedigree (Figure 1A), US was carried out in week 20 of gestation (II 2). Dilated bilateral ventricles were observed, 20 mm in the left and 19 mm in the right lateral ventricle, and severe hydrocephalus was diagnosed (Figure 1B).

In the second pedigree (Figure 3A), US was carried out in week 25 of gestation (II b). The bilateral ventricles were severely enlarged, with a width of 30 mm in the right and 25 mm in the left lateral ventricle (Figure 3C). In addition, a separation width of almost 2.5 mm was found in the inferior of the cerebellar vermis (Figure 3D).

3.2 | Identification of compound heterozygous variants

The karyotype analysis of G-banding showed a standard chromosomal pattern. No duplication or deletion copy numbers variants were identified in the CMA (see Supporting information, Fig. CMA and Fig. karyotype).

In the first pedigree, the variants of c.1A>G p.(Met1Val) and c.1151+1G>A were identified in the *B3GALNT2* (NM_152490) gene (Figure 4), neither of which were recorded in the gnomAD-exome database, the Clinvar databases or the HGMD databases (PM2). The phenotype was in accordance with the genotype (PP4). The variant of c.1151+1G>A, with an additional nucleotide insertion at the canonical

splicing sites, was predicted to impair protein function (PSV1). The variant c.1A>G, with a single nucleotide change at the initiation codon, led to an deficiency of protein (PSV1). According to the ACMG and the AMP guidelines,[17] both of the mutations were predicted to be pathogenic.

In the second pedigree, the variants c.1052 T>A p.(L351*) and c.1068dupT p.(D357*) were identified in the *B3GALNT2* (NM_152490) gene (Figure 5), neither of which were recorded in the Clinvar databases or the HGMD databases (PM2). The premature stop codon introduced by c.1052 T>A p.(L351*) was predicted to impair protein function (http://www.pathogenic varianttaster.org) (PSV1). The variant c.1068dupT p.(D357*) introduced a termination codon, leading to the termination of the encoding protein (PSV1). It was predicted to be harmful with Mutation Taster (http://www.path-ogenic varianttaster.org). According to the ACMG and the AMP guidelines,[17] both of the mutations were predicted to be likely pathogenic.

4 | DISCUSSION

In the present study, WWS was prenatally diagnosed on the basis of the fetal imaging and the identification of pathogenic mutations in the *B3GALNT2* gene. Our findings expand the mutation spectrum of the *B3GALNT2* gene and provide new insights into the prenatal diagnosis for WWS.

Compared with the acquired hydrocephalus, CH was reported to be enriched in the genetic causes,[18] 40% of which was caused by the disruptions of genes.[19] The *LICAM* gene, for example, is the mostly reported pathogenic gene leading to CH.[18] Generally, CH serves as the specific symptom of a syndrome and is usually accompanied with other abnormalities. WWS, for example, is a common clinical syndrome with the symptom of hydrocephalus.[20]

WWS is on the extreme severe end of the dystroglycanopathies,[21] which are disorders characterized by congenital muscle dystrophies. WWS was first described by Walker[11] and Warburg,[9] with the clinical characteristics of hydrocephalus,
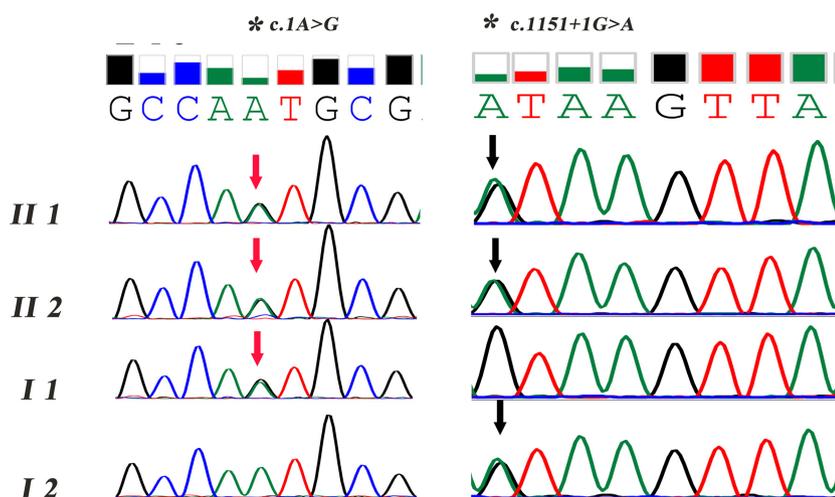
FIGURE 4 Sanger sequencing of the compound heterozygous variants in the *B3GALNT2* gene in the first family. The red arrow refers to the variant of c.1A>G. The black arrow refers to the variant of c.1151+1G>A

FIGURE 5 Sanger sequencing results of the compound heterozygous variants in the *B3GALNT2* gene in the second family. The red arrow refers to the variant of c.1068dupT. The black arrow refers to the variant of c.1052 T>A
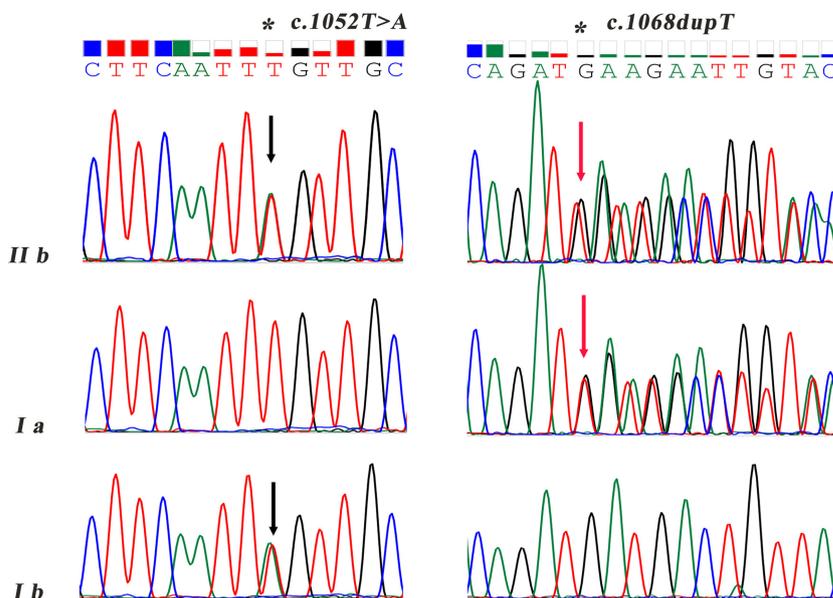
lissencephaly, eye anomalies and retinal detachment.[10] Subsequently, muscular dystrophy was described, which was suggested to be added into the diagnosis criterion of WWS.[7,10,12] To date, the diagnosis criterion of WWS has not come to a consensus. The diagnosis of WWS mainly depends on the clinical manifestations and the US or MRI after birth.[13] Lissencephaly, cerebellar malformation, hydrocephalus, hypoplasia of midline structures, retinal malformation, cataract, microphthalmia, anophthalmia, coloboma and muscular dystrophy are common clinical manifestations.[7,9–13] It is reported that the patients with WWS usually die in infancy.[10] Therefore, the early detection and diagnosis of WWS is necessary and it is favorable for the prevention of birth defects.

With the advancement in WES, studies regarding pathogenic mutations leading to WWS are increasing. However, observations about the association between the *B3GALNT2* gene and WWS are quite rare. To date, the *B3GALNT2* gene has been identified in 10 patients who were diagnosed with WWS, four of whom were observed to have hydrocephalus.[5,6,22–24] The *B3GALNT2* gene, located on chromosome 1q42.3, is composed of 12 exons. Consisting 500 amino acids in the open read frame, it encodes a specific enzyme for which the transcripts are mostly restricted to the skeletal muscle and adipose tissue.[25] Therefore, disruptions of the *B3GALNT2* gene may lead to the muscular dystrophy and brain malformations. Research about the *B3GALNT2* gene leading to hydrocephalus has been reported in the mammal animals. Horses, for example, were the most frequent reported animals and variants in the *B3GALNT2* gene were confirmed to be pathogenic mutations.[2,3,26]

The variety of clinical manifestations and the limited recognition of its pathogenic genetic mechanism usually result in the underestimation of the identification of WWS. Cormand et al.[10] considered that prenatal severe hydrocephalus might be a signal of WWS. Subsequently, Ho et al.[23] proposed the importance of the prenatal MRI for the early identification of the brain–eye–muscle disease, including WWS. Jin et al.[19] recommended that WES should be

performed for routine clinical tests when CH is newly diagnosed. Therefore, when severe hydrocephalus is observed prenatally, prenatal WES is necessary for the early diagnosis of WWS.

In conclusion, the present study reported two families with the *B3GALNT2* gene mutation leading to WWS, which expand the spectrum. However, the mechanisms regarding mutations in the *B3GALNT2* gene leading to cortical dysplasia remain unknown. Further studies are being carried out to investigate their pathogenicity and molecular mechanisms.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

MD conceived the study. MD and PW participated in its design. PJ drafted the manuscript. MW, LZ and WZ collected the samples. YFX collected clinical data. MC and YQ carried out the WES. YQX helped to revise the manuscript. All authors have read and approved the final version of the manuscript submitted for publication.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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