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ORIGINAL ARTICLE

Analysis of L1CAM gene mutation and imaging appearance in three Chinese families with L1 syndrome: Three case reports

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Abstract

Background: The molecular mutations of the *L1CAM* gene and the imaging appearances of four fetuses with L1 syndrome from three independent Chinese families with a history of hydrocephalus were reported in this study. Two of the three are novel L1CAM variants.

Methods: Results of clinical and imaging examinations of three Chinese families were collected. Fetal samples were collected by puncture, genomic DNA was extracted, whole-exome sequencing was performed, and the *L1CAM* gene mutation sites were verified by PCR and Sanger sequencing.

Results: In this case report, we described the imaging appearance and investigated the mutations of the *L1CAM* gene in three Chinese families with a history of L1 syndrome; these included two nonsense mutations (c.262C>T and c.261C>G) and one splice-site mutation (c.524-1G>A). Two of these three are novel *L1CAM* variants: c.262C>T and c.261C>G. The results of the sonographic images of the affected fetuses showed severe hydrocephalus. Bilateral lateral ventricles were dilated in the fetuses with c.262C>T and c.261C>G mutations. The left ventricle was about 14 mm wide and the right was about 14 mm in the fetus with c.262C>T mutation. The left ventricle was about 24.9 mm wide and the right was about 23.9 mm in the fetus with c.261C>G mutation. The ultrasound examination of the fetus with c.524-1G>A mutation showed that the third ventricle (7.5 mm wide) was raised, and the fourth ventricle was communicated with the cisterna magna. The parents requested termination of the above pregnancy.

Conclusion: The current study emphasizes the importance of combining family history, prenatal ultrasonography, and *L1CAM* mutation testing positive for the diagnosis of the L1 syndrome.

KEYWORDS

fetal ultrasound, gene mutation, L1 syndrome, *L1CAM*

1 | INTRODUCTION

L1 syndrome is an X-linked recessive rare disease that includes four neurological conditions as follows: X-linked hydrocephalus (HSAS, OMIM 307000), mental retardation, adducted thumbs, shuffling gait, aphasia syndrome (MASA syndrome, OMIM 303350), X-linked complicated spastic paraplegia type 1 (SPG1, OMIM 303350), and X-linked agenesis of the corpus callosum (X-linked ACC, OMIM 304100) (Gauntner et al., 2021; Norman et al., 2021). Since the main features of L1 syndrome include *C*orpus callosum hypoplasia, mental *R*etardation, *A*dducted thumbs, *S*pastic paraplegia, and *H*ydrocephalus, this condition is termed CRASH syndrome. (Gauntner et al., 2021) The incidence of this disease is 1/3000–25,000 in the live-birth male babies, and the related pathogenic gene is the L1 cell adhesion molecule (*L1CAM*) gene (Bousquet et al., 2021). However, the pathogenetic mechanism underlying the L1 syndrome is yet to be elucidated, and the treatment requires shunting of the cerebrospinal fluid as needed (Itoh & Fushiki, 2015). Importantly, the severity of the L1 syndrome varies from severe hydrocephalus and prenatal death (HSAS) to a milder phenotype (MASA syndrome) or isolated agenesis of the corpus callosum (Adle-Biassette et al., 2013).

The *L1CAM* gene is located on band Xq28, encoding a transmembrane glycoprotein of the immunoglobulin (Ig) superfamily. The gene consists of 29 exons, and the first exon is noncoding (Christaller et al., 2017). The 200–220 kDa protein contains one short conserved cytoplasmic domain, one single-pass transmembrane domain, and 11 distinct domains, that is, five fibronectin type III (FN III) repeats and six Ig-like domains at the extracellular surface (Figure 1) (Linneberg et al., 2019). The L1CAM protein has key functions in the development of the nervous system and is also involved in the malignant progression of neoplasia and the metastatic cascade (Grońska-Pęski et al., 2020). Hitherto, >300 variants of the *L1CAM* gene have been published in the Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=L1CAM). The types of mutations include missense, nonsense, splice site, insertions, and deletions. These variants were distributed across exons without hot spots. The phenotypes of L1 syndrome are complex and changing. A genotype–phenotype correlation of the *L1CAM* gene was confirmed. The L1 syndrome children with a truncating mutation were more likely to perish before the age of 3 years than those with a missense mutation (Vos et al., 2010). Ferese et al. reported a novel *L1CAM* mutation (c.1267+5delG) causing the loss of exon 10 in the extracellular domain in a female carrier who underwent two voluntary pregnancy terminations due to fetal hydrocephalus, whereas Maarten et al. described a 56-year-old proband who exhibited a mild learning disability and carried an L1CAM mutation (c.998C>T; p.Pro333Leu) which can lead to the structure of the fourth immunoglobulin-like domain in the protein (Ferese et al., 2016; Otter et al., 2017). A moderate phenotype with mild intellectual disability (ID) and X-linked partial corpus callosum agenesis (CCA) has been related to L1CAM in two families which, respectively, carried c.3226A>C (p.Thr1076Pro) and c.719C>T (p.Pro240Leu) missense variations in the extracellular domain (Basel-Vanagaite et al., 2006; Bousquet et al., 2021).

This differential pathogenicity could be attributed to the varied mutation sites in the *L1CAM* gene being different. If the mutations occur in the key residues crucial for the structure of the L1CAM protein, the phenotype may be severe. Although the mutation has little effect on the structure and function of the protein, it might cause a mild phenotype.

In this study, we reported the mutations of the *L1CAM* gene and the imaging appearances of four fetuses with L1 syndrome from three independent Chinese families with a history of hydrocephalus. Of these three, two are novel L1CAM variants.

2 | CASE REPORT

2.1 | Family 1

The case was a 34-year-old female (II.4). The nonconsanguineous couple denied a family history of genetic disease. The pedigree is shown in Figure 2a. In the second pregnancy (III.3), the ultrasound examination at 17^{+6} weeks

FIGURE 1 Structural schematic of L1CAM protein

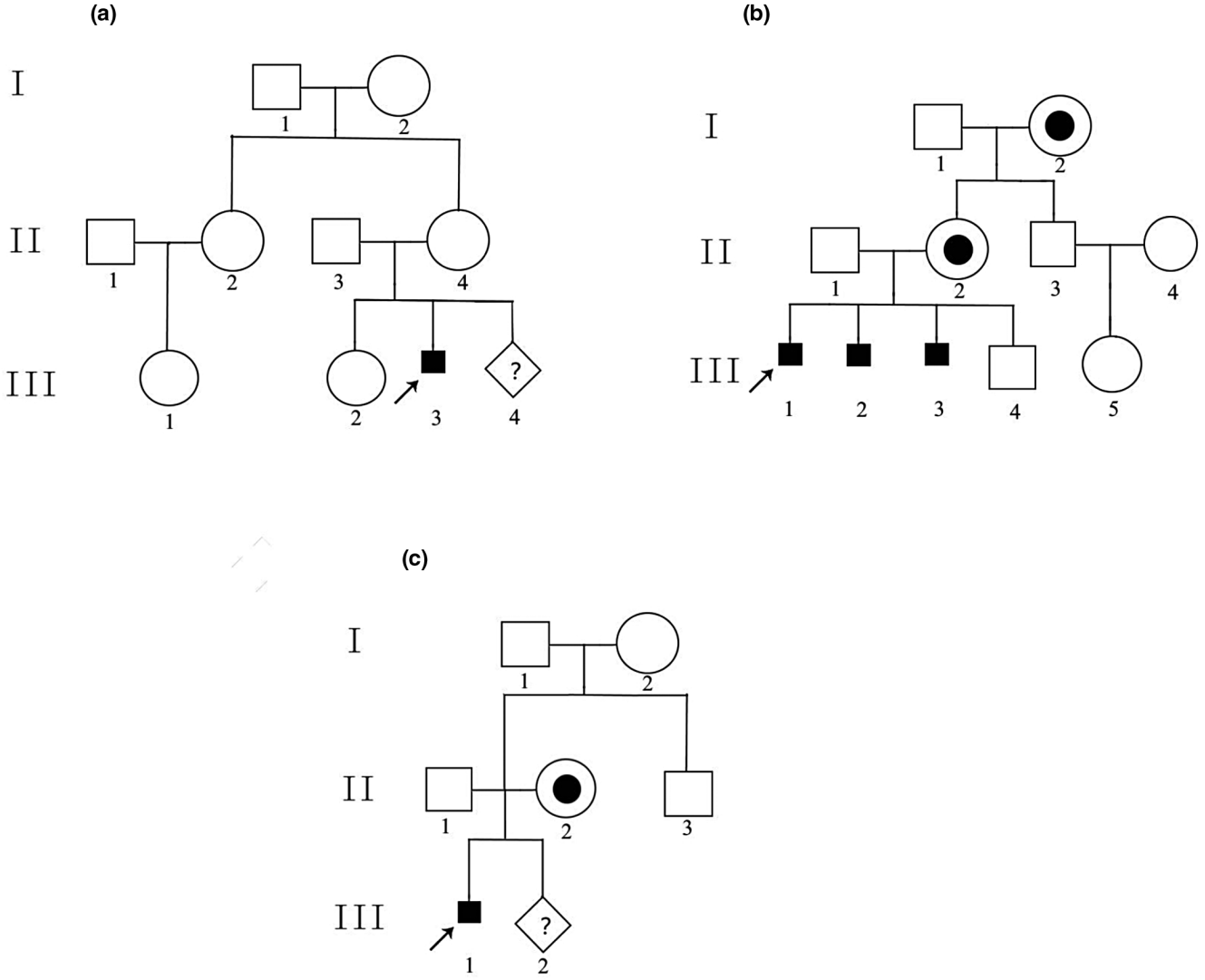

FIGURE 2 Pedigrees of three Chinese families with the L1 syndrome. (a) Family 1, (b) family 2, and (c) family 3.

of gestation identified a male fetus, and the observations were as follows. The bilateral lateral ventricles were dilated in the fetus. The left and right ventricles were about 14 mm (Figure 3a,b). The parents requested termination of pregnancy. *L1CAM* gene mutation was NM_000425.3: c.262C>T (p.Q88*) in the apoblema (III.3). The sequence analysis of the aborted fetus detected a C>T base substitution at the nucleotide 262 (c.262C>T) in exon 4 (Figure 4a). This modification replaced the glutamine at codon 88 with a stop signal (p.Q88*) yielding a truncated protein. The mother (II.4) and the third female fetus (III.4) did not carry the c.262C>T mutation on the *L1CAM* gene (Figure 4a).

2.2 | Family 2

The case was a 29-year-old female (II.2) with four previous pregnancies, of which three males (III.1, III.2, and III.3) were terminated due to severe hydrocephalus, and one was a healthy male (III.4). The nonconsanguineous couple denied a family history of genetic disease. The pedigree is shown in Figure 2b. The ultrasound examination at 24^{+1} weeks of gestation in second pregnancy identified a male fetus (III.2). The ultrasound examination showed that the third ventricle (7.5 mm wide) was raised, and the fourth ventricle was communicated with the cisterna magna (Figure 3c,d). In her third pregnancy, the ultrasound examination at 23^{+4} weeks gestation identified a male fetus (III.3, Figure 2b), and the observations were as follows (Figure 3e). The cerebellar vermis was slightly smaller. The transparent septum was missing. The third ventricle (about 6.4 mm wide) was raised. The left ventricle was about 13 mm wide and the right was about 12 mm. The longitudinal fissure of the brain was widened. The posture of the hands was abnormal (adducted thumbs) (Figure 3f). Since X-linked hydrocephalus was suspected, an *L1CAM* gene analysis of the patient (III.3) was performed to identify the

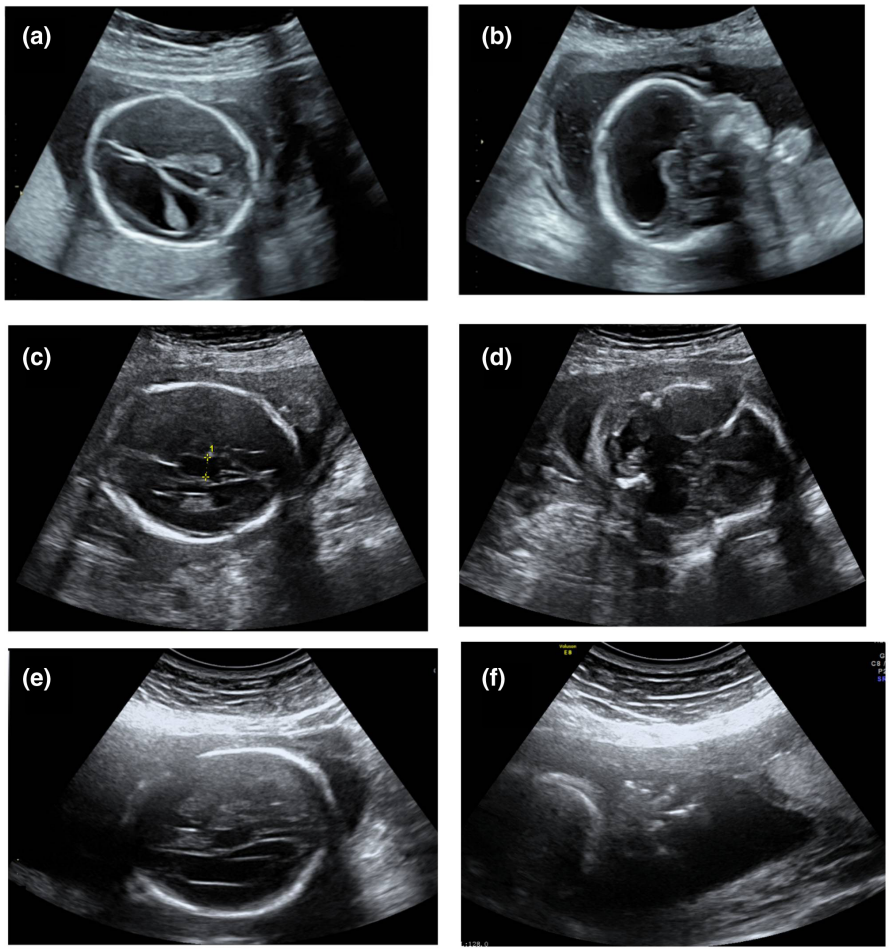

FIGURE 3 Sonography image of the affected fetuses in families 1 and 2. (a,b) Severe hydrocephaly of fetus (III.3) in family 1, (c,d) ultrasonography view of the fetus (III.2) in family 2, (e,f) ultrasonography view of the fetus (III.3) in family 2.

cause of fetal malformations and provide counseling for future pregnancies. The *L1CAM* gene mutation was NM_000425.3:c.524-1G>A. The sequence of the *L1CAM* gene showed a G>A substitution at the nucleotide 524–1 (c.524-1G>A) (Figure 4b). The splicing mutation was inherited from the mother (II.2). Finally, the woman gave birth to a healthy male baby (III.4) by the third generation of test-tube baby technology. Her mother (I.2) was a c.524-1G>A heterozygous carrier of the *L1CAM* gene and had a healthy son (II.3).

2.3 | Family 3

The case was a 30-year-old female (II.2). The nonconsanguineous couple denied a family history of genetic disease. The pedigree is shown in Figure 2c. In the first pregnancy (III.1), the ultrasound examination at 24^{+3} weeks of gestation identified a male fetus, and the observations were as follows. The bilateral lateral ventricles were dilated, and the choroid plexuses showed a tear-shaped change in the fetus. The left ventricle was about 24.9 mm wide and the right was about 23.9 mm. The parents requested termination of pregnancy. *L1CAM* gene mutation was NM_000425.3:c.261C>G (p.Y87*) in the female (II.2). The sequence of the *L1CAM* gene of this woman (II.2) showed a heterozygous C>G substitution at the nucleotide 261 (c.261C>G) in exon 4 (Figure 4c). This modification replaced the tyrosine at codon 87 with a stop signal (p.Y87*) yielding a truncated protein. The mother (I.2) and the second male fetus (III.2) did not carry the c.261C>G mutation on *L1CAM* gene (Figure 4c).

2.4 | Molecular analysis

Genomic DNA was extracted from the aborted fetus and peripheral leukocytes from the mothers and the grandma of the patients, according to standard protocols (Gao et al., 2020). Whole-exon sequencing was used to analyze the gene variation and screen the candidate gene, followed by PCR and Sanger sequencing to confirm *the L1CAM* gene. The gnomAD data were utilized.

3 | DISCUSSION

Herein, we described the imaging results in three families with the genetically confirmed L1 syndrome and reported

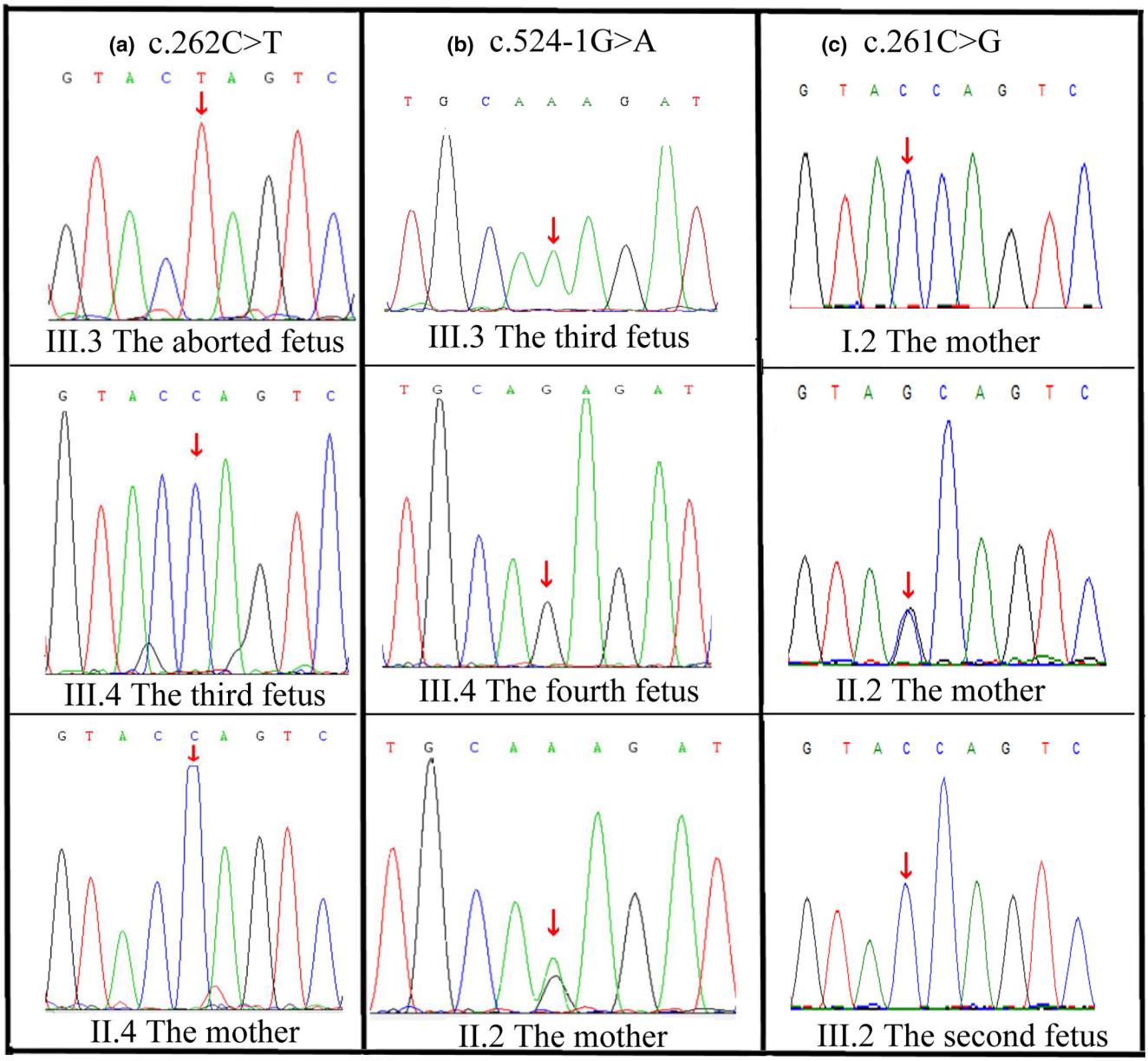

FIGURE 4 Partial DNA sequences in *L1CAM* of three families. (a) Family 1, (b) family 2, (c) family 3.

three mutations in the *L1CAM* gene: two nonsense mutations (c.262C>T and c.261C>G) and one splice-site mutation (c.524-1G>A). Two of these three are novel L1CAM variants: c.262C>T and c c.261C>G. In the present study, we enriched the clinical data of the c.524-1G>A variation in the *L1CAM* gene.

The fetus (III.3, Figure 2a) in family 1 possessed a nonsense mutation (c.262C>T; p.Q88*) in exon 4 that replaced the glutamine at codon 88 with a stop signal. This mutation could affect the structure of the L1CAM protein because the peptide chain synthesis is terminated prematurely. Moreover, this mutation is extremely rare in the normal population and has not yet been reported in the HGMD database. The bioinformatics analysis results of this site were all "unknown" by the protein function prediction software (SIFT, PolyPhen_2, and REVEL). Initially, the mutation was determined to be a suspected pathogenic variation according to ACMG guidelines (PVS1+PM2_supported). Subsequently, it was identified as a pathogenic mutation, giving rise to a protein of 88 amino acids instead of 1257 amino acids because the truncated protein lost its biological function. Furthermore, most of the nonsense mutations are known to be pathogenic (Kong et al., 2020).

The female carrier and the fetus (II.2 and III.3, Figure 2b) in family 2 possessed a splicing mutation (c.524-1G>A) near exon 6. As assessed by online software tools, the mutation sites affect the mRNA splicing

(Human Splicing Finder and NetGene2). This mutation does not belong to the polymorphic site and is infrequent in the normal population. This site is a pathogenic variation and has been reported in the HGMD database according to ACMG guidelines (PVS1+PM2_supported). Finckh et al. reported a 1-year-old boy who carried the mutation (c.524-1G>A), and the clinical manifestations were hydrocephalus and adducted thumbs (Finckh et al., 2000).

The female (II.2, Figure 2c) carrier in case 3 possessed a nonsense mutation (c.261C>G; p.Y87*) in exon 4 that replaced the tyrosine at codon 87 with a stop signal. This mutation could affect the structure of the L1CAM protein because the peptide chain synthesis is terminated prematurely. Moreover, this mutation is extremely rare in the normal population and has not yet been reported in the HGMD database. The bioinformatics analysis results of this site were all "unknown" by the protein function prediction software (SIFT, PolyPhen_2, and REVEL). Initially, the mutation was determined to be suspected pathogenic variation according to ACMG guidelines (PVS1+PM2_supported). Subsequently, it was identified as a pathogenic mutation, giving rise to a protein of 87 amino acids instead of 1257 amino acids because the truncated protein lost its biological function. Furthermore, most of the nonsense mutations are known to be pathogenic (Kong et al., 2020).

In conclusion, the present study identified three mutations in the *L1CAM* gene in three Chinese families with a history of L1 syndrome; of these, two were novel mutations. The current data confirmed the importance of combining family history, prenatal ultrasonography, and *L1CAM* mutation testing to diagnose the L1 syndrome.

AUTHOR CONTRIBUTIONS

KXD designed the study. GSS analyzed and interpreted the data and wrote the manuscript. ZXC and ZGY analyzed and interpreted the data. DP provided technical support for the analysis and critical revision of the manuscript. All authors have read and approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no competing interest.

DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Ethics Committee for Scientific Research and Clinical Trials of the First Affiliated Hospital of Zhengzhou University (KS-2018-KY-36). All the participants and parents signed informed consent forms.

CONSENT TO PUBLICATION

Informed consent for publication was obtained from all the participants and the parents.

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