Identification of a novel heterozygous missense mutation of SEMA3E (c.1327G>A; p. Ala443Thr) in a labor induced fetus with CHARGE syndrome

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Abstract
Background: CHARGE syndrome is a complex multisystem genetic disease. We aimed to find the potential gene mutation in the labor induced fetus with CHARGE syndrome.

Methods: Genomic DNA was extracted from the fetal thigh muscle tissue and the peripheral blood of his parents. The resulting exomes were sequenced using whole exome sequencing (WES) followed by the selection of the candidate causative mutation genes. The deleteriousness of the identified variants was predicted. Analysis of multiple alignment of protein sequences and protein conserved domains was performed by online software. Finally, Sanger sequencing was applied for validation of the identified variants in the WES.

Results: After sequencing and bioinformatics filtering, a heterozygous missense mutation of SEMA3E (c.1327G>A; p. Ala443Thr) was found in the fetus, while the mutation was absent in his parents. Genotyping results showed that the mutation cosegregated fully with definite CHARGE phenotypes between the fetus and his parents. This change was located in the Sema superfamily and highly conserved across different species. Sanger validation result was consistent with the WES analysis.

Conclusion: Our investigations suggested that the heterozygous missense mutation of SEMA3E (c.1327G>A; p. Ala443Thr) may be a potential causal variant in the fetus with CHARGE syndrome.

KEYWORDS
CHARGE syndrome, fetus, heterozygous missense mutation, SEMA3E, whole exome sequencing
1 | INTRODUCTION

CHARGE syndrome, a rare autosomal dominant syndrome, has been estimated to occur in 1:10,000 newborns in the world (Jongmans et al., 2006). The disorder is characterized by major clinical manifestations including ocular coloboma, congenital heart defects, choanal atresia, retardation of growth, genital hypoplasia and ear abnormalities and additional less frequent clinical anomalies such as cleft lip/palate, facial asymmetry (a broad forehead and prominent nasal bridge), esophageal anomalies, feeding difficulties, cranial nerve dysfunction, defects in neural crest migration, hypothalamo-hypophyseal dysfunction, agenesis of the semicircular canals, limb deformity, developmental delay and hyperactive behavior (Blake et al., 1998; Blake & Prasad, 2006; Graham, Beth, Rosner, Dykens, & Visootsak, 2005; Issekutz, Graham, Graham, Prasad, Smith, & Blake, 2005; Lalani, Hefner, Hefner, Belmont, & Davenport, 2012; Lanson, Green, Green, Roland, Lalwani, & Waltzman, 2007; Okuno et al., 2017; Song et al., 2011; Tellier et al., 1998).

The mutation in the chromodomain helicase DNA binding protein 7 (CHD7) (located on chromosome 8q12.1) gene was the most common in patients with CHARGE syndrome. It is estimated that 15% missense mutations, 10% splice site mutations, 5% chromosomal and exonic deletions and chromosomal rearrangements are found in the CHD7 gene variants in CHARGE syndrome (Zentner, Layman, Layman, Martin, & Scacheri, 2010). In addition, the novel monoallelic frameshift mutation of CHD7 (c.4656dupT), de novo variant in exon 37 of CHD7 (c.8016G>A) and intronic mutations of CHD7 (c.5405-7G>A, c.5405-17G>A, c.5405-13G>A and c.5405-18C>A) are also found in patients with CHARGE syndrome (Legendre et al., 2018; Ungaro et al., 2018; Xu, Shi, Shi, & Zhu, 2018). Besides common CHD7 gene variants, the mutations of elongation factor Tu GTP binding domain containing 2 (EFTUD2), lysine methyltransferase 2D (KMT2D), semaphorin 3E (SEMA3E) (OMIM# 214800) and arginine-glutamic acid dipeptide repeats (RERE) duplication could also cause CHARGE syndrome (Badalato et al., 2017; Jordan et al., 2018; Lehalle et al., 2014; Luqueti et al., 2013; Sanlaville & Verloes, 2007).

It is noted that the various anomalies in CHARGE syndrome could be life-threatening and approximately 30% of affected children die before 5 years of age (Zentner et al., 2010). Survival of those affected children is tightly associated with age (Hale, Niederriter, Niederriter, Green, & Martin, 2016; Hsu et al., 2014). As CHARGE syndrome significantly resembles other patterns of anomalies, genetic testing such as mutation screening of potential candidate genes should be emphasized as a useful method in clinical diagnosis. It is worth mentioning that whole exome sequencing is a valuable tool to explore the genetic basis of diseases (Bamshad et al., 2011), which may be helpful in clinical diagnosis. In this study, we investigated an labor induced fetus with CHARGE syndrome. Using whole exome sequencing, we identified a novel heterozygous missense mutation (c.1327G>A; p. Ala443Thr) in SEMA3E, which may be another causative mutation of CHARGE syndrome. Our results may provide a new genetic basis for understanding the pathology mechanism of CHARGE syndrome.

2 | MATERIAL AND METHODS

2.1 | Ethical compliance

Our study was approved by the ethics committee of the local hospital.

2.2 | Sample collection

In this study, a pregnant woman (29 years old) with G1P0 was recruited. According to the diagnosis criteria, the fetus was diagnosed with CHARGE syndrome on prenatal examination of the pregnant woman. The pregnant woman was normal and had no family history or history of adverse contact. Moreover, prenatal pregnancy examination was performed during the pregnancy. In addition, her husband also had a normal phenotype. In order to explore the pathogenesis of CHARGE syndrome, the pregnancy was terminated. Whole exome sequencing of the fetal thigh muscle tissue and peripheral blood (2 ml) of his parents was performed.

2.3 | Exome sequencing

Genomic DNA from the fetal thigh muscle tissue of and peripheral blood of his parents was extracted using DNeasy Blood & Tissue Kit (DP348) (Qiagen). DNA content and quality was determined using Nanodrop and agarose gel electrophoresis, respectively. Genomic DNA was broken into random fragments and purified. The Roche SeqCap EZ MedExome Kit was used to capture genome-wide exons. After enrichment of quality control, the captured fragments were used for genomic library preparation. An Illumina Hiseq XTen sequencer was used for double side high flux PE150 sequencing. The average sequencing depth is 100× and more than 95% of the area reached more than 20× of the coverage. The original sequencing data were presented in FASTQ format. After basic data analysis including image recognition, filtering connection sequences and decontamination, the data were processed as follows: (a) Using Burrows-Wheeler Alignment (BWA) (http://bio-bwa.sourceforge.net/bwa.shtml) BWA software (version 0.7-6a), short reads and the reference genome (hg19) (http://hgdownload.soe.ucsc.edu/goldenPath/hg19/bigZips/) (NG_021242.2) were compared to determine the position of short sequences in the
genome; (b) The SAMtools software (version 0.1.18) was used to classify and convert the short sequences; (c) Picard (http://picard.sourceforge.net/) software (version 1.91) was utilized to remove redundant information and noise generated during sequencing; (d) The sites (SNV and InDel) in sequencing data were obtained by Genome Analysis Toolkit (GATK) software (version 2.6-4) (McKenna et al., 2010); (e) The functional annotation of these mutation sites were formed by Annovar software (version 2013 August 23).

2.4 | Bioinformatics analysis

All mutations were filtered by ExAC, 1000 Genomes Project and ESP6500 ordinary public database. According to the variation in genes corresponding to genetic pattern of the disease, the variation could be classified as autosomal dominant, autosomal recessive, X-linked dominant and X-linked recessive. For autosomal dominant or X-linked dominant mutation loci, variation with frequency less than 0.01 in the database was reserved; for the autosomal recessive or X-linked recessive mutation loci, variation with a frequency less than 0.05 in the database was reserved. After removing the synonymous mutation loci, the remaining variation loci within 20bp of exons were sorted by combining the harmful score predicted by SIFT, polyphen2 and other software. The possible pathogenic mutation loci were screened out according to the disease phenotype and family information.

2.5 | In silico analysis

Multiple alignments of protein sequences in different species was analyzed based on the tool (https://www.ncbi.nlm.nih.gov/tools/cobalt/cobalt.cgi?LINK_LOC=BlastHomeLink). The protein conserved domains were further identified using

**FIGURE 1** Color ultrasonography of the fetus. (a) Color ultrasonography of the fetus in early pregnancy; the reverse reflux of venous catheter of the fetus; (b–i) Color ultrasonography of the fetus in the second trimester. (b) A cystic dark area was observed in both choroid plexuses of the fetus; (c) the left ulna and radius were visible; (d) the right ulna was visible; (e) the hands were curving; (f) the left foot and the heart were observed; (g) mirror right ventricular was observed; (h) an atrioventricular septal defect was observed; (i) bilateral radial dysplasia and left foot pronation posture was observed.
the Conserved Domain Search Service (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

2.6 | Mutation validation and cosegregation analysis

In order to validate potential causative variants in the fetus and his parents, Sanger sequencing was carried out using an ABI 3730xl sequencer (Applied Biosystems company). In addition, segregation analysis was performed for all family members. The interpretation and evidence grading of genetic variation was performed according to the latest editor of the American society for medical genetics and genomics (ACMG) standard and guidelines. Primer pairs used to amplify fragments encompassing individual variants were designed by Primer 5.

3 | RESULTS

3.1 | Clinical feature of fetus

In the color ultrasonography in early pregnancy, reverse reflux of venous catheter of the fetus was observed (Figure 1a). In the ultrasonography in prenatal diagnosis in the second trimester, a cystic dark area was observed in both choroid plexuses of the fetus (Figure 1b), and the larger dark area was about 0.7 × 0.4 cm. The left ulna was visible, and the radius was short measuring about 0.35 cm (Figure 1c). The right ulna was visible, the radius was not shown (Figure 1d), and the hands were hooked (Figure 1e). During the examination, the left foot was in the same plane with the left tibiofibula bone, the heart was located in the right thoracic cavity, the apex of the heart was toward the right, the atrial septum and the upper ventricular septum were not detected, and only one set of atrioventricular valves was observed. No vertebral bones in the left side of the fetal spine were observed (Figure 1f). In the second color ultrasound examination of the fetus, mirror right ventricular (Figure 1g) with an atrioventricular septal defect (Figure 1h) was observed. Bilateral radial dysplasia, left foot pronation posture (Figure 1i), left coccyx dysplasia, and bilateral choroid plexus cysts were observed. The above clinical feature of the fetus was in line with the diagnostic criteria of CHARGE syndrome.

3.2 | Genetic and bioinformatics analysis

In this study, we did not find any other known gene mutations for CHARGE syndrome, such as CHD7 in the genome of the fetus. Interestingly, a heterozygous missense mutation of SEMA3E (c.1327G>A; p. Ala443Thr) was found in the fetus. However, the mutation was not detected at this locus in either parent, suggesting that the mutation was a new mutation in the fetus. The mutation frequency was not found in the ExAC, 1000 Genomes Project and ESP6500 ordinary public database, indicating the rarity of the mutation. In addition, predictive soft PROVEAN/SIFT, Polyphen2 and FATHMM indicated that the mutation was harmful and destructive to protein function (Figure 2).

3.3 | In silico analysis

The c.1327G>A mutation in SEMA3E resulted in p. Ala443Thr change. Moreover, the multiple alignment of

![FIGURE 2](image-url) The harmful prediction of the mutation of SEMA3E by software of PROVEAN/SIFT (a), Polyphen2 (b) and FATHMM (c)
protein sequences and protein conserved domains analysis showed that the amino acid in 443 locus of SEMA3E protein sequence was located in the domain of Sema superfamily (Figure 3a), and it was highly conserved across species of human, rhesus, rat, dog, elephant, *Xenopus laevis* and zebra fish (Figure 3b).

**FIGURE 3** (a) The affected amino acid residue was highly conserved across species. (b) Conserved domains in SEMA3E protein. Arrow represents the mutation site.

**FIGURE 4** Sanger validation results of *SEMA3E* variants in the fetus (a) and his parents (b). Arrow represents the mutation site.
3.4 Sanger sequencing

In order to further validate the heterozygous missense variants of c.1327G>A mutation in SEMA3E, Sanger sequencing was performed. The results showed that the c.1327G>A mutation in SEMA3E was found in the fetus (Figure 4a). However, the mutation was not found in his parents (Figure 4b). The Sanger validation result was consistent with the WES analysis.

4 DISCUSSION

Although most patients carry CHD7 gene mutations, some other genes are also associated with CHARGE syndrome. It was shown that (semaphorin) 3E (SEMA3E) was interrupted by a de novo balanced translocation [t(2;7)(p14;q21.11)] in a CHARGE syndrome patient (Lalani et al., 2004). In this study, we first found a heterozygous missense mutation (c.1327G>A; p. Ala443Thr) in the gene of SEMA3E in labor induced fetus with CHARGE syndrome, which may be another potential causative gene mutation of SEMA3E for CHARGE syndrome. In addition, we also validated the mutation in the fetus and his parents using Sanger sequencing, which showed that the SEMA3E (c.1327G>A; p. Ala443Thr) mutation was cosegregated between the fetus and his parents. Our result indicated that the novel mutation of SEMA3E may be valuable in illustrating the pathological mechanism of CHARGE syndrome.

SEMA3E, contains 13 conserved cysteine residues and three potential N-glycosylation sites, was first identified in metastatic cell lines (Christensen et al., 1998). The expression of SEMA3E was found in the eye (retina), nervous system and bone (osteoclasts/osteobalsts) and lung tissues (Christensen et al., 1998; Ryynänen et al., 2017; Sun et al., 2017). Several reports have demonstrated the role of SEMA3E in angiogenesis. It is reported that SEMA3E plays a crucial role in vascular patterning, such as formation of the first embryonic blood vessel (Gu et al., 2005; Meadows et al., 2012). Gitler, Lu, Lu, & Epstein, 2004 and Gu et al., 2005 found that SEMA3E in labor induced fetus with CHARGE syndrome. In addition, we also validated the mutation in the fetus and his parents using Sanger sequencing, which showed that the SEMA3E (c.1327G>A; p. Ala443Thr) mutation was cosegregated between the fetus and his parents. Our result indicated that the novel mutation of SEMA3E may be valuable in illustrating the pathological mechanism of CHARGE syndrome.

SEMA3E plays a key role in regulating synaptic connectivity and axonal growth in the central nervous system (Bellon et al., 2010; Chauvet et al., 2007; Ding, Oh, & Oh, 2011). Exposure of developing hippocampus subicular neurons to SEMA3E could promote axonal growth in mouse (Bellon et al., 2010). In addition, it has been indicated that SEMA3E can promote the survival of the maturing hypothalamus (Cariboni et al., 2015). Interestingly, abnormalities in the nervous systems caused by SEMA3E are found in patients with heart defects (Silversides et al., 2012). In addition, SEMA3E is related to idiopathic hypogonadotropic hypogonadism disorders (Topaloglu, 2017). In addition, SEMA3E also play roles in angiogenesis and central nervous system in lower vertebrate animals, such as zebra fish. Lamont RE et al found that Sema3e acts autonomously and nonautonomously in angioblasts to modulate interactions among themselves (Lamont, Lamont, & Childs, 2009). Moreover, loss of Sema3e results in delayed exit of angioblasts from the dorsal aorta in intersegmental vessels formation. Dell AL et al reported that elevated cyclic adenosine monophosphate (cAMP) levels increased the sensitivity of retinal axons to Sema3e, and consequently facilitated retinal axon crossing in the chiasm (Dell, Fried-Cassorla, & Torres-Vázquez, 2011). In addition, SEMA3E is also associated with heart and bone development. Copy number variants of SEMA3E are found in patients with heart defects (Silversides et al., 2012). SEMA3E knockout leads to the cardiovascular abnormalities in patients with CHARGE syndrome (Gay et al., 2011). In addition, SEMA3E plays a crucial role in regulating bone homeostasis in osteoblasts (Ryynänen et al., 2017). In this study, several heart abnormalities including mirror right ventricular and atrioventricular septal defect and bone abnormalities including short radius, curving hands, left foot pronation posture and no visible vertebral bones in the left side of the fetal spine were found. It is indicated that SEMA3E may be associated with heart and bone development.

In the molecular genetics level, mutations of SEMA3E have been found in CHARGE syndrome. By sequencing, a missense mutation of SEMA3E was revealed in 72 patients with CHARGE syndrome (Lalani et al., 2004). The de novo missense mutation S703L of SEMA3E has also been uncovered in CHARGE syndrome patients (Damien & Alain, 2007). In addition, nonsynonymous SNPs including G623C, G603T, C1062T, G1272A, A2149G, A2211G in SEMA3E were seen in patients with CHARGE syndrome (Lalani et al., 2004). Silversides CK found that SEMA3E was involved in regulating the NOTCH pathway in CHARGE syndrome (Silversides et al., 2012). In this study, we first found a novel heterozygous missense mutation (c.1327G>A; p. Ala443Thr) in SEMA3E in labor induced fetus with CHARGE syndrome.
Moreover, the mutation frequency was not found in the ExAC, 1000 Genomes Project and ESP6500 ordinary public database, indicating the rarity of the mutation. The mutation was highly analogous across species of human, rhesus, rat, dog, elephant, *Xenopus laevis* and zebra fish. In addition, predictive soft PROVEAN/SIFT, Polyphen2 and FATHMM indicated that the mutation was harmful and destructive to protein function. It is indicated that the novel heterozygous missense mutation (c.1327G>A; p. Ala443Thr) in SEMA3E may be another potential causative factor for CHARGE syndrome.

In other words, our findings reported here show that SEMA3E (c.1327G>A; p. Ala443Thr) may be another pathogenic factor for CHARGE syndrome. Our results may be helpful in understanding the potential pathological mechanism of CHARGE syndrome. However, there is a limitation of our study. The underlying signaling pathway mechanism is not investigated, and further animal model or cell culture experiments are needed.

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CONFLICT OF INTEREST
None.

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