Whole exome sequencing revealed a heterozygous elongation factor Tu GTP-binding domain containing 2 (EFTUD2) mutation in a couple experiencing recurrent pregnancy loss

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To the Editor: Recurrent pregnancy loss (RPL) is defined as the failure of two or more clinically recognized pregnancies.[1] Both parental and embryonic/fetal factors are associated with RPL. Parental factors include balanced chromosome rearrangements, maternal antiphospholipid syndrome, uterine anomalies, and hormonal or metabolic disorders.[2] Of the examined products of conception (POC), approximately 60% of early pregnancy losses result from sporadic chromosomal abnormalities in embryos, specifically numeric chromosome errors. However, up to 50% of RPL cases remain unexplainable by known causes. Here, we report a couple who have experienced four consecutive clinical pregnancy losses within 10 weeks of gestation. Peripheral karyotypic analyses of the 33-year-old female and 36-year-old male were normal. Based on these findings, further genetic examination was performed. We obtained peripheral blood samples from the couple, semen samples from the male partner, and the last three miscarried embryonic tissues (POC-2, POC-3, and POC-4).

To detect embryonic genetic factors of RPL, chromosomal microarray analyses (CMA) were successively performed using the three miscarried embryonic tissues. These analyses yielded negative results. Furthermore, trio-WES was performed on POC-4 and on peripheral blood samples from the partner. A novel de novo heterozygous nonsense mutation was identified (c.1012G→T) in exon 12 of the EFTUD2 gene (NM_004247.4), which could result in structural changes in the EFTUD2 protein (p. E338*). WES was subsequently performed on POC-3, and the same mutation was detected. The presence of the mutation in embryonic tissues from all three miscarriages was confirmed by Sanger sequencing [Figure 1A (a-c)].

The mutation was not detected in peripheral blood samples of the partner. Gonadal mosaicism was suspected, as all POC detected the same mutation. Exome sequencing identified the same mutation (c.1012G→T) in the EFTUD2 gene in 13.5% of the male partner’s sperm cells (ref [bias-field method]/[alternative method] alt:...
pregnancy loss; WES: Whole exome sequencing.

The mosaic mutation was confirmed by Sanger sequencing of the sperm [Figure 1A (d)] but was not found in the male partner’s saliva.

To understand the potential impact of the c.1012G>T mutation on EFTUD2 function, in silico analysis was performed using Protein Variation Effect Analyzer (PROVEAN, [http://provean.jcvi.org/index.php], version 1.1.3). The nonsense mutation (c.1012G>T) results in a change in glutamic acid to a premature stop codon (UAA) at amino acid position 338 (p.E338∗), producing a truncated EFTUD2 protein that lacks its C-terminal 634 amino acids. Wild type EFTUD2 protein (NP_004238.3), composed of 972 amino acids, contains six vital domains (the Protein Families [Pfam] database [http://pfam.xfam.org]): (a) 116 kDa U5 small nuclear ribonucleoprotein component N-terminus (amino acids 4–110); elongation factor Tu GTP-binding domain (amino acids 129–377); elongation factor Tu domain 2 (amino acids 491–566); elongation factor G, domain III (amino acids 586–648); elongation factor G, domain IV (amino acids 707–823); and elongation factor G, domain V-like (amino acids 826–914). Amino acid 338 is located within the elongation factor Tu GTP-binding domain, before translation elongation factor Tu-like domain 2. The mutated protein contains the 116 kDa U5 small nuclear ribonucleoprotein component N-terminus and an aberrant elongation factor Tu GTP-binding domain [Figure 1B]. PROVEAN predicted the EFTUD2 mutation (p.E338∗) to be pathogenic (PROVEAN: deleterious, with a score of −2333.598).

The EFTUD2 gene is located on chromosome 17q21.31. It encodes U5-116 kDa, a highly conserved GTPase component of the major spliceosome. Several studies have reported that EFTUD2 haplesinsufficiency is linked to mandibulofacial dysostosis, Guion-Almeida type (MIM # 610536), a rare syndrome with a wide spectrum of congenital anomalies, characterized by malar and mandibular hypoplasia, microcephaly, micrognathia, dysplastic ears with hearing loss, cleft palate, choanal atresia, and facial asymmetry. However, there is a paucity of studies that have evaluated the function of EFTUD2 in spontaneous miscarriage.

Since the couple has experienced recurrent abortions, a possible explanation for the difference between outcomes for our patients and previously reported patients is that the truncating protein (p.E338∗) may induce embryonic lethality, which can be considered the most severe phenotype caused by EFTUD2 mutations in humans. We used a zebrafish model to confirm the effects of loss of EFTUD2 gene function in vivo. Messenger RNAs (mRNAs) encoding wild type (wt) and mutant human EFTUD2 were synthesized and injected into zebrafish embryos. The human EFTUD2 coding region sequence was obtained from Sino Biological Inc. (Catalog Number HG14427-G, Beijing, China). When zebrafish embryos...
were injected with several doses of mRNA, wt EFTUD2 mRNAs produced significantly higher rates of embryonic death/deformities at 24 h per fertilization (hpf) than EFTUD2 c.1012G>T mutation in the human EFTUD2 gene causes loss of function. Based on clinical symptoms, we investigated brain and heart development during embryogenesis by marker analyses using whole-mount in situ hybridization. The hindbrain neuron marker pax2a decreased significantly with injection of ~30 pg of wt EFTUD2 mRNA at the indicated stages. Heart development was also affected by wt EFTUD2. Cardiac marker myl7 showed that wt EFTUD2 mRNAs induced looping defects. A small head phenotype was also observed in embryos injected with wt EFTUD2 mRNAs. Embryos injected with the same doses of EFTUD2 p.E338T mutant mRNAs displayed little alteration in brain and heart development [Figure 1C (b)]. Taken together, these results demonstrate that the EFTUD2 c.1012G>T mutation causes loss of gene function in EFTUD2.

Because the novel EFTUD2 c.1012G>T mutation potentially correlates with RPL, the couple received in vitro fertilization (IVF) with pre-implantation genetic testing (PGT) to prevent the mutation. One embryo sample without the mutation (c.1012G>T) in the EFTUD2 gene was selected for transfer, and a successful pregnancy was confirmed by human chorionic gonadotropin and ultrasound examination. In the second trimester, amniocentesis was performed at 18 gestational weeks with negative results by CMA for chromosome abnormality and by Sanger sequencing for the EFTUD2 gene mutation (c.1012G>T). At present, the fetus is at 24 gestational weeks, and ultrasound abnormalities have been excluded by detailed second trimester fetal anomaly scans.

In conclusion, we have identified a novel EFTUD2 c.1012G>T mutation in a non-consanguineous Chinese couple, which potentially correlates with RPL. WES is useful for determining the etiology of unsolved RPL cases. This EFTUD2 gene proved useful information for genetic counseling, leading the couple to receive IVF with PGT to prevent the mutation. As our data are based on only one family, further functional tests and investigations are needed to determine EFTUD2 function in human embryonic/fetal development.

Acknowledgements

The authors thank the couple for their participation in this study.

Funding

This work was supported by grants from the Technology Research and Development Program of Science and Technology Department of Sichuan Province (Nos. 2017SZ0125, 2021YFS0026, and 2020ZYD007).

Conflicts of interest

None.

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How to cite this article: Yang M, Sun H, Liu Y, Hu T. Whole exome sequencing revealed a heterozygous elongation factor Tu GTP-binding domain containing 2 (EFTUD2) mutation in a couple experiencing recurrent pregnancy loss. Chin Med J 2022;135:1108–1110. doi: 10.1097/CMJ.0000000000001667.