Prenatal Diagnosis of Ellis–van Creveld Syndrome by Targeted Sequencing

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To the Editor: Ellis–van Creveld (EvC) syndrome is a rare, autosomal recessive skeletal dysplasia with the prevalence of 1/60,000 approximately.[1] It is characterized by short limbs, short ribs, postaxial polydactyly, dysplastic nails/teeth, and congenital heart defects (CHD) which were observed in about 60% of affected individuals. Mutations in genes *EVC* or *EVC2* have been identified in two-thirds of patients with EvC syndrome.

A 24-week-old male fetus was obtained by means of therapeutic abortion with multiples anomalies such as short humerus, hexadactyly, and CHD including atrial-ventricular septal defect [Figure 1a and 1b], total anomalous pulmonary venous connection [Figure 1c], and persistent superior left vena cava [Figure 1d] detected by ultrasound and fetal echocardiography scan. Postmortem autopsy and X-ray findings were consistent with those seen by prenatal examinations [Figure 1e and 1f]. Both the parents and his sister have no phenotypic symptoms similar to those of the fetus.

With informed consent, umbilical cord of the fetus was obtained during the autopsy. The genome was extracted using QIAamp DNA Mini Kit. Chromosomal aneuploidy and microdeletions/microduplications above 100 kb were detected first by whole genome sequencing (~1×) on Illumina HiSeq 2500 (PE50). With no chromosomal anomalies identified, single nucleotide variations and indels in all exons and ± 10 bp in the intron/exon boundaries of 143 CHD-related genes were then detected by targeted sequencing on Illumina HiSeq (PE100). In addition, about 405.94 Mb of data was mapped to target regions with a mean depth of 401.94.

During data analysis, two novel mutations c.1626_1630dupGCTCC (p.P544Rfs*5) (paternal)/c.2783-3C>A (maternal) in *EVC* gene were identified. The frameshift, leading to a truncated protein, was predicted to be disease causing by Mutation Taster program. The latter was considered to be a candidate variant as the site was highly conserved in vertebrates and the variant was predicted to be damaging by both Human Splicing Finder and Mutation Taster program. Moreover, familial analysis revealed that the unaffected parents and sister were all mutation carriers [Figure 2].

The encoding protein of *EVC* acts as a positive mediator of sonic hedgehog (Shh) signaling pathway which is indispensable for normal endochondral growth and skeletal development. The study of atrioventricular septation defect phenotype of $Shh^{-/-}$ mutant mouse embryos demonstrated that the Shh signaling is important in the septation of the mammalian heart into four chambers.[2] During mouse embryonic development, *EVC* expressed in the secondary heart field, including both the outflow tract and the dorsal mesenchymal protrusion, and in mesenchymal structures of the atrial septum and the atrioventricular cushions. Therefore, mutations in this gene might result in skeletal and cardiac hypoplasia.

Clinical symptoms manifested in the proband, including short humerus, postaxial polydactyly and heart malformation overlap with the characteristics of EvC syndrome. *EVC* compound heterozygous mutations identified in the patient was in accordance with the autosomal recessive inheritance pattern of EvC syndrome. Both of the two mutations were novel and presuming to be damaging. Moreover, the mutations were found to cosegregate with disease in the family. Therefore, the two *EVC* mutations identified were probably responsible for the disease phenotype in the affected fetus. Since the first two abortions were carried out at the request of the pregnant woman and the clinical information and samples of them were not available, we cannot get more evidence for segregation.

EvC syndrome is a subtype of the short-rib thoracic dysplasia is implicated with many genes like *EVC/EVC2/SRTD1/IFT80/*

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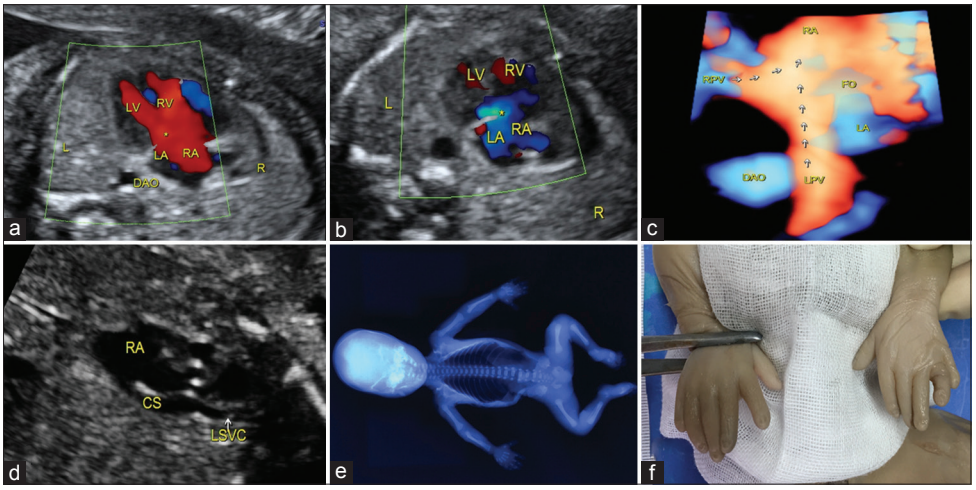

Figure 1: (a-f) Complete atrioventricular septal defects during diastole (a) and systole (b); (c) spatiotemporal image correlation image of total anomalous pulmonary venous connection (type II); (d) persistent superior left vena cava; (e) whole body X-ray shows skeletal dysplasia; (f) postaxial polydactyly. LA: Left atrium; LV: Left ventricle; RA: Right atrium; RV: Right ventricle; DAO: Descending aorta; LPV: Left pulmonary vein; RPV: Right pulmonary vein; FO: Oval foramen; CS: Coronary sinus.

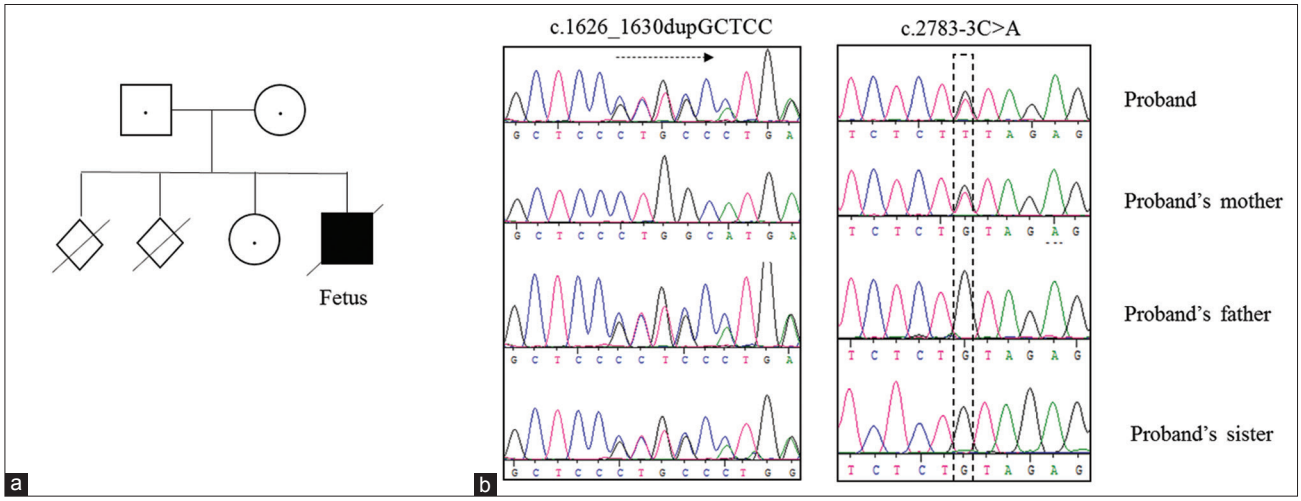

Figure 2: The c.1656_1630dupGCTCC and c.2783-3C>A mutation identified in the family. (a) The pedigree (b) the proband carried the compound heterozygous mutation c.1626_1630dupGCTCC/c.2783-3C>A, his father and sister carried the heterozygous mutation c.1626_1630dup, while his mother carried the heterozygous mutation c.2783-3C>A.

DYNC2H1/TTC21B/WDR19/NEK1/WDR35/WDR60/IFT140/IFT172/WDR34/CEP120/KIAA0586. Different clinical diagnoses such as Smith-Lemli-Opitz syndrome and Hydrolethalus syndrome exist since clinical symptoms of these diseases involve similar cardiac and skeletal defects. The clinical and genetical heterogeneities of diseases increase the difficulty in diagnosing clinically. It proved that detecting of chromosomal anomalies and genetic variations was a powerful tool to provide more information for diagnosis.[3]

We have identified inherited gene mutations associated with EvC syndrome in a fetus with CHD by targeted sequencing, highlighting the value of molecular analysis in the diagnosis and clinical management of CHD. With this information, preconceptional and prenatal genetic counseling to the couple, as well as a medical assessment of other family members, can be conducted more effectively. However, the molecular mechanism underlying the pathogenesis of EvC syndrome remains under investigation. Functional study on the two mutant alleles of the *EVC* gene is warranted.

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Conflicts of interest

There are no conflicts of interest.

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