Novel Compound Heterozygous Variants of *DYNC2H1* Causing The Short Rib-Polydactyly Syndrome, Type III With Situs Inversus Totalis in a Fetus

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Research Article

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Abstract

Background

Short-rib thoracic dysplasia 3 with or without polydactyly (SRTD3) is an autosomal recessive disorder. SRTD3 presents clinically with the narrow thorax, short ribs, shortened tubular bones, and acetabular roof abnormalities. Clinical signs of SRTD3 vary among individuals. Mutations of *DYNC2H1* were reported to cause SRTD3.

Methods

We performed a detailed clinical prenatal sonographic characterization of the fetus with SRTD3. Whole-exome sequencing was used to identify causative variants in the trio family. The identified variants were validated by Sanger sequencing and mass spectrometry in the families. Multiple computational tools were used to predict the harmfulness of the two variants.

Results

We presented with the prenatal sonographic image of the fetus with SRTD3, including abnormal rib curvature, narrow thorax, bilateral hypoplastic lungs, bilateral polydactyly, syndactyly and fetal visceral situs inversus with mirror-image dextrocardia. We revealed novel compound variants of *DYNC2H1* (NM_001080463.1: c.11504T > G (p.Ile3835Arg) and c.2106 + 3A > T). Various statistical methods predicted that the variants would cause harmful effects on genes or gene products.

Conclusion

It is the first time that fetal SRTD3 with situs inversus totalis in a Chinese family. Our study not only revealed two novel compound heterozygous Dynein Cytoplasmic 2 Heavy Chain 1 (*DYNC2H1*) variants, expanding the genetic spectrum of SRTD3 but also raising the new phenotype. Our study helps add experience to further our expertise in prenatal counseling for SRTD3.

Introduction

Short-rib thoracic dysplasia 3 with or without polydactyly (SRTD3, MIM:613091) is an autosomal recessive disorder. SRTD3 presents clinically with the narrow thorax, short ribs, shortened tubular bones, and acetabular roof abnormalities. Clinical signs of SRTD3 vary among individuals. Non-skeletal manifestation also involved including cleft lip and palate and some major organs such as the brain, eyes, heart, kidneys, liver, pancreas, intestinal abnormalities, and genitalia. Some fetuses died in the neonatal period due to severe restriction of the thorax secondary to respiratory insufficiency, while others survive. There are many SRTD subtypes (SRPS 1–17). The four lethal SRTD are SRTD type I–IV. SRTD types I–IV

are mainly characterized by short limb deformities, thoracic stenosis, often combined with polydactyly, and multiple anomalies of other organs. Each of them has its typical features and also common overlapping phenotype. The prenatal phenotype differ and have variable expressivity. Therefore, it is a challenge to give a timely and accurate diagnosis [1]. Sonographic examinations gave clues and genetic tests help to give a molecular diagnosis.

Methods

Patient and Sonographic examinations

A 22-year-old woman was referred to our department for routine sonographic examination at 22.4 gestational weeks (GW). Systematic prenatal sonographic examinations including two-dimensional, three-dimensional ultrasound and echocardiography were obtained to a final diagnosis by at least two chief physicians. GE Volusion E10 was used to perform the sonographic examination with the probe of 1-5MHz. These sonographic scans were all performed using an GE Volusion E10 machine, with 1-5MHz sector transducer.

Genetic testing

The Whole-exome sequencing (WES)-Trio was performed in the trio-family. In this method, genomic DNA was collected from blood in parents and umbilical cord blood in the fetus. First, DNA was broken and the library was prepared. Then, the exons of the target gene and the DNA in the adjacent shear region were captured and enriched by the BGI V4 chip. Finally, the MGISEQ-2000 sequencing platform is used for variant detection. The average sequencing depth of the target region was $\geq 180X$, and the percentage of loci with an average depth of $>20X$ in the target region was $>95\%$. Sequenced fragments were aligned with the UCSC hg19 human reference genome by BWA to remove duplicates. Base Quality Score Recalibration, SNV, InDel, and genotype detection were performed using GATK. ExomeDepth was used for copy number variation detection at the exon level. The variants were named according to the Human Genome Variation Society (HGVS) nomenclature. Variation annotation and screening were conducted based on clinical information of subjects, population database, disease database, and biological information prediction tool. Variation pathogenicity was classified according to the American College of Medical Genetics and Genomics (ACMG), Association for Molecular Pathology (AMP) Sequence Variation Interpretation Guidelines, and the Clingen Sequence Variation Interpretation Working Group and the Association for Clinical Genomic Science (ACGS), and other detailed interpretation of these guidelines.

Variants validation

DYNC2H1 variant c.2106+3A>T (NM_001080463.1) was validated by Sanger sequencing using the Forward primer: TTTGCCAGACTGCCTTATCA and the Reverse primer: AGACTGAGGAGGGGAGGCTA. *DYNC2H1* variant c.11504T>G (NM_001080463.1) was validated by mass spectrometry.

Results

Patient and sonographic findings

This study was approved by the Ethics Committee of Maternal and Child Health Hospital of Hubei Province and informed consent was obtained from parents.

A 22-year-old woman was referred to our department for routine sonographic examination at an ultrasonic gestational age of 20.6 weeks and 22.4 weeks from the last menstrual period (LMP). This was the second pregnancy of the woman. She had an early pregnancy loss in the first pregnancy. The couple was healthy and non-consanguineous. The sonographic examination detected multiple anomalies. Measurement value (-2 standard deviation (SD) / +2 SD) of the biparietal diameter (BPD) was 5.7 cm (4.9 cm/ 6.1cm); head circumference (HC) was 21.7 cm (18.3 cm/ 21.8 cm); abdominal circumference (AC) was 16.7 cm (15.7 cm/ 19.6 cm), femur length (FL) was 3.3 cm (3.3 cm/ 4.1 cm); humerus length (HL) was 2.5 cm (32.1cm/ 40.5 cm) respectively. (Fig. 1) Both HL and FL values were below - 2SD. The fetus showed abnormal rib curvature, narrow thorax, bilateral hypoplastic lungs, bilateral polydactyly, hallux valgus angle on the foot and syndactyly. (Fig. 1) The fetus was situs inversus totalis with mirror-image dextrocardia. The bilateral humerus was curved. (Fig. 1)

The equal echo of approximately 0.4*0.3 cm in size was seen on the lateral aspect of the little fingers on both sides. A strong osseous echo could be seen inside. Ribs were short and curved. The thoracoabdominal junction was markedly depressed and the thorax was markedly reduced ($3.4/5.18 = 0.66$). (Fig. 1) The thoracoabdominal transverse diameter ratio was 0.66. The size of the left lung is 2.7*1.1cm and the right lung is 1.6*1.0cm. Both fetal kidneys collecting systems are separated by about 0.4cm. Echoes of 6 toes were seen in the left foot. The first toe and second toe of the right foot are widely spaced and the fourth and fifth toes appear to be syndactyly. Tibial length was 2.7 cm (-2.8 SD); ulna length was 2.6 cm (-3.2 SD). The final US diagnosis was short-rib thoracic dysplasia (Narrow thorax, broken ribs with curvature, short limb deformities, polydactyly, hallux valgus angle), situs inversus totalis, mirror-image dextrocardia. (Fig. 1)

Genetic Findings

Two heterozygous variants of the *DYNC2H1* gene associated with the subject's phenotype in SRTD3 were detected, NM_001080463.1: c.11504T > G (p.Ile3835Arg) and c.2106 + 3A > T. Both variants were classified as "variants of uncertain significance (VUS)". No pathogenic chromosomal CNV variants above 1M and LOH variants above 5M associated with the phenotypes of the proband were detected through the analysis of high-throughput data information. c.11504T > G (p.Ile3835Arg) was detected in the father and the fetus in the trio-family. c.11504T > G variant was located in exon 79 of the *DYNC2H1* gene and caused the alteration of isoleucine to arginine at the amino acid position 3835. It was classified to be VUS (PM2 + PP3), with the frequencies of 0.000013 in GnomAD, 0.00019 in GnomAD - EAS. This variant was not found in the normal control population in ESP, 1000, and EXAC databases. Various statistical methods, including conservative prediction and evolutionary prediction, predicted that the variant would

cause harmful effects on genes or gene products. Computer software tools including SIFT, Mutation Taster, Condel predicted the missense variant was pathogenic. All the three tools (PhyloP Vertebrates, PhyloP Placental Mammals, GERP++) showed this locus was a conserved one. c.2106 + 3A > T was detected in the mother and the fetus in the trio-family. No frequency was found in the ESP, 1000, EXAC, and GnomAD databases. All the three tools predicting splicing site effect (SpliceAI, dbSCSNV_RF, dbSCSNV_ADA) showed the variant causing the harmful effect. Sanger sequencing was validated in the family. (Fig. 2)

Discussion

Many genes have been reported to relate to SRTD3, such as *DYNC2H1*, *DYNC2LI1*, *WDR19*, *WDR34*, *WDR35*, *WDR60*, *IFT140*, *TTC21B*, *TCTEX1D2*, *IFT80*, *IFT172*, *EVC1*, *EVC2*, *KIAA0586* and *CEP120*, *INTU*, *ICK*, *NEK1*, and *C21ORF2* [2]. Among the above genes, *DYNC2H1* is the most common one associated with the SRTD3 disease. *DYNC2H1* encodes a dynein protein that participating in ciliary intraflagellar transport. Mutations of *DYNC2H1* will lead to dysfunction of primary cilia causing a heterogeneous spectrum of conditions. *DYNC2H1* is the pathogenic gene of the SRTD3 disease. SRTD3 disease was clinically and genetically heterogeneous. There is significant variability in the course and severity of the thoracic phenotype, both between siblings with the same *DYNC2H1* allele and between individuals with different alleles, suggesting that the *DYNC2H1* phenotype may be influenced by modified alleles, non-genetic or epigenetic factors [3]. More details concerning how each variant causes its phenotype remained to be revealed further by investigating the mechanism of the gene function. Among all the cases of reported SRTD, Okamoto et al once reported a Japanese patient carried a c.5682_5683 delAA and c.9010C > T showed an extremely severe phenotype including shortness of the ribs and long bones, severe thoracic hypoplasia, polydactyly, ventriculomegaly, macrocephaly, polyhydramnios, visceral heterotaxia, a congenital heart defect and hypoplasia of the iliac bones. In our case, the Chinese fetus carried a missense variant and the other variant might cause splicing of the transcript. Neither craniocerebral malformations nor craniofacial abnormalities were found in the fetus. Situs inversus totalis was presented in our case.

SRPS III generally presented prenatally with extremely shortened long bones and ribs, round metaphyseal ends and lateral spikes, a small and narrow thorax, preaxial and postaxial polydactyly, sometimes presented with bowing femora, genital and multiple malformations [4].

The compound heterozygous variants of autosomal recessive inheritance pattern accorded with the SRTD3 disease. On all these counts, the compound heterozygous variants in the *DYNC2H1* gene may be the cause of this patient with SRTD3 with polydactyly. Through sonographic examination, the fetus was diagnosed with typical anomalies of SRTD3, narrow thorax, abnormal bones, polydactyly, interestingly, situs inversus totalis was also presented. As far as we know, this is the first time that fetal SRTD3 with situs inversus totalis and mirror-image dextrocardia in a Chinese family.

Our study not only revealed two novel compound heterozygous *DYNC2H1* variants, expanding the genetic spectrum of SRPS Type III, but also for the first time, raising the new phenotypic of the disease. The specific sonographic findings and the molecular diagnosis help add experience to further our expertise in prenatal counseling for SRTD3. The Next-generation sequencing is an effective method of prenatal diagnosis of a genetic skeletal disorder.

Declarations

Conflicts of Interest: The authors have declared no conflicts of interest.

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Availability of data and materials

The novel variants revealed during the study were submitted to ClinVar database (<https://www.ncbi.nlm.nih.gov/clinvar/>) under Accession Numbers SCV001806774 and SCV001806775. The datasets used and analyzed during the current study are available from the corresponding author upon request.

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- ## Figures

Figure 2

Compound heterozygous variants in *DYNC2H1* in a family with SRTD3. A. Familial pedigree. B. NM_001080463.1: c.11504T>G (p.Ile3835Arg) and c.2106+3A>T were confirmed in family members by Sanger sequencing and mass spectrometry.