

# Identification of novel compound heterozygous mutations of the *DYNC2H1* gene in a fetus with short-rib thoracic dysplasia 3 with or without polydactyly

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**SUMMARY** A prenatal sonograph revealed a 26-week-old fetus with short limbs and a narrow chest in a 23-year-old woman with a history of fetal skeletal dysplasia. A single nucleotide polymorphism-based chromosomal microarray (CMA) indicated a normal karyotype, and no chromosomal segments with abnormal copy numbers were noted in the fetus. Whole exome sequencing identified compound heterozygous mutations in the *DYNC2H1* gene responsible for a lethal type of bone growth disorder, short-rib thoracic dysplasia 3 with or without polydactyly (SRTD3), and revealed a missense mutation c.515C>A (p. Pro172Gln) of paternal origin and a missense mutation c.5983G>A (p. Ala1995Thr) of maternal origin. These variants were further confirmed by Sanger sequencing. To the extent known, the c.515C>A (p. Pro172Gln) mutation is novel for SRTD3, and the site is conserved across species. This study found a novel mutation of the *DYNC2H1* gene for SRTD3 and it has increased the number of reported cases and expanded the spectrum of mutations causing this rare disease.

**Keywords** short-rib thoracic dysplasia 3 with or without polydactyly, *DYNC2H1*, compound heterozygous mutations

## 1. Introduction

Short-rib thoracic dysplasia 3 with or without polydactyly (SRTD3) covers a range of autosomal recessive or digenic recessive skeletal dysplasia characterized by shortened limbs, a narrow trunk, and associated visceral abnormalities with or without polydactyly (1).

Currently, short-rib polydactyly syndromes (SRPSs) have been classified into short-rib thoracic dysplasias with or without polydactyly types 1-17 (SRTD1-17). Short-rib thoracic dysplasia 3 with or without polydactyly (SRTD3; 613091) is caused by homozygous or compound heterozygous mutations in the dynein heavy chain, isotype 1B (*DYNC2H1*) gene, which encodes a protein involved in ciliary intraflagellar transport (2,3). Currently, more than 140 mutations in the *DYNC2H1* gene have been identified in SRTD3.

Reported here is a case of a Chinese woman with three consecutive pregnancies that were diagnosed with SRTD3 without polydactyly according to clinical and ultrasound results revealing novel compound

heterozygous mutations in *DYNC2H1*.

## 2. Patients and Methods

### 2.1. Fetus with SRTD3 and samples

A woman in her third pregnancy at 26 weeks of gestation was seen at Zibo Maternal and Child Health Hospital in 2015. A fetus with abnormal dysplasia had developed in her first and second pregnancies. An ultrasound of the third pregnancy revealed a maximal depth of amniotic fluid of 3.6 cm, a biparietal diameter of 6.4 cm, a femur length of 2.8 cm, and a humerus length of 1.6 cm. The fetal chest is bilaterally symmetrical but narrow. In addition, a prenatal ultrasound also indicated that the long bones of the lower extremities were clearly short and the epiphyses were irregular. Abnormalities in the brain, liver, or kidneys were not noted. The parents of the fetus with SRTD3 are healthy and have no family history of genetic diseases or dysplasia.

The pregnancy was terminated after diagnosis, and amniotic fluid cells of the fetus and whole blood

samples of the parents were collected after obtaining informed consent from the parents. This study was approved by the ethics committee of the Shandong Medical Biotechnological Center.

## 2.2. DNA extraction

Genomic DNA was extracted from fetal amniotic fluid cells and whole blood cells using a blood DNA extraction kit (Omega Bio-tek, Norcross, GA, USA). DNA purity was measured with the NanoDrop® 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), and the concentration of extracted DNA was measured using the Qubit 3.0 Fluorometer (Thermo Scientific, Waltham, MA, USA).

## 2.3. Genome-wide copy number analysis

Genomic alterations of fetal amniotic fluid cells were used to analyze single nucleotide polymorphism by chromosomal microarray following the provided standard protocol (Affymetrix, CA, USA). Purified DNA was first fragmented and biotin-labeled and subsequently hybridized and scanned. Data were analyzed using the software Chromosome Analysis Suite (Affymetrix, CA, USA).

## 2.4. Whole exome sequencing (WES)

A DNA libraries was constructed using the Illumina TruSeqDNA sample preparation kit (Illumina Inc. San Diego, CA, USA). Exome capture was performed using the Agilent Custom sureselect Enrichment Kit (Agilent, Santa Clara, CA, USA). Massive parallel sequencing was performed with the Illumina Genome Analyzer IIX instrument (Illumina Inc. San Diego, CA, USA). Raw data were assessed to generate clean reads, and the reference human genome (GRCh37/hg19) was mapped using the Burrows-Wheeler Aligner (BWA). The genome analysis toolkit (GATK), samtools, and Picard tools were used to remove duplicates and false mutations introduced by library construction and to recalibrate map quality scores. Single nucleotide variants (SNVs) and small insertion-deletions (InDels) were detected with GATK and Varscan. Numerous databases were used to annotate the identified SNP and InDel variants. The pathogenicity of the detected SNP variants was assessed using the tools PolyPhen-2, SIFT, and PROVEAN.

## 2.5. Sanger sequencing

Probable mutations identified by WES in the fetus and parents were verified using Sanger sequencing. The PCR primers used to amplify *DYNC2H1* exons 4 and 38 are shown in Table 1. Two hundred unrelated healthy controls were subjected to Sanger sequencing to verify

**Table 1. Primer sequences used to amplify *DYNC2H1* exons 4 and 38**

Primer name	Sequence (5'-3')
DYNC2H1-4F	TCAAATATTTTGCTGCTCTGC
DYNC2H1-4R	CATTTTAAAACAGAAGAGAGGCTGT
DYNC2H1-38F	GGCAATACCTTCCACTGAAGAAA
DYNC2H1-38R	CCCCCTCCAAAGTAAAATATCAAAT

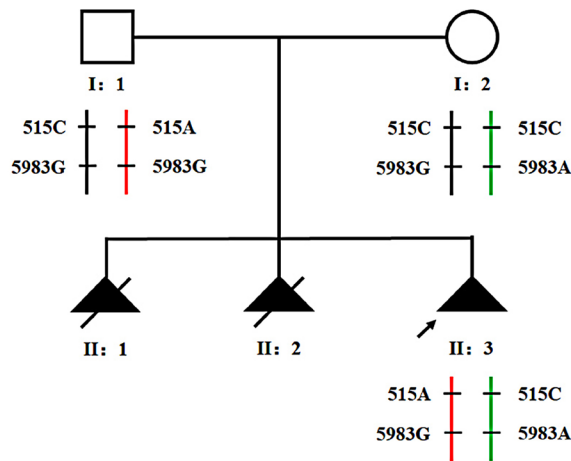
the identified variants.

## 3. Results and Discussion

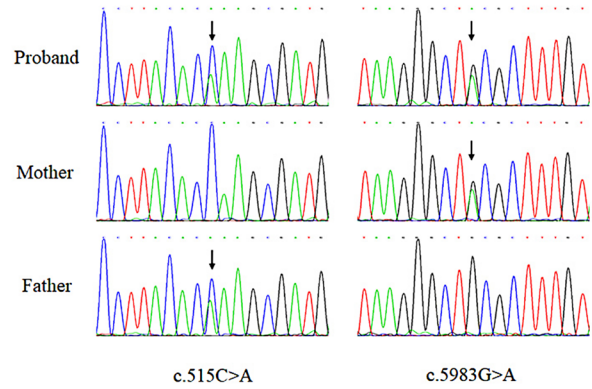
SRTD3 represents a type of severe autosomal recessive or digenic recessive fetal skeletal dysplasia characterized by shortened limbs, a narrow thorax, and with or without polydactyly. Signs of polydactyly were not noted in the current case. Moreover, this fetus with SRTD3 reflected an obvious family history, and similar symptoms were noted during two previous pregnancies.

Chromosomal microarray (CMA) analysis indicated that the karyotype of the fetus was normal (46, XX), and no chromosomal segments with abnormal copy numbers were noted in the whole genome. WES of the fetus revealed a mean target coverage of 76.5%, a total sequencing depth of 100×, and a target region sequencing depth of 35×-50×. In addition, coverage of the targeted bases for > 10 reads was 92.19%, that for > 20 reads was 86.22%, and that for > 30 reads was 78.81%. Two compound heterozygous variants c.515C>A and c.5983G>A in the *DYNC2H1* (NM\_001377) gene were screened out by filtering out known variants in the relevant database using an autosomal recessive inheritance model. Sanger sequencing confirmed the finding of *DYNC2H1* mutations in the fetal sample from WES; the father carried the c.515C>A mutation while the mother carried the c.5983G>A mutation, both of which were heterozygous mutations (Figures 1 and 2).

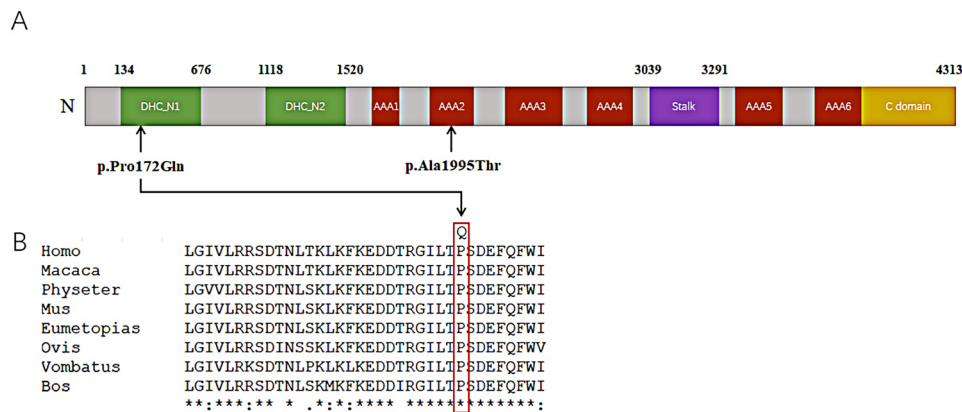
The c.515C>A mutation in the *DYNC2H1* gene was located in exon 4, and the c.5983G>A mutation was located in exon 38; both were missense mutations. The c.515C>A mutation will give rise to a substitution of a glutamine for a proline at amino acid 172 (Pro172Gln), and the c.5983G>A mutation causes a substitution of a threonine for an alanine at amino acid 1995 (Ala1995Thr). The c.515C>A mutation affects the highly conserved proline residues in the N-terminal region 1 (DHC\_N1) (Figure 3). The pathogenicity of this mutation was predicted using a series of online pathogenesis prediction programs; it was "Probably Damaging" according to polyphen-2, "Affect Protein Function" according to SIFT, and "Deleterious" according to PROVEAN. Moreover, the c.515C>A (Pro172Gln) missense mutation was not present in the Human Gene Mutation Database (HGMD), the Exome Aggregation Consortium (ExAc), ClinVar, or



**Figure 1.** Pedigrees of the family members with SRTD3 and mutation analysis of the *DYNC2H1* gene. The proband (II:3) is indicated with a black arrow.



**Figure 2.** A DNA sequence chromatogram of the family members indicating that compound heterozygous mutations (c.515C>A and c.5983G>A) of the *DYNC2H1* gene in the proband (II:3) were respectively inherited paternally and maternally.



**Figure 3.** (A) Schematic diagram of the *DYNC2H1* protein and the locations of the mutations detected in the proband. (B) Conservation analysis of *DYNC2H1* indicated that the proline residue at position 172 is highly conserved in various species.

1000 genomic databases. No such mutation was found in 200 unrelated healthy controls according to Sanger sequencing. Thus, c.515C>A in the *DYNC2H1* gene may be a novel pathogenic mutation.

*DYNC2H1*, the gene responsible for SRTD3, is located at chromosome 11q22.3 and encodes a large cytoplasmic dynein protein involved in the structure and function of cilia (4,5). It consists of an N-terminal tail (DHC\_N1), a linker domain (DHC\_N2), six identifiable AAA-ATPase domains, a stalk between AAA domains 4 and 5 in the microtubule binding domain (stalk MTBD), and a C-terminal tail (C domain) (6). The c.515C>A (p. Pro172Gln) variant is located in the DHC\_N1, which along with DHC\_N2 is considered to be the tail domain of a dynein heavy chain. This domain is considered to be the motile element of the dynein heavy chain, directing its movement along the dynein microtubules (7). A recent study reported that disruption of the dynein-2 tail domain can eliminate the ciliary localization of dynein-2, suggesting its important role in the ciliary entry of dynein-2 (8). The c.5983G>A(Ala1995Thr)

variant is located in the AAA2 domain. This variant was first reported in Chinese SRTD3 cases, and three cases of this variant have been reported overseas, one of which is homozygous mutation and two of which are heterozygous mutations ([c.195G>T] + [c.5983G>A] and [c.5983G>A] + [c.10594C>T]) (9).

Currently, a total of 93 SRTD3 cases caused by *DYNC2H1* mutations have been reported (3,9-13). Shortened limbs and a narrow chest are common in these cases, but polydactyly, irregular epiphyses, and visceral abnormalities are not found in all cases. A total of 147 pathogenic mutants of the *DYNC2H1* gene have been identified for SRTD3, including 92 missense mutations, 21 nonsense mutations, 22 insertion/deletion mutations, and 12 splicing mutations. When these mutations were mapped on the domain of dynein protein, 16 mutations were located in the DHC\_N1, 9 were located in the DHC\_N2, 62 were located in the six AAA+ domains, 6 were located in the stalk MTBD, and 11 were located in the dynein heavy chain. In all cases, most of those mutations were compound heterozygous mutations, and only four were homozygous mutations.

In summary, the current study involving WES identified two missense mutations of *DYNC2H1* in a fetus with SRTD3, including a novel c.515C>A (p. Pro172Gln) mutation with probable pathological significance. This finding expands the spectrum of *DYNC2H1* mutations found in patients with SRTD3 and it also provides helpful information to better understand the molecular function of the *DYNC2H1* gene.

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