



Novel *carbohydrate sulfotransferase 3* mutation causing spondyloepiphyseal dysplasia with congenital joint dislocations in a Chinese family

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Abstract: Spondyloepiphyseal dysplasia with congenital joint dislocations (SEDCJD) is a rare autosomal recessive disease caused by mutations in the gene *carbohydrate sulfotransferase 3* (*CHST3*). One SEDCJD proband diagnosed by X-ray and physical examination from a Chinese family was recruited in our study. Genetic testing using the whole exome sequencing (WES) and Sanger was performed for the proband. The target mutation in the proband's family members and 50 healthy individuals were checked by using Sanger sequencing. A novel homozygous mutation (c.626C>A; p.Pro209Gln) in the *CHST3* gene was identified. The family members who have one heterozygous mutation in *CHST3* didn't show any skeletal abnormality. The mutation was not discovered in the 50 healthy individuals. Our study suggested that the homozygous mutation c.626C>A in *CHST3* was responsible for SEDCJD. Our study contributed to the further expansion of the *CHST3* mutation spectrum, and demonstrated the genotype-phenotype relationship between mutations in the *CHST3* gene and clinical findings of SEDCJD.

Keywords: Spondyloepiphyseal dysplasia with congenital joint dislocations (SEDCJD); *carbohydrate sulfotransferase 3* (*CHST3*); mutation

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Spondyloepiphyseal dysplasia with congenital joint dislocations (SEDCJD; OMIM 143095) is an autosomal recessive skeletal dysplasia with clinic features such as severe progressive kyphoscoliosis, arthritic changes with joint dislocations, genu valgum, cubitus valgus, mild brachydactyly, camptodactyly, microdontia, and normal intelligence. The affected individuals may receive an initial clinical diagnosis of Larsen syndrome (OMIM 245600) or humerospinal dysostosis (1). SEDCJD is usually evident at birth. The dislocations were aggravated year by year, and the features of spondyloepiphyseal dysplasia become

apparent during grow-up. These will lead to arthritis changes of the hip and spine, rigid kyphoscoliosis, and trunk shortening (2). Congenital valvular heart disease has also been reported in several patients with SEDCJD (2-6). Our proband had heart problem with mitral regurgitation, and has had a son, who died of congenital heart disease at the age of 7 months. It is likely that involvement of cardiac valvular connective tissue is an important feature of SEDCJD.

SEDCJD was caused by *carbohydrate sulfotransferase 3* (*CHST3*, OMIM 603799) mutations. *CHST3* spans more

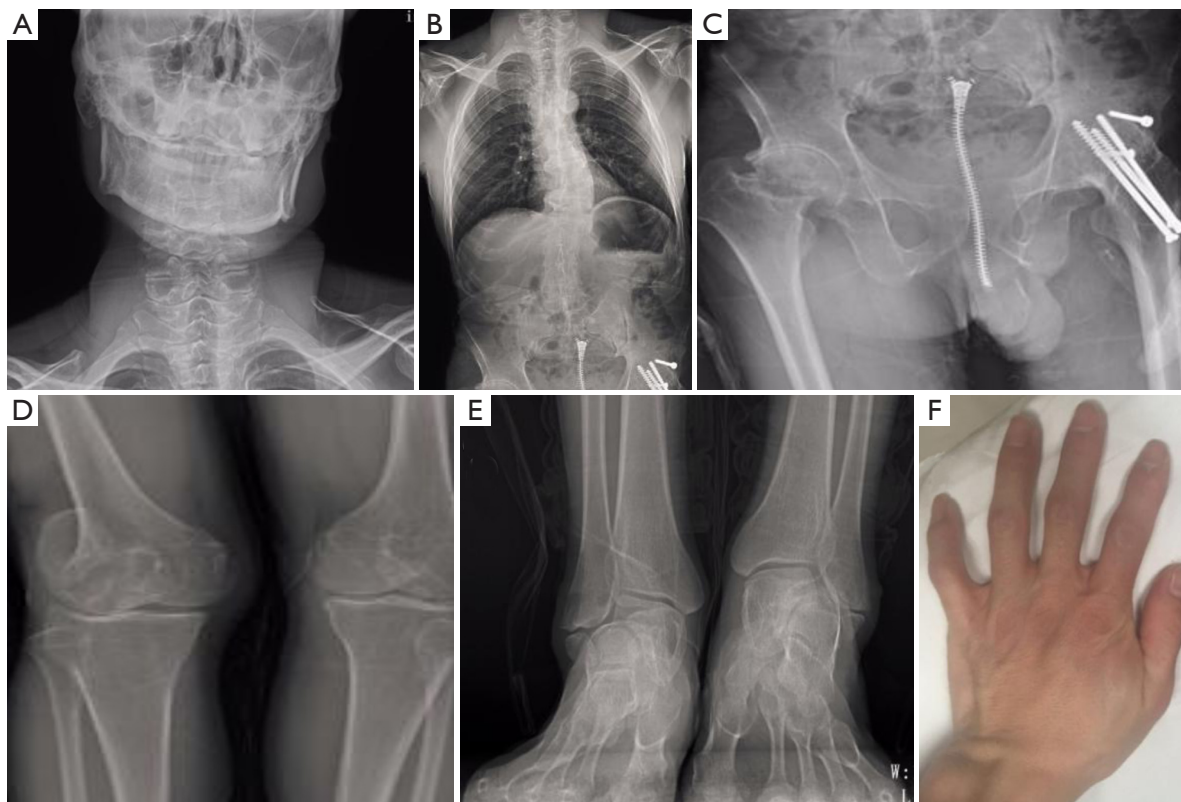


Figure 1 Clinical features of the proband. (A-D) radiographs at age 35; (G) photograph of the hand at age 35. (A) Head. Note a high palate, short neck; (B) thoraco-lumbar spine. Note fusion of vertebral bodies, severe scoliosis and narrowed intervertebral space; (C) pelvis. Note short and broad femoral neck and severe osteoarthritic and destructive changes in the hip joints; (D) note enlarged femoral epiphyses and irregular and small epiphyses of the tibia; (E) clubfoot was noted and the joint space is narrow; (F) hand. Thumbs appeared short with broad.

than 20 kb with three exons, encodes human C6ST, which consist of a core protein that distributed on the surfaces of most cells and the extracellular matrix in virtually every tissue (4). In this study, we presented the clinical manifestations and radiographic features of one Chinese SEDCJD patient, whom we found a novel *CHST3* missense mutation.

The 35-year-old male proband is the only child born to healthy but consanguineous parents (cousins) of Chinese Han descent. There was no family history of SEDCJD or other skeletal diseases. The hip joint dislocations and elbow subluxations of the proband were observed at birth. At the age of 10, he began to suffer from severe hip joint pain and had to rely on wheelchair. At the age of 15, his stature appeared to be significantly shorter than his peers. Short trunk with intervertebral disc degeneration and rigid thoracic kyphoscoliosis were noted. The proband has a rigid lumbar spine with flexibility less than 30%. At the

age of 19, the proband underwent treatment of the femoral head osteonecrosis by using bone flap with blood pedicle and cannulated screw internal fixation. Progression of knee epiphyseal changes, osteopenia, short and broad thumbs (*Figure 1*) and a lordotic gait were also present at this time. Neurological and other examinations were within the normal range. His height was 145 cm (-3.8 SD).

X-ray imaging at 35 years revealed a high palate and short neck (*Figure 1A*). A fusion of vertebral bodies, severe scoliosis and narrowed intervertebral space were noted (*Figure 1B*). There were significant bilateral hip joint destruction and generalized osteopenia. Short femoral neck and severe osteoarthritis observed (*Figure 1C*). The femoral epiphyses were enlarged, irregular and small epiphyses of the tibia were noted (*Figure 1D*). Clubfoot was discovered and the joint space was narrow (*Figure 1E*).

In retrospect, the radiographic findings of the proband were quite identified with the previous reports of SEDCJD

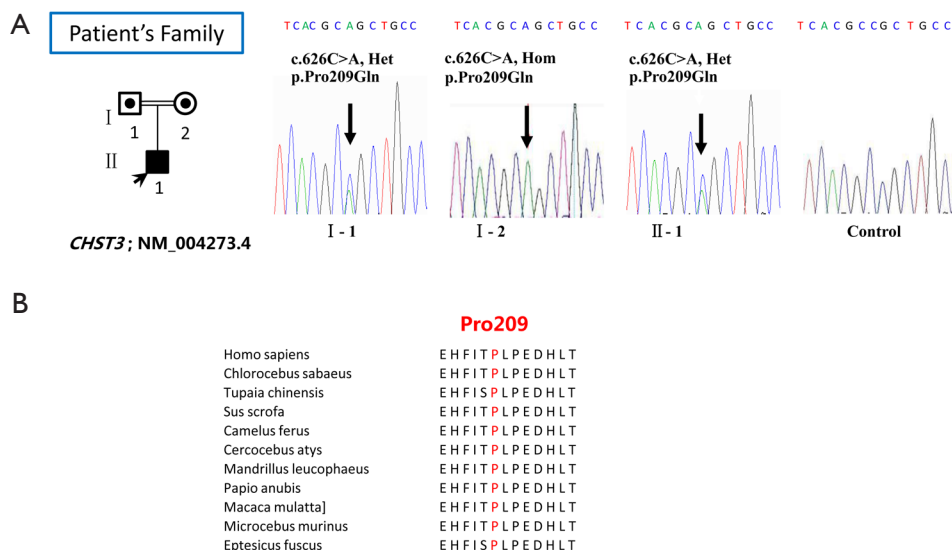


Figure 2 Pedigree of the family with spondyloepiphyseal dysplasia with congenital joint dislocations (SEDCJD) and amino acid sequence alignment of *carbohydrate sulfotransferase 3* (*CHST3*). (A) Pedigree of the family with autosomal recessive transmission and DNA sequencing of the *CHST3* gene in the family for mutation c.626C>A. The proband (II-1) is a homozygote for the mutation and his parents are carriers; (B) amino acids alignment in different species around the mutation (c.626C>A; p.Pro209Gln) found in the family is evolutionally conserved.

(6-8); however, because of the closure of growth plates and secondary changes related to the aging, definite diagnosis was difficult. Therefore, we performed molecular analysis of the proband. After the written informed consent, genomic DNAs from peripheral blood of the proband and his family was extracted using a QIA-GEN DNA minikit (Qiagen, Hilden, Germany).

The whole exome sequencing (WES) in the proband was conducted. Briefly, genomic DNA was divided into smaller fragments of 200–250 bp by ultrasonic instrument (Covaris LE220, Massachusetts, USA). Subsequently, Ampure Beads (Beckman Coulter, California, USA) purification was used to add poly A/joint reaction in the end of the purified DNA fragments. The gene-trapping chip (Roche NimbleGen, Madison, USA) was used to hybridize with the purified DNA fragments of proband. After hybridization, captured DNAs was sequenced on Illumina HiSeq2500 Analyzers (Illumina, San Diego, USA) and read on Illumina Pipeline software (version 1.3.4). We used BWA v0.59 (9) to align sequence reads to the human genome reference (build 37) and removed duplicated reads from subsequent analyses. Sequences variants were identified by compared to the NCBI reference sequence (NM 004273.4) and annotated by ANNOVAR (<http://www.openbioinformatics.org/annovar>). The 20× coverage for the RefSeq coding region was 97.47%

(Figure S1).

One novel missense mutations (c.626 C>A; p.Pro209Gln) in exon 3 of *CHST3* (Figure 2A) was discovered in the proband. This mutation was not previously reported and was not found in sequencing data projects (Exome Aggregation Consortium Exome <http://exac.broadinstitute.org/>). One cytosine-ribonucleotide altered to adenine-ribonucleotide in codon 626 and caused the change of reading frame from proline to glutamine. SIFT and Polyphen-2 (10) both predicted the mutations to have severe damage to the *CHST3* protein function.

We performed Sanger sequencing for the proband's family and confirmed the mutation c.626C>A; p.Pro209Gln (Figure 2A) which was highly conserved among diverse species (Figure 2B). The proband's parents are healthy, and they are carrier of this missense mutation. All this suggested that mutation c.626C>A was pathopoiesis. The inbreeding caused the proband's deformity, a homozygote missense mutation of *CHST3*.

Thus, one novel *CHST3* mutation in an adult case of SEDCJD was discovered in this study. Our study performed the WES, which allows us to get molecular results faster especially when traditional specific diagnosis takes a long time to sequence a large gene. The exome will give us possibility to analyze the first candidate gene and check

the presence of mutations in other genes at the same time (11,12). This study has further expanded the *CHST3* mutation spectrum, and thus contributed to the early detection of this disease, providing significant benefits to the patient and his families, and being aware of the growing amount of SEDCJD patients for future clinic diagnosis.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/aoj.2017.01.02>). QJ serves as an Editor-in-Chief of *Annals of Joint* from Mar 2016 to Feb 2021. DS serves as an unpaid Executive Editor-in-Chief of *Annals of Joint* from Mar 2016 to Feb 2021. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this manuscript and any accompanying images.

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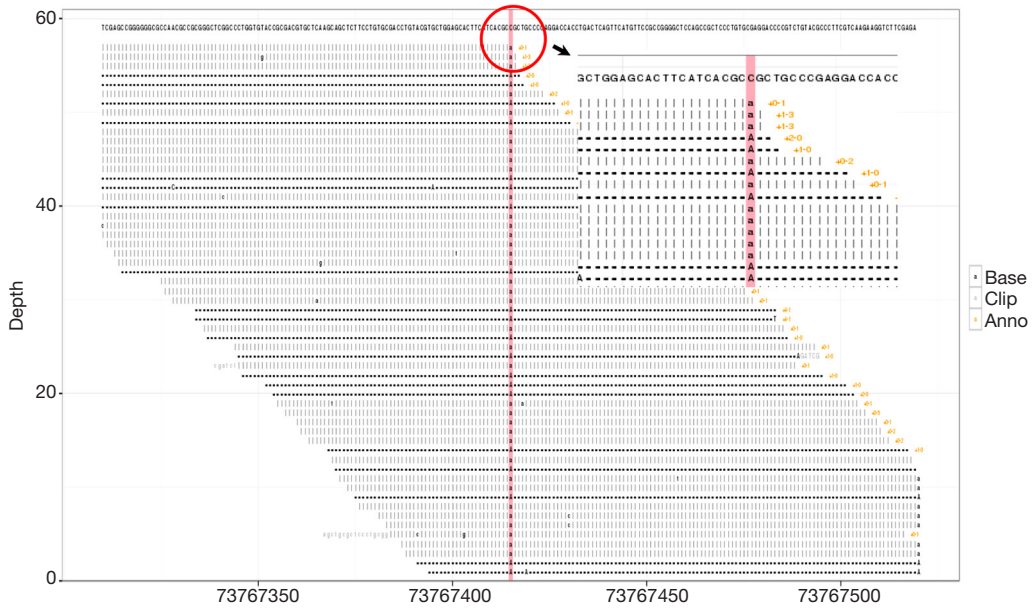


Figure S1 The WES screenshots of coverage at positions c.626C>A of gene *carbohydrate sulfotransferase 3 (CHST3)* in the patient. The 20× coverage for the RefSeq coding region was 97.47%.