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A novel insertion mutation in the *SEDL* gene results in X-linked spondyloepiphyseal dysplasia tarda in a large Chinese pedigree

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ABSTRACT

Background: Spondyloepiphyseal dysplasia tarda (SEDT) is an X-chromosome linked primary skeletal dysplasia characterized by a disproportionate short-trunked short stature, dysplasia of the large joints and flattened thoracic and lumber vertebral bodies. The objective of this study is to describe a large Chinese SEDT family with a milder phenotype and describe the molecular and clinical findings.
Methods: Eight affected males of the family were diagnosed with SEDT according to their clinical and radiological features. Direct DNA sequencing of the *SEDL* gene was performed. RT-PCR experiments on total RNA from blood lymphocytes were performed to confirm the defect on the *SEDL* gene. A short summary of all currently known *SEDL* gene mutations is presented.
Results: DNA sequencing revealed that all the affected males carried an insertion mutation (c.370-371insA) unreported previously, predicted to result in frameshifts and generate a premature stop codon (p. S124fsX127). The identical mutation was also observed in a 10-year old presymptomatic boy of the family. Eight female carriers had the typical sequencing chromatograms of heterozygotes.
Conclusions: Identification of the novel insertion mutation (c.370-371insA) in this SEDT family enables carrier detection and presymptomatic/prenatal diagnosis, but also the detailed molecular and clinical features will be useful for extending the evidence for genetic and phenotypic heterogeneity in SEDT.

1. Introduction

X-linked spondyloepiphyseal dysplasia tarda (SEDT; MIM 313400) is a well-defined, primary skeletal dysplasia that predominantly affects the spinal vertebral bodies and epiphyses during skeletal growth. SEDT was first reported by Jacobsen (1939), and its estimated prevalence is 1.7 per million [1–3]. The condition is not evident at birth, the usual age of presentation being after the first decade of life. A common presenting feature in a male is having a disproportionate (short-trunk) short stature, with or without back pain. Other characteristic clinical features include a broad chest with mild sternal protrusion and limitation of joint motion at the hips and elbows. The most characteristic radiological signs are seen in the lateral view of the thoraco-lumbar spine; comprising generalized platyspondyly, narrowing of intervertebral disc spaces, and pathognomonic superior and inferior 'humps' involving the posterior two-thirds of the flattened vertebral bodies [1–5].

The *SEDL* gene in Xp22 causing SEDT was identified by Gedeon et al. [2]. It is a small gene, which escapes X-inactivation, composed of 6 exons spanning approximately 20 kb of genomic DNA. The coding region is 420 bp in size and encompassed by exons 3–6. The gene encodes a 140-amino acid protein (Sedlin) with homology to genes in yeast, *Drosophila*, *Caenorhabditis elegans*, mouse, and rat. The yeast homolog was characterized as a member of a large multiprotein complex called TRAPP (transport protein particle), which has a role in the targeting and fusion of the endoplasmic reticulum (ER) to Golgi transport vesicles with their acceptor compartment [6]. An interaction of Sedlin with chloride intracellular channel proteins was described by Fan et al. [7]. *SEDL* gene has at least 7 pseudogenes in the human genome: one SEDLP1 on chromosome 19q13.4; one SEDLP2 on chromosome 8q13.3; and 5 SEDLP3–SEDLP7, pseudogenes on chromosome Yq11.23 [1]. The function of human Sedlin protein is still unclear. Based on the previous studies of the yeast homolog p20, it was hypothesized that the *SEDL* gene product might have a key role in trafficking chondrocyte proteins from ER to Golgi complexes and therefore be essential for maintaining homeostasis of the cartilage extracellular matrix [1,6].

In this study, we describe a large Chinese SEDT family with a milder phenotype and identified a novel insertion mutation in the *SEDL* gene. Identification of a defect in the *SEDL* gene in this Chinese kindred enables carrier detection and presymptomatic/prenatal

Abbreviations: SEDT, spondyloepiphyseal dysplasia tarda; TRAPP, transport protein particle; ER, endoplasmic reticulum.

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diagnosis. We believe our new data could contribute to fill in the *SEDL* gene mutation map that is far from saturation.

2. Materials and methods

2.1. Clinical report

The proband (case IV20), a Chinese male, born in 1989, was referred for genetic counseling for short stature, knowing our recent achievements in genetic evaluation and prenatal diagnosis of genetic skeletal dysplasia [8–11]. On examination at age 20 years, he is 153 cm tall (<2SD) and 51 kg weight (<1SD) with an arm span of 166.5 cm and an upper to lower body segment ratio of 0.72. Short stature was noted in late childhood (age 11 years). Serum concentrations of growth hormone and thyroid hormone were normal. X-ray radiographs show typical changes of platyspondyly with hump-shaped central portions of the vertebral bodies and the epiphyses were irregular with flattening of the femoral heads and relatively short femoral necks. The diagnosis of SEDT was based on radiological features and a three-generation family history (suggestive of X-linked inheritance).

The pedigree of the large SEDT family is shown in Fig. 1. All 8 affected males were diagnosed in the second decade of life when they were investigated for short stature. They had disproportionate short stature with short trunks. Head circumferences were all within the normal range and there were no dysmorphic facial features. Intelligence was normal. Affected males appeared to be a milder clinical condition, with no hip pain or back pain and no evidence of kyphosis or scoliosis. The carrier females in the pedigree were all of normal stature and did not complain of joint pain.

2.2. Mutation analysis

All protocols were approved by the Institutional Review Board and the Committee on Ethics of Research Involving Human Subjects of the Nanjing University Medical Center, Nanjing, China. Blood samples were obtained with informed consents from 8 affected individuals, 60 unaffected relatives of the family and 100 controls (unrelated healthy subjects, 50 males and 50 females). Genomic DNA was extracted from blood samples using Wizard™ Genomic DNA Purification Kit (Promega, Madison, WI) according to the manufacturer's instructions, and stored at −80 °C. In each subject, exons 3, 4, 5 and 6 (containing the coding sequence) of SEDL and adjacent splice sites were amplified by PCR under the following conditions: 95 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 60 s. The specific primer pairs are listed in Supplementary Table 1. PCR products were sequenced in both directions and sequencing reactions were performed with Big-Dye terminators and an ABI 3730 automated sequencer. All of the sequences were compared with the normal sequences of NCBI databases.

RT-PCR experiments were performed to further confirm the defect on *SEDL* gene. Total RNA from blood lymphocytes was extracted using Trizol reagent (Invitrogen, Carlsbad CA). RNA integrity was confirmed by direct visualization of 18S and 28S rRNA bands after agarose gel electrophoresis. The purified RNA samples (0.5 mg) were then reverse-transcribed using the SuperScript first-strand synthesis system (Invitrogen) and oligo-dT18. Subsequently, PCR was performed using primers listed in Supplementary Table 1. RT-PCR products were sequenced in both directions.

3. Results

Genomic DNA sequencing of all the affected males in this family revealed an insertion of a single nucleotide of A, in nucleotides 370 and 371 of the cDNA starting from the first A in the start codon (i.e. c.370-371insA). This mutation might result in frameshifts and generate premature stop codon (p.S124fsX127), thus a loss of the protein function can be predicted. This mutation (c.370-371insA) appeared to be novel, which is unreported previously in literature.

The identical mutation was also observed in a 10-year old boy in the family (IV10), who has no apparent change in his spine right now. The proband's mother and other 7 female carriers had the typical sequencing chromatograms of heterozygotes, showing clear-cut peaks before the insertion point and ambiguous peaks beginning at the insertion position. No other sequence changes were observed in the coding region or flanking intron sequences of the members of the family and the c.370-371insA mutation was not found in the 100 healthy controls. The above genomic DNA sequencing results were confirmed by RT-PCR experiment. RT-PCR sequencing results were shown in Supplementary Fig. 1.

4. Discussion

Mutations in *SEDL* gene are responsible for the vast majority of cases of SEDT. *SEDL* gene mutations are spread along the entire length of the 4 SEDL exons and their flanking introns. Examples include point mutations, splice alterations, an insertion, many deletions and several complex deletions/insertions [3]. Including the novel c.370-371insA mutation described in the present study, 47 different sequence variations (Fig. 2, Supplementary Table 2) have been described in patients with SEDT in various ethnic groups since its identification [2], including deletions (23/47), 9 splice-site mutations, 7 nonsense mutations, 6 missense mutations, and 2 insertion mutations [2,4,5,12–

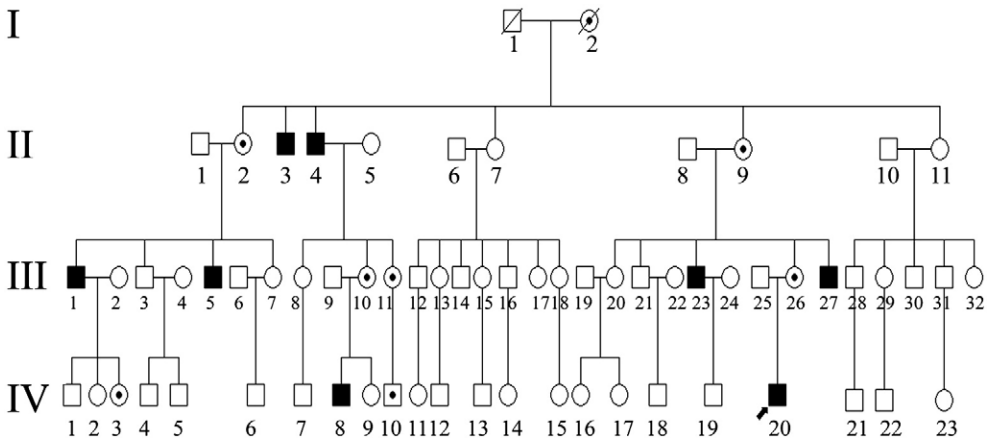

Fig. 1. Pedigree of a large Chinese SEDT family. The arrow indicates the proband (IV20). All the open boxes represent healthy males and open circles represent healthy females. Black boxes represent affected males. Boxes or circles with a crossing line indicates that the person has already died. All the circles with a dot in the middle indicate the status of carrier, including an asymptomatic 10-year old boy (IV10).