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A novel nonsense mutation in the sedlin gene (*SEDL*) causes severe spondyloepiphyseal dysplasia tarda in a five-generation Chinese pedigree

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ABSTRACT. Spondyloepiphyseal dysplasia tarda (SEDT) is an X-linked recessive osteochondrodysplasia characterized by disproportionately short stature and degenerative joint disease. The objective of this study was to describe a novel nonsense mutation in the sedlin gene (*SEDL*) causing severe SEDT in a large Chinese pedigree. The clinical features of all affected individuals and female carriers were presented. Four affected males of the family were diagnosed with SEDT according to their clinical and radiological features. Direct DNA sequencing of *SEDL* was performed. Reverse-transcription polymerase chain reaction (RT-PCR) experiments of

total RNA from blood lymphocytes were performed to confirm the defect in *SEDL*. DNA sequencing revealed that all of the affected males carried a nonsense mutation (c.61G>T) in *SEDL* that has not been previously reported. The c.61G>T mutation resulted in a premature translation termination codon (GAG>TAG) at amino acid position 21 (p.E21*), and was predicted to initiate the degradation of mutant transcripts through the nonsense-mediated mRNA decay pathway. Two female carriers showed typical sequencing chromatograms of a heterozygote. Following genetic counseling, individual IV7 gave birth to a healthy baby. Therefore, identification of the novel nonsense mutation (c.61G>T) in the SEDT family enables carrier detection, genetic counseling, and prenatal diagnosis. The detailed genotype/phenotype descriptions contribute to the *SEDL* mutation spectrum. The continued identification of mutations in SEDT patients will greatly aid further elucidation of the role of the sedlin protein in normal bone growth.

Key words: Spondyloepiphyseal dysplasia tarda; *SEDL* gene; Nonsense mutation

INTRODUCTION

Spondyloepiphyseal dysplasia tarda (SEDT; MIM 313400) is an X-linked skeletal disorder characterized by short stature, a short trunk, and precocious osteoarthritis (Gedeon et al., 1999; Savarirayan et al., 2003). The condition is not evident at birth, but rather presents most commonly after the first decade of life. Diagnosis is usually based on the characteristic vertebral body dysplasia, comprising platyspondyly and a central hump (Mumm et al., 2000; Christie et al., 2001). The causative gene of SEDT was successfully identified in 1999 and was designated as *SEDL* (Gedeon et al., 1999). *SEDL* consists of six exons that span approximately 22 kb of genomic DNA and encodes a 140 amino acid protein, sedlin (Savarirayan et al., 2003). The function of sedlin has not been well-established, but recent studies have postulated that it may play a role in the compartments involved in vesicular transport from the endoplasmic reticulum (ER) to the Golgi (Jang et al., 2002; Liu et al., 2010; Zong et al., 2011). The 420-bp coding region of *SEDL* is organized into four exons (exon 3 to exon 6) and three introns. The non-coding exons consist of exons 1 and 2, the 5' portion of exon 3, and the 3' portion of exon 6 (Gecz et al., 2003). More than 40 different *SEDL* mutations have been described in various ethnic groups since the gene was first identified; however, genotype-phenotype correlations for *SEDL* mutations remain unknown (Gedeon et al., 2001; Grunebaum et al., 2001; Mumm et al., 2001; Shaw et al., 2003; Fiedler et al., 2004; Xia et al., 2009). In the present study, we report a novel nonsense mutation in *SEDL* that causes severe SEDT in a large Chinese pedigree. Molecular genetic testing of the *SEDL* gene enables carrier detection and presymptomatic/prenatal diagnosis for SEDT. Identification of the novel mutation will also be useful for understanding the molecular mechanisms underlying SEDT.

MATERIAL AND METHODS

Clinical report

The present study was approved by the Institutional Review Board of Nanjing University Medical Center, and informed consent was obtained from all participants. A 30-year-old Chinese male of short stature (case IV20, the proband) was referred for genetic counseling because of our recent achievements in the molecular diagnosis of genetic skeletal dysplasia (Xia et al., 2007; Cui et al., 2008; Xia et al., 2008a,b). Upon examination, his height was 147 cm (25th percentile), and his arm span was 165 cm; his trunk was disproportionately short, and his chest was barrel-shaped. The patient complained of moderate pain in weight-bearing joints and mild back pain. Facial features were unremarkable, and the results of neurological examination were within normal limits. Short stature was noted in late childhood (age 10). Serum concentrations of growth hormone and thyroid hormone were normal. Radiography of the lumbar vertebrae and epiphyses revealed the typical characteristic features of SEDT. Platyspondyly with hump-shaped central portions of the vertebral bodies was noted, and the epiphyses were irregular with flattening of the femoral heads (Figure 1). Family history also revealed five other affected males on the maternal side. The five-generation Chinese SEDT pedigree is shown in Figure 2. There was no male-to-male transmission of the short stature phenotype, suggesting an X-linked recessive mode of inheritance. The diagnosis of SEDT was based on radiological features and on the inheritance pattern of the family.

Clinical features of all of the affected individuals and female carriers in the SEDT family are summarized in Table 1. Affected male family members appeared to show a severe clinical condition, with disproportionate short-trunked short stature, hip or back

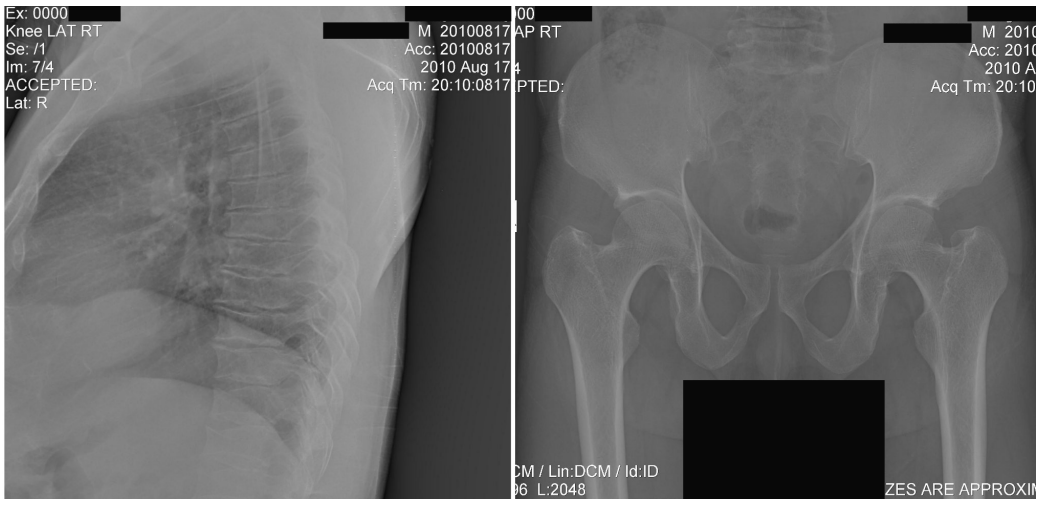

Figure 1. Radiographs of the lateral lumbosacral spine (left) and pelvis (right) of the proband (case IV5) from the large Chinese SEDT pedigree. Radiographs revealed platyspondyly with superior and inferior humping of the vertebral bodies; the pelvis was somewhat narrow, and the femoral necks were short.

pain, and obvious evidence of thoracolumbar scoliosis. The female carrier in the pedigree (III16) complained of mild joint pain. Three individuals in the family (IV7, IV10, and IV11), who were at-risk SEDT female carriers, wanted to have molecular genetic testing before pregnancy. All individuals who participated in the study were provided with extensive genetic counseling before testing.

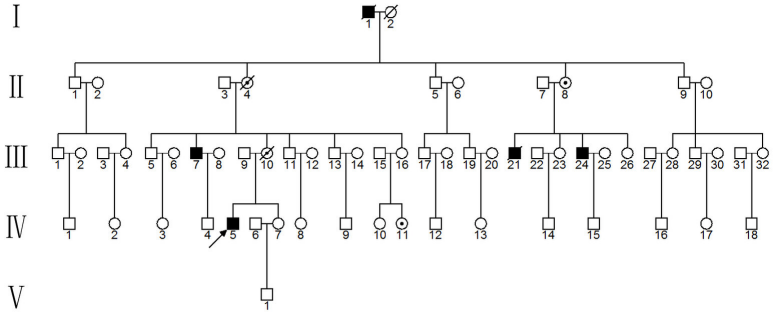

Figure 2. Pedigree of a large Chinese SEDT family. The arrow indicates the proband (IV5). All open boxes represent healthy males and open circles represent healthy females. Filled boxes represent affected males. Boxes or circles with a crossing line indicate that the person has already died. All circles with a dot in the middle indicate the status of carrier.

Table 1. Clinical features of all affected individuals and female carriers in the SEDT family.

Cases	Gender	Age (yr)	Onset (yr)	Adult height (cm)	Arm span (cm)	Back pain	Hip pain	Barrel chest	Thoracolumbar scoliosis	Platyspondyly
Patient No.										
Proband (IV5)	M	30	10	147	165	Mild	Moderate	Yes	Moderate	Mild
III7	M	58	8	138	162	Severe	Severe	Yes	Severe	Severe
III13	M	54	9	140	163	Severe	Severe	Yes	Severe	Severe
III24	M	39	10	143	167	Moderate	Severe	Yes	Severe	Moderate
Carrier No.										
III16	F	53	No	165	163	No	Mild	No	No	No
IV11	F	24	No	169	166	No	No	No	No	No

DNA sequencing

Blood samples were obtained with informed consent from the five affected individuals (except I1, who was deceased), 58 unaffected relatives of the family, and 200 controls (unrelated healthy subjects, 100 males and 100 females). Genomic DNA was extracted from blood samples using the Wizard™ Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to manufacturer instructions, and was 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 polymerase chain reaction (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 Table 2. PCR products were sequenced in both directions and sequencing reactions were performed using the Big-Dye terminator sequencing kit and an ABI 3730 automated sequencer. All of the sequences were compared with the normal sequences in the National Center for Biotechnology Information (NCBI) database.

Reverse transcription (RT)-PCR experiments

RT-PCR experiments were performed to further confirm the defect in the *SEDL* gene. Total RNA from blood lymphocytes was extracted using Trizol reagent (Invitrogen, USA). RNA integrity was confirmed by direct visualization of the 18S and 28S rRNA bands after agarose gel electrophoresis. The purified 0.5 mg RNA samples were then reverse-transcribed using the SuperScript first-strand synthesis system (Invitrogen) and oligo-dT18. Subsequently, PCR was performed using the primers listed in Table 2. RT-PCR products were sequenced in both directions.

Table 2. Primer sequences for amplying *SEDL* gene.

Fragments	Primers (5'→3')	PCR products (bp)
Exon 3	F: GAATTCTACACTTCCCATTAGTC R: TATCTGTCCAGATCTTCCAGTTC	265
Exon 4	F: GCAAATGTTAATCTGTGGTTGC R: TGACTGTGAAGTCTACAGACT	289
Exon 5	F: TACTGTATCAACGAACTGGTTG R: GTCGAATCCTTTCTCATCAGAA	203
Exon 6	F: CAGAAACTTAAGATTTGTCAGC R: TGACATGAGACAGAATGTACTA	326
cDNA	F: GAGATCCACAGAGCTAAACG R: CACAAAAGTTTTCCAGGCTA	600

RESULTS

Genomic DNA sequencing of all affected males in this family revealed a single nucleotide substitution (c.61G>T) in exon 3 of *SEDL*. The c.61G>T mutation creates a premature termination codon (GAG>TAG) at amino acid position 21 (p.Glu21Stop, p.E21*), which is predicted to initiate degradation of mutant transcripts through the nonsense-mediated mRNA decay (NMD) pathway. The nonsense mutation (c.61G>T) appeared to be novel, as it has not been previously reported in the literature or databases.

The sequencing chromatograms of two females (III16 and IV11 in Figure 2) were shown to be heterozygous for the substitution, confirming their carrier status. Therefore, individual IV11 would be at risk of having children affected with SEDT, whereas individuals IV7 and IV10 would not. Following genetic counseling, IV7 has since given birth to a healthy baby.

No other sequence changes were observed in the coding region or flanking intronic sequences of the members of the family, and the c.61G>T mutation was not found in the 200 healthy control subjects tested. The nonsense mutation (c.61G>T) identified in *SEDL* is illustrated in the schematic diagram (Figure 3A), and the typical sequencing chromatograms of a healthy control, an SEDT patient, and a female carrier are shown in Figure 3B, C, and D, respectively. The above genomic DNA sequencing results were confirmed by RT-PCR experiments. RT-PCR sequencing results are shown in **Figure S1**.

DISCUSSION

X-linked SEDT is a rare disease with an estimated prevalence of 1.7 per 1,000,000 individuals (Xia et al., 2009). Various types of mutations in the *SEDL* gene, including dele-

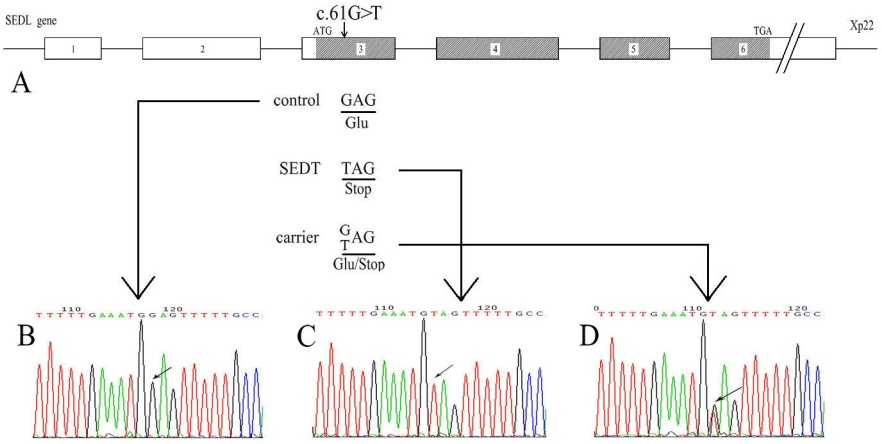

Figure 3. Nonsense mutation (c.61G>T) identified in the family is illustrated in the schematic diagram (**A**). The human *SEDL* gene consists of six exons that span approximately 20 kb of genomic DNA and encodes a 140 amino acid protein. The 420-bp coding region (hatched boxes) is encompassed by exons 3-6, and the untranslated regions (open boxes) consist of exons 1, 2, the 5' portion of exon 3, and the 3' portion of exon 6. The start (ATG) and stop (TGA) codons in exons 3 and 6, respectively, are indicated. The typical sequencing chromatograms of healthy control, SEDT patient, and female carrier are shown in **B. C.** and **D.** respectively.

tions, splice-site mutations, nonsense mutations, missense mutations, and insertions, are responsible for most cases of SEDT (Xiao et al., 2003; Xiong et al., 2009). To our knowledge, although more than 40 different sequence variations across *SEDL* have been reported to date, the genotype-phenotype correlation for *SEDL* mutations remains unclear. Gedeon et al. (2001) suggested that there might be a trend of decreasing severity within the group of patients according to the location of their mutations, 5'→3', along the *SEDL* gene. In contrast, based on their clinical findings, Shaw et al. (2003) proposed the hypothesis that any mutations would cause a complete loss-of-function effect of the sedlin protein, and the resulting clinical outcomes might be generally the same. The identification of further novel mutations in individuals with SEDT will provide additional evidence for or against the two contrasting hypotheses. In the Chinese family evaluated in the present study, a novel nonsense mutation (c.61G>T) in the 5' end of *SEDL* caused a severe SEDT phenotype. Additionally, four other mutations located upstream of intron 3 in *SEDL*, which were identified in five unrelated families, also appeared to result in a severe outcome. A summary of the 5' mutations and the corresponding phenotypes is presented in Table 3 (Gedeon et al., 1999, 2001; Matsui et al., 2001; Gao et al., 2003). Therefore, the trend of increasing severity towards the 5' end of the open reading frame (ORF) noted by Gedeon et al. (2001) was also apparent in the reported cases, including the current study.

In this study, the novel nonsense mutation (c.61G>T) in exon 3 of the *SEDL* gene can be predicted to create a premature translation termination codon (PTC). PTCs have been shown to initiate the degradation of mutant transcripts through the NMD pathway to prevent the possible toxic effects of truncated peptides (Huang and Wilkinson, 2012; Kervestin and Jacobson, 2012; Lin et al., 2012). The nature of the nonsense mutation suggests that the *SEDL* product, sedlin, would not be produced in the affected individuals. Thus, the severe clinical

phenotypes might be caused by the complete loss-of-function effect of the sedlin mutation, which needs to be further investigated.

Table 3. Mutations identified located upstream intron 3 in the 5' end of SEDL gene and the corresponding phenotypes of SEDT patients.

No.	Gene region	Nucleotide change	Predicted consequence	Ethnic origin	Clinical features			Reference
					Adult height (cm)	Onset (yr)	Severity	
1	Intron 2	IVS2-2 A>C	Splicing variant	French	NA			Gedeon et al., 2001
2	Intron 2	IVS2-2 A>C	Splicing variant	Chinese	140	8	Progressive back pain, severe scoliosis, short neck, barrel chest	Gao et al., 2003
3	Intron 2/ Exon 3	in2/ex3 (del1763bp)	Gross	Japanese	145	NA	back pain, paravertebral muscle tenderness	Matsui et al., 2001
4	Exon 3	Exon3del	Gross	Australian	139.3	6.5	scoliosis, hip pain referred to knees, back pain, arms extend to knees	Gedeon et al., 2001
5	Exon 3	c.53-54delTT	p.F18*	Australian	137-160	11	barrel chest, kyphoscoliosis, and osteoarthritis affecting the femoral heads	Gedeon et al., 1999
6	Exon 3	c.61G>T	p.E21 *	Chinese	147	10	Back pain, hip pain, scoliosis, barrel chest	Present study

Mutation numbering is based on cDNA sequences. +1 corresponds to the A of the ATG translation initiation codon. Amino acids are numbered from the translation initiator methionine. *Stop codon. NA = not available.

The actual function of the sedlin protein is not known, although it is speculated that it might participate in ER-to-Golgi transport as part of a novel, highly conserved multi-protein TRAPP complex, which functions at various steps in intracellular transport (Scrivens et al., 2009; Venditti et al., 2012). Recently, several investigations have aimed to gain insight into the function of the sedlin protein. Choi et al. (2009) suggested that the sedlin mutations S73L, F83S, and V130D cause SEDT by sedlin protein misfolding, whereas the D47Y mutation may influence normal TRAPP dynamics. Jeyabalan et al. (2010) argued that sedlin was present in the nucleus, forms homodimers, and that SEDT-associated mutations cause a loss of interaction with the transcription factors *c-myc* promoter-binding protein 1 (MBP1), pituitary homeobox 1 (PITX1), and steroidogenic factor 1 (SF1). Venditti et al. (2012) demonstrated that sedlin controlled the ER export of procollagen by regulating the Sar1 cycle, which might explain the defective chondrogenesis underlying SEDT. In short, SEDT might be caused by derangement in chondrogenesis, reflecting the inability of chondrocytes to properly secrete extracellular matrix components (Venditti et al., 2012).

In conclusion, identification of the novel nonsense mutation (c.61G>T) in the SEDT family has enabled carrier detection, genetic counseling, and prenatal diagnosis. The detailed genotype/phenotype descriptions contribute to completion of the *SEDL* mutation spectrum. The continued identification of mutations in SEDT patients will greatly assist in the further elucidation of the role of the sedlin protein in normal bone growth.

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Supplementary material

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