RESEARCH NOTE

A novel RNA-splicing mutation in *TRAPPC2* gene causing X-linked spondyloepiphyseal dysplasia tarda in a large Chinese family

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Introduction

X-linked spondyloepiphyseal dysplasia tarda (SEDT; OMIM 313400) is a rare osteochondrodysplasia that occurs in affected individuals between 3 and 12 yr of age. Clinical features include short trunk, barrel-shaped chest and disproportionate short stature. Radiological abnormalities may become evident between 10 and 14 yr of age and include platyspondyly with hump-shaped central and posterior portions of the vertebrae, narrow disc spaces and moderate epiphyseal dysplasia of the long bones, which may be associated with osteoarthritis. Female heterozygous carriers are clinically and radiographically normal. SEDT is caused by mutations in the *TRAPPC2* gene, (trafficking protein particle complex 2), which spans a genomic region of ~20 kb in Xp22. In a large Chinese SEDT family, we screened all the six exons of the *TRAPPC2* gene and identified a novel RNA-splicing mutation (IVS4+1A>G). We also demonstrated that the mutation induced splice pattern change from AT/AC to GT/AG. As a result, the first seven nucleotides of exon 5 were spliced out from the transcript. The prediction of the amino acid sequence showed that the seven nucleotides deletion of the transcript caused frame shift and led to premature translation termination, causing loss of two alpha helices. The results of our study expand the spectrum of the gene mutations associated with SEDT, and will help further to elucidate the role of this protein in the etiology of this form of osteochondrodysplasia.

X-linked spondyloepiphyseal dysplasia tarda mutations in the *TRAPPC2* gene previously identified as *SEDL* gene, have been confirmed to cause SEDT (Gedeon *et al*. 1999). This gene contains six exons, spanning about 20 kb of genomic DNA in Xp22. The coding region is 420 bp in size and encompassed by exons 3, 4, 5 and 6. The coding region yields a 140 amino acid protein referred to as TRAPPC2 protein (Gecz *et al*. 2000; Mumm *et al*. 2001). The function of TRAPPC2 protein is still unclear. Based on the previous studies of the yeast homologue, it was postulated that TRAPPC2 protein might have a role in the endoplasmic reticulum (ER) to golgi vesicular transport compartments. Forty-four different mutations of the *TRAPPC2* gene in various ethnic groups have been reported since its identification (Shaw *et al*. 2003; Fiedler *et al*. 2004). In this paper, we report a large Chinese family with SEDT and identified a novel RNA-splicing mutation in the *TRAPPC2* gene.

Materials and methods

Patients

The proband of this family is 38-year-old man who sought medical attention because of chronic pain in weight-bearing joints. The proband's height is 135 cm and his arm span is 155 cm. The length of his parents were normal, with his father being 170 cm and his mother 157 cm tall. His trunk was disproportionately short and his chest was barrel shaped. Facial features were unremarkable and the results of the neuroendocrinological examination were within the normal limits. Radiographs revealed platyspondyly with superior and inferior humping of the vertebral bodies, narrow pelvis and short femoral necks. On the other hand, family history also revealed 17 other affected males. The pedigree of this large SEDT family is shown in figure 1, indicating typical X-linked recessive inheritance pattern. This study is

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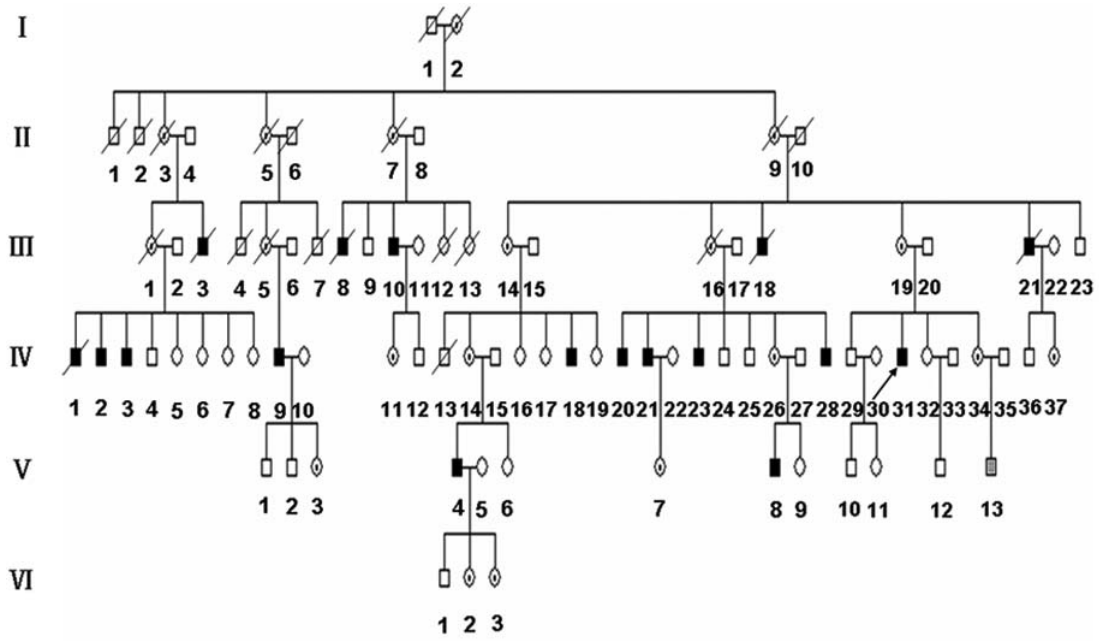

Figure 1. Pedigree of the SEDT family. The arrow indicates the proband (IV31). All the open boxes represent healthy males and open circles represent healthy females. Black boxes represent affected males. Boxes or circles with a crossing line indicates that the person has already dead. All the circles with a dot in the middle indicate the status of carrier. Grid box represents asymptomatic patient (V13).

approved by the Third Military Medical University Review Board and informed consent was obtained from all human subjects.

RNA and DNA Isolation

Blood samples were collected from 29 members of this family, with the consent of the participants. The subjects included were, III: 14, 19, 20; IV: 2, 3, 4, 5, 9, 11, 14, 18, 20, 21, 23, 26, 28, 31, 34, 37; V1: 3, 4, 7, 8, 9, 11, 13; VI: 1, 2, 3, we also collected blood samples from 50 controls (general healthy population). Genomic DNA was extracted from whole blood using the Qiagen blood kit (Qiagen, Hilden, Germany). Lymphocytes from the family were harvested using a ficoll-paque gradient. A 5-ml aliquot of blood/PBS (1:1) was layered on 3 ml of the ficoll-paque (TBD, Tianjing, China) and the mixture was centrifuged for 20 min at 400 g. The lymphocyte-rich fraction was transferred to a clean centrifuge tube and washed in fresh PBS. Total RNA was isolated from approximate 5×10^6 cells using the Tiangen (Invitrogen, Beijing, China) Trizol kit.

Mutation detection

In each subject, four exons (including exon/intron boundaries) of *TRAPPC2* gene were amplified using the polymerase chain reaction (PCR), which was performed with Master Mix (Tiangen, Beijing, China) under the following conditions: 95°C for 5 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s. The specific primer pairs are as follows: exon 3 with 617-bp product, 3F (5′-cgtgctgctactaactggtcc-3′) and 3R (5′-tggcttctatcagtctgtggg-3′); exons 4 and 5 with 862-bp product, 45F (5′-tccccacaagaaaaccatctat-3′) and 45R (5′-taagggagttacttcaataggc-3′); exon 6 with 619-bp product, 6F (5′-tgccattagttttgccacaga-3′) and 6R (5′-cagcctggcaacagagtgaga-3′). PCR product was purified using an ultra clean PCR purification kit (OMEGA, Colorado, USA) and sequenced in ABI 3130 sequencer using a Big Dye 3.1 terminator ready reaction kit (ABI, California, USA lot no. 0610066). DNA sequences were analysed using the vector NTI 10.3 software package (download from www.invitrogen.com, Invitrogen, California, USA).

Expression analysis

With the isolated total RNA, the first-strand cDNA was synthesized using RT kit (Takara, Dalian, China) and PCR was carried out with primers designed to amplify the region from exon 1 to 6 of *TRAPPC2* gene. The specific primer pair was: F (5′-CTTCCGCGGAAACTGACATTGC-3′) and R (5′-GAGTATACACCATTGTGGTGACATC-3′). β-Actin was amplified as positive control. PCR was performed with Master Mix (Tiangen, Beijing, China) 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 PCR product was purified using an ultra clean PCR purification kit (OMEGA, Colorado, USA) and sequenced in ABI 3130 sequencer using a Big dye 3.1 terminator ready reaction kit (ABI) manufacturer, city, country. DNA sequences were analysed using the vector NTI 10.3 software package (download from www.invitrogen.com, Invitrogen, California, USA).

Results

Direct sequencing of all the affected males in this family revealed an A>G transition in the first nucleotide of the intron 4 splice-donor site (shown in figure 2,a). The identical mutation was also observed in a nine-year-old boy in the family (V13), who has no apparent change of his spine right now. The mother, sister, aunt, niece and maternal grandmother of the proband are the carriers, who were shown to be A/G heterozygous. No other sequence changes were observed in the coding region or flanking intron sequences of the members of the family. No mutation in the *TRAPPC2* gene was detected in the 50 healthy controls.

Previous studies have revealed that there are two *TRAPPC2* transcripts in normal tissues and the predominant *TRAPPC2* transcript lacks exon 2 (Gedeon *et al*. 1999; Gecz *et al*. 2000). To assess the effect of this IVS4+1A>G mutation on splicing, exon 1 to 6 of *TRAPPC2* gene was amplified from the total RNA of the proband's lymphocytes, using RT-PCR. Two *TRAPPC2* transcripts were observed. Sequence analysis revealed that the intron 4 splice-donor sites changed from AT to GT, and the splice recipient also changed from AC to AG, that lies in exon 5. As a result, the first seven nucleotides of exon 5 were spliced out (figure 2,b) from the transcripts.

After the prediction of the amino acid sequence, we found that this seven nucleotides deletion of the transcript caused frame shift and led to premature translation termination (figure 3,a). The translation product contained 86 amino acids and the last six amino acids differed from FIMLHD to LLCFMT. With several programmes including PDB, HNNC and predator predicting the secondary structure of this

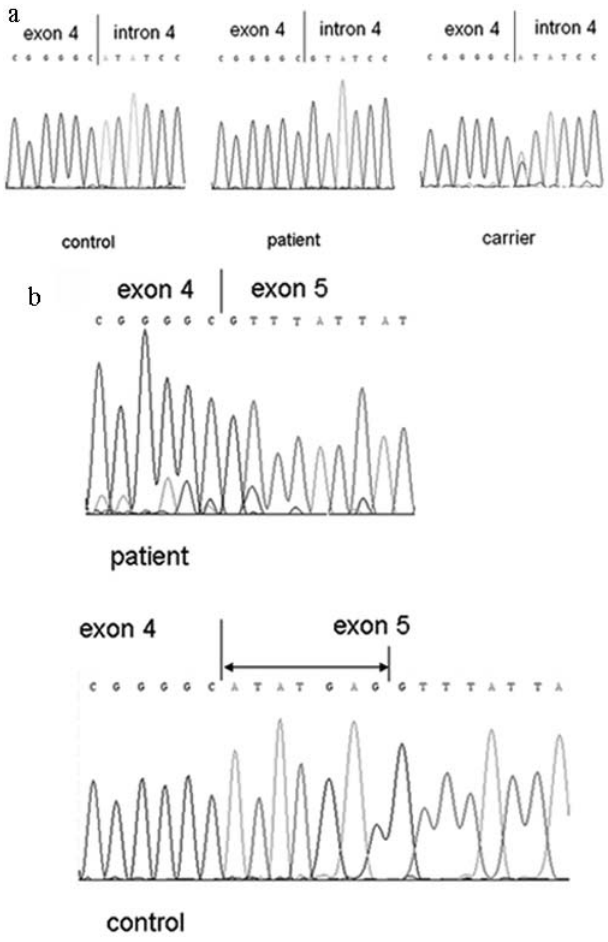

Figure 2. (a) The chromatograms of DNA sequence showing mutation in *TRAPPC2* genomic DNA. The first nucleotide of the intron 4 is A in the normal controls, G in the patients and A/G in the female carriers. (b) The cDNA sequencing across the splice junctions, intron/exon boundaries are indicated. IVS4+1A>G mutation causes the transcript of the patients lacking seven nucleotides of exon 5, the controls are normal.

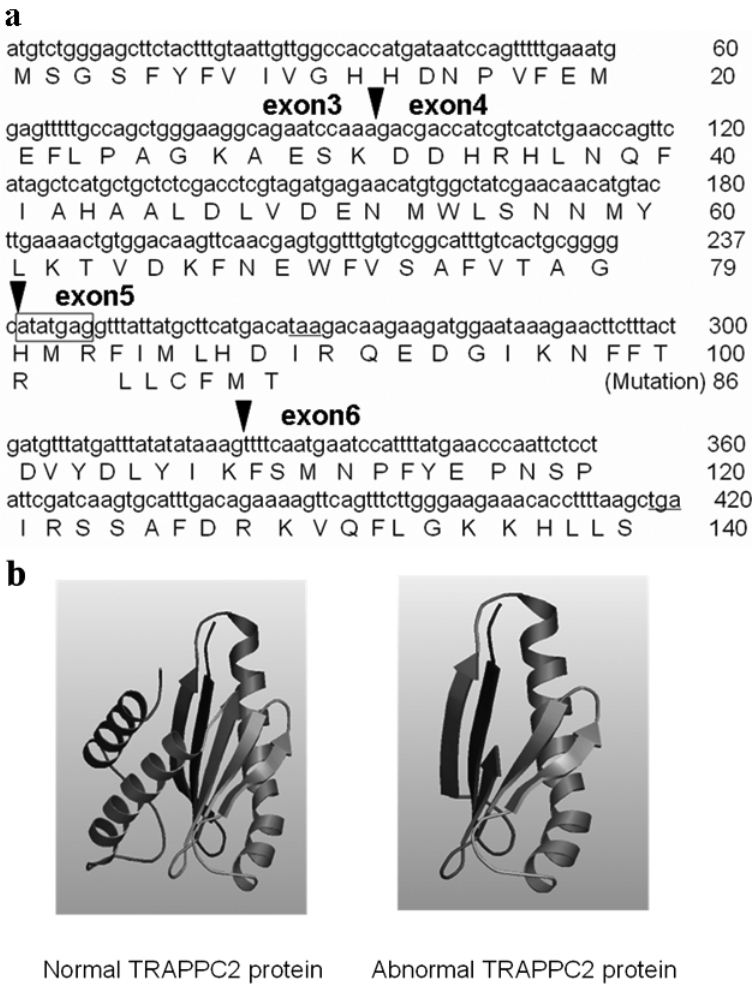

Figure 3. (a) mRNA and amino acid sequence of TRAPPC2 protein. Exon–intron boundaries are indicated with arrowheads. The seven nucleotides deletion of the transcript is boxed. The stop codons created by these frame shift mutations are underlined. (b) The secondary structure of TRAPPC2 protein was predicted. The normal TRAPPC2 protein is on the left and the abnormal on the right.

protein, the results showed a complete N-terminal of TRAPPC2 protein, but with the loss of two alpha helices (figure 3,b). The important protein-binding motif locates between the two alpha helices, so the function of TRAPPC2 protein is disrupted.

Discussion

Mutations in the *TRAPPC2* gene have been confirmed to cause SEDT. Since the first three mutations in *TRAPPC2* were reported in the year 1999, with the inclusion of this study, 45 different sequence variations have been described so far in patients with SEDT of different ethnic origins. These mutations have included nonsense mutations, missense mutations, deletions, insertions and putative splicing errors. Among the known SEDL mutations, eight splicing aberrations have been found (Shaw *et al*. 2003), including IVS2-2A>G, IVS2-2A>C, IVS3+5G>A, IVS4+4T>C, IVS4-4–11del, IVS4-9–12del, IVS5-2A>C, IVS5-2–3del and IVS5-4–10delTCTTTCCinsAA.

TRAPPC2 gene is composed of six exons with translation starting in exon 3. The sequence of splice site for four of the five introns conforms to the GT/AG consensus sequences, however, the splice site between exons 4 and 5 displays a non-canonical splice site sequence AT/AC (Gecz *et al*. 2000; Gedeon *et al*. 2001). The non-canonical splice site sequence is very rare in the human genome and only a few genes containing these rare-splice sites have been identified. Shaw *et al*. (2003) have reported a splice mutation in the 5′ splice donor site of intron 4 of *TRAPPC2* gene (IVS4+4T>C) in a patient with SEDT. The splice mutation resulted in the ignorance of splice site and exon 5 was spliced out from the transcript (Shaw *et al*. 2003). In this study, we found an A>G transition in the first nucleotide of intron 4 in the affected males of SEDT, which changed the splice donor site of in-

tron 4 from AT to GT. The sequencing analysis of the cDNA indicated that the mutation resulted in an alternative splicing and the new splice acceptor site AG is located in the exon 5. The splice pattern changed from noncanonical AT/AC to the canonical GT/AG. As a result, the first seven nucleotides of exon 5 were spliced out from the RT-PCR product. Concerning the protein prediction of the transcript, we found that this seven nucleotides deletion of the transcript caused frame shift and led to the premature termination of RNA translation. The translation product contained only 86 amino acids, which was almost the same with the anterior part of normal TRAPPC2 protein except the last six amino acids. The prediction of the secondary structure of the TRAPPC2 protein showed that the mutation would significantly alter the protein structure and cause the loss of two alpha helices, which might disrupt the functional interactions with a partner protein (Jang *et al*. 2002). TRAPPC2 is a component of the transport protein particle, which is mainly involved in ER to golgi vesicle transport. Because of the loss of the function of TRAPPC2 protein originating from the X chromosome, the mutation in the *TRAPPC2* gene could cause abnormal cellular transport of cartilage proteins.

It is an important question, whether there is a correlation between the sequence variation and severity of the phenotype (Christie *et al*. 2001). In this research, all the adult patients have apparent kyphosis and ankle pain between the age 15 to 18, and the pain gradually gets aggravated with age. The radiographs of the big joints have also shown apparent degenerative changes of the hips, ankles and knees. Comparing the symptoms of the affected males in this family with the SEDT patients reported earlier, no significant difference was observed. Therefore, there is still no clear quantitative genotype–phenotype correlation for *TRAPPC2* gene mutations known so far (Gecz *et al*. 2003).

As far as we know, this Chinese family is one of the two largest SEDT families in the world; the other one was reported by Tiller *et al*. (2001). Identification of the defect in the *TRAPPC2* gene in this Chinese family may be helpful to carriers' detection and prenatal diagnosis. The nine-year-old boy (V13) in this family showed no apparent change in the radiograph of his spine. However, through genomic DNA and transcript sequencing, he may be diagnosed to be a patient. It would be desirable that any preventive treatment could be made to him in order to alleviate the severity of the disease; however, it needs to be further studied.

Acknowledgements

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