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Novel and recurrent *COL2A1* mutations in Chinese patients with spondyloepiphyseal dysplasia

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ABSTRACT. Spondyloepiphyseal dysplasia (SED) is an autosomal dominant skeletal dysplasia characterized by short stature, abnormal epiphyses and flattened vertebral bodies. SED is mainly caused by mutations in the gene encoding the type II procollagen α-1 chain (*COL2A1*). We looked for mutations in *COL2A1* in three unrelated Chinese families with SED. Putative mutations were confirmed by RFLP analysis. We identified three missense mutations (p.G504S, p.G801S and p.G1176V) located in the triple-helical domain; p.G801S and p.G1176V are novel mutations. The p.G504S mutation has been associated with diverse phenotypes in previous studies. Our study extends the mutation spectrum of SED and confirms a relationship between mutations in the *COL2A1* gene and clinical findings of SED.

Key words: Type II collagen; *COL2A1*; Spondyloepiphyseal dysplasia

INTRODUCTION

Spondyloepiphyseal dysplasia (SED) is a group of rare inherited chondrodysplasia disorders characterized by short-trunk dwarfism combined with epiphyseal disorder of the spine and limbs (Spranger et al., 1994). SED impairs skeletal growth and causes ocular and otolaryngological abnormalities, such as myopia, retinal detachment, sensorineural deafness, cleft palate, etc. (Jung et al., 2004). The SED spectrum ranges in severity from lethal SED, including achondrogenesis type II and hypochondrogenesis (ACG2 and HCG, MIM 200610), through SED congenita (SEDC, MIM 183900) to late-onset SED (SED tarda or SEDT, MIM 184100) (Nishimura et al., 2005). SED is a genetically heterogeneous disorder, most often inherited in an autosomal dominant manner, but autosomal recessive (Omani type, MIM 608637) and X-linked forms (MIM 313400) have been reported (Bar-Yosef et al., 2004; Nishimura et al., 2005; Unger et al., 2010). To date, at least eight related causative genes have been identified, including *COL2A1* (collagen II α-1), *TRAPPC2* (trafficking protein particle complex 2), *CHST3* [carbohydrate (chondroitin 6) sulfotransferase 3], *PAPSS2* (3'-phosphoadenosine 5'-phosphosulfate synthase 2), *WISP3* (WNT1-inducible signaling pathway protein 3), *ACAN* (aggrecan), *TRPV4* (transient receptor potential cation channel, subfamily V, member 4), and *MATN3* (matrilin 3) (Faiyaz ul Haque et al., 1998; Fiedler et al., 2003; Borochowitz et al., 2004; Liao et al., 2004; Thiele et al., 2004; Gleghorn et al., 2005; Nishimura et al., 2005, 2010).

Most autosomal dominant SED cases are caused by mutations in *COL2A1*, the gene encoding the type II procollagen α-1 chain, which is the main structural protein of cartilage and bone. *COL2A1* is >30 kb with 54 exons and encodes 1487 amino acids containing a triple-helical domain formed by ~330 Gly-X-Y triplets, and the most common type of mutation is a substitution in the triple-helical glycine residue of the type II procollagen α-1 chain (Nishimura et al., 2005).

Although a large number of mutations in *COL2A1* have been reported, the identification of gene mutations in Chinese patients with SED is rare (Xia et al., 2007; Zhang et al., 2011). We identified two novel mutations (c.2401G>A, p.G801S and c.3527G>T, p.G1176V) and one recurrent mutation (c.1510G>A, p.G504S) at the Gly position of the Gly-X-Y triplets of *COL2A1* in Chinese patients with SED. Identification of the disease-causative mutation is consistent with the clinical diagnosis of SED and provides information for genetic counseling of other family members.

MATERIAL AND METHODS

Patients and clinical study

The probands of three Chinese families with SED were referred from the Department of Developing Pediatrics of Shengjing Hospital for genetic testing. All probands had X-ray examinations (lumbar vertebrae and pelvis), and we collected peripheral venous blood samples and radiographs from the patients and available family members with informed consent and approval of the China Medical University Institutional Review Board. Genomic DNA was extracted using a genomic DNA extraction kit (TaKaRa, Japan).

Mutation analysis

The probands from each family were screened for mutation in the *COL2A1* gene. More-

over, DNA sequence analysis of *TRAPPC2* was done for the proband of family 1 and *WISP3* for the proband of family 2. Putative mutations were confirmed by duplicate PCR amplification and sequencing of the affected exons from genomic DNA of the patients and their family members. To confirm the pathogenicity of the novel p.G1176V and p.G801S mutations, *Afl*III and *Aor*51H I restriction sites were introduced into the mutant allele by PCR using mismatch primers, respectively. Restriction analysis was then done with all of the available family members and in 60 unrelated Han Chinese as normal controls. The PCR primers and restriction enzymes used for restricton analysis are given in Table 1 and other primers for sequencing are available on request.

Table 1. Primers and restriction enzymes used for mutation confirmation.

Proband	Sequence change	Restriction enzyme	Primer sequence (5'-3')	Length (bp)
1	c.3527G>T (p.G1176V)	*Afl*III	F-gtcggtccctctggcaaacatg R-aggataaaggatgccatcactg	220
2	c.2401G>A (p.G801S)	*Aor*51H1	F-ggtccctggtcctgtctctg R-ccgggactcaccttagcgc	249

RESULTS

Clinical findings

The clinical and radiographic features of each proband were reviewed by at least two clinical geneticists and radiologists. Clinical histories and examination findings were systematically recorded and skeletal radiographs were reviewed; details of the clinical information for the probands are presented in Table 2.

Table 2. Clinical informations of the three probands from the Chinese families with spondyloepiphyseal dysplasia.

	Proband 1	Proband 2	Proband 3
Family history	Negative	Positive	Positive
Age	12 years	11 years	11 months
Gender	Male	Female	Female
Stature (cm)	124	118.5	65.8
Weight (kg)	28	NA	9.2
Onset	At birth	5-6 years	None
Birth length	NA	NA	NA
Birht weight	NA	3.0	3.6
Myopia	None	None	None
Retinal detachment	None	None	None
Vitreoretinal degeneration	None	None	None
Hearing impairment	None	None	None
Cleft palate	Yes	None	None
Brachydactyly	None	None	None
Scoliosis	Yes	None	None
Kyphosis	Yes	None	None
Lumbar lordosis	Yes	None	None
Platyspondyly	Yes	Yes	None
Osteoporosis	Yes	Yes	None
Acetabular roof	Flattening	Flattening	Normal
Femoral neck	Normal	Short, wide	Normal
Femur shaft	Normal	Normal	Normal
Femoral head	Flattening, edge unsharpness	Flattening	Normal
Neck-shaft angle	Normal	Decreasing	Normal
Capital femoral epiphyses	Dysplasia	Dysplasia	Normal

NA = not available.

The proband (II1) of family 1 was a 12-year-old boy who was born to healthy nonconsanguineous parents (Figure 1A). His growth was markedly delayed and he had pain in both knees and hip joints with a waddling gait. Physical examination revealed a disproportionately shortened trunk and lumbar lordosis. The patient's height (124 cm), arm span (130.8 cm), arm/height ratio (1.05), head circumference (53.6 cm) and body weight (28 kg) were measured. The patient had a cleft lip, for which he underwent plastic surgery. His gestation and birth history were unremarkable and his intelligence, hearing and eyesight were normal. Radiography of his spine showed moderate scoliosis, kyphosis, lumbar lordosis, and platyspondyly (Figure 2A). X-ray of his pelvis revealed dysplasia of bilateral capital femoral epiphyses and flattening of the acetabular roof (Figure 2B). His skull, femur shaft, tibio-fibula, humerus, radio-ulna, and metacarpals were normal.

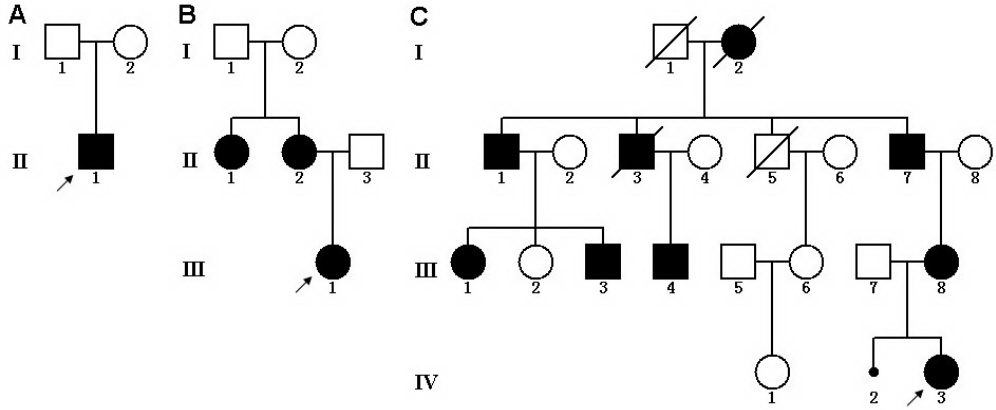

Figure 1. Pedigrees of the three families that took part in this study. Patients with spondyloepiphyseal dysplasia are shown by darkened symbols. Arrows indicate the probands. **A.** Family 1. **B.** Family 2. **C.** Family 3.

The proband (III1) of family 2 was an 11-year-old girl with short-trunk dwarfism (118.5 cm heigh) and short neck (Figure 1B). Her birth weight was 3.0 kg but her birth length had not been recorded. She suffered from growth retardation after birth, and her parents noticed that she had lumbar deformity at the age of 5-6 years. Her cranio-facial features, hearing, vision, and intellectual development were normal. She had a positive family history, her mother and maternal aunt also had short stature with short neck, and each was 140 cm tall. Radiography of her spine showed irregular vertebrae and osteoporosis (Figure 2C). Her pelvic radiograph showed abnormalities of the capital femoral epiphyses, flattening of the acetabular roof, shortening of the femoral neck and decreasing neck-shaft angle (Figure 2D).

The proband (IV3) of family 3 was first seen at the age of 11 months for genetic counseling because her mother (137 cm heigh) and maternal grandfather (140 cm heigh) were diagnosed with spondyloepiphyseal dysplasia (Figure 1C). The patient's height was 65.8 cm and her body weight was 9.2 kg. Her birth weight was 3.6 kg but her birth length had not been recorded. Upon physical examination at 27 months, she had short stature (80.5 cm), but no other abnormality was observed. The patient had a positive family history; at least nine family members had short stature. Radiographs of her spine and pelvis at the age of 11 months did not show obvious abnormality (Figure 2E and F).

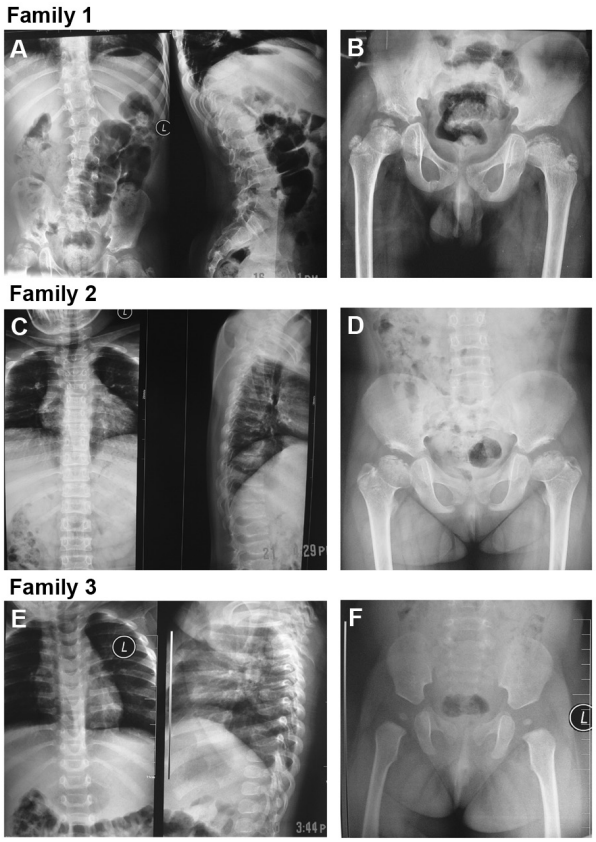

Figure 2. Radiograph of the probands' spine and pelvis. **A.** Family 1, spine. **B.** Family 1, pelvis. **C.** Family 2, spine. **D.** Family 2, pelvis. **E.** Family 3, spine. **F.** Family 3, pelvis.

Mutation detection

Three unrelated Chinese patients affected with SED underwent mutation analysis by direct sequencing of the PCR-amplified DNA fragments spanning 54 coding exons and flanking intronic sequences of the *COL2A1* gene; three heterozygous missense mutations were identified. In family 1, a novel missense mutation, c.3527G>T (p.G1176V), in exon 50 was found in the proband (Figure 3A). In family 2, we identified a novel missense mutation, c.2401G>A (p.G801S), in exon 36 in the proband (Figure 3B), her affected mother and her maternal aunt. In family 3, a recurrent missense mutation, c.1510G>A (p.G504S), in exon 23 was found in the proband (Figure 3C). Her affected mother, maternal grandfather and unaffected maternal grandmother were also analyzed for mutations in *COL2A1*; her mother and grandfather had the same heterozygous *COL2A1* mutation (p.G801S), but her grandmother did not carry this sequence variant. All mutations resulted in substitutions of highly conserved glycine residues for bulkier amino acids in the Gly-X-Y triple-helical region. By restriction analysis, both of these two novel missense mutations were found in affected individuals but were not detected in normal family members or control individuals. We did not find any changes in *TRAPPC2* or *WISP3*.

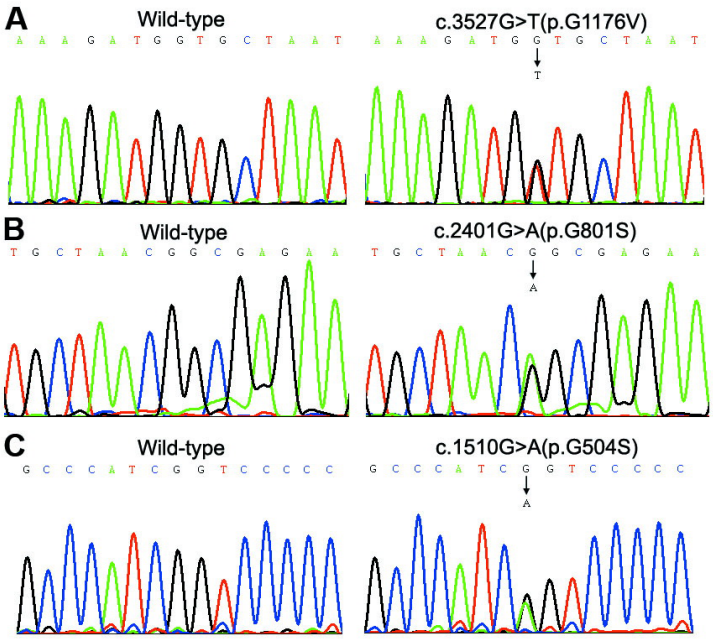

Figure 3. Genetic analyses of *COL2A1* in the spondyloepiphyseal dysplasia families. The arrows in **A**, **B** and **C** indicate the heterozygous mutation site in families 1, 2 and 3, respectively.

DISCUSSION

Over 300 mutations of the *COL2A1* that are responsible for SED and more than 20 kinds of other skeletal dysplasias have been reported, and most of those affecting the triple helix are glycine substitutions, which are widely distributed in *COL2A1*, with no particular hot spot. We report here three unrelated Chinese families with heterozygous missense mutations of the *COL2A1* that changed the glycine residues of Gly-X-Y regions. Two mutations resulted in substitutions of glycine to serine (p.G801S and p.G504S) and the other mutation changed glycine to valine (p.G1176V). To the best of our knowledge, this is the first report of the mutations G801S and G1176V, whereas the recurrent mutation p.G504S was reported earlier by other groups (Tiller et al., 1995; Nishimura et al., 2005; Xia et al., 2007). The glycine residues in the repeating Gly-X-Y triplet were highly conservative; replacement with bulkier amino acids may interfere with the triple-helical integrity and/or assembly of the protein into fibrils.

Although it is difficult to elucidate the accurate genotype-phenotype correlations in SED because of the wide range of phenotypic variation and age-dependent phenotypic transitions, a few specific genotype-phenotype relationships have been described (Nishimura et al., 2005; Kannu et al., 2012). Since the collagen triple helix folds in a C- to N-terminal direction, it has been suggested that mutations in the C-terminal domains are more likely to produce severe phenotypes than mutations in the N-terminal domains (Byers et al., 1991). The results of our study in the probands were consistent with this rule; the proband of family 1 (p.G1176V) showed the severest phenotype, next was the proband of family 2 (p.G801S), and the proband of family 3 (p.G504S) had the mildest. However, there are several examples of the same

substitution for glycine at the same position that produce diverse phenotypes, where variable phenotypic expression occur both within families and between families. Earlier studies suggested that the identical mutation G504S could lead to different phenotypes, including SEDC, SEDC-M (SEDC with mild coxa vara), SEDT, and SEMD-STR (spondyloepimetaphyseal dysplasia, Strudwick type), among others (Tiller et al., 1995; Nishimura et al., 2005; Xia et al., 2007). Williams et al. (1995) reported a mutation that changed glycine to serine at position 1176, which caused SED with precocious osteoarthritis, similar to the phenotype of family 2 in our study, which was caused by G1176V substitution. G801 substitution has not been reported before, G795 and G804 were localized close to G801; therefore, when they were substituted for arginine and alanine, it would give rise to the severe phenotypes ACG2 and HCG, respectively (Freisinger et al., 1994; Körkkö et al., 2000). Moreover, patients with SED can differ greatly in their clinical manifestations and outcomes, and thus, much effort has been made to elucidate the underlying mechanisms of genotype-phenotype correlations.

The proband of family 1 and two affected individuals (II2 and II3) of family 2 were born to healthy nonconsanguineous parents. In the absence of parental DNA, we could not determine if the mutations (p.G1176V and p.G801S) arose *de novo* or were inherited from a parent who was a mosaicism, but we highly suspect a germline mosaic status for mutation p.G801S in one of their parents. The reason is that two siblings carried the same mutation and that their parents showed a "normal" phenotype. It is important to consider the impact of mosaicism when counseling families with newly discovered *COL2A1*-related disorders.

In summary, we identified two novel mutations (c.3527G>T, p.G1176V and c.2401G>A, p.G801S) and one recurrent mutation (c.1510G>A, p.G504S) in the Gly-X-Y regions of the type II procollagen α-1 chain in three Chinese families with SED, and extended the mutation spectrum of SED. SED is a heterogeneous disorder and diagnosis solely on the basis of the clinical features is difficult; therefore, molecular diagnosis is helpful for confirming the clinical diagnosis and for genetic counseling.

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