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# Mutation of the Type X Collagen Gene (*COL10A1*) Causes Spondylometaphyseal Dysplasia

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## Summary

**Spondylometaphyseal dysplasia (SMD) comprises a heterogeneous group of heritable skeletal dysplasias characterized by modifications of the vertebral bodies of the spine and metaphyses of the tubular bones. The genetic etiology of SMD is currently unknown; however, the type X collagen gene (*COL10A1*) is considered an excellent candidate, for two reasons: first, Schmid metaphyseal chondrodysplasia, a condition known to result from *COL10A1* mutations, shows a significant phenotypic overlap with SMD; and, second, transgenic mice carrying deletions in type X collagen show SMD phenotypes. Hence, we examined the entire coding region of *COL10A1* by direct sequencing of DNA from five unrelated patients with SMD and found a heterozygous missense mutation (Gly595Glu) cosegregating with the disease phenotype in one SMD family. This initial documented identification of a mutation in SMD expands our knowledge concerning the range of the pathological phenotypes that can be produced by aberrations of type X collagen (type X collagenopathy).**

## Introduction

Spondylometaphyseal dysplasia (SMD [MIM 184250, MIM 184252, MIM 184253, MIM 184255, MIM 313420, and MIM 602271]) is a group of heritable skeletal dysplasias characterized by modifications of the vertebral bodies of the spine and metaphyses of the tubular bones. The clinical hallmark of SMD is short stature mainly due to shortening of the trunk and occasionally associated with micromelia and deformities of extremities, such as coxa vara and genu varum (Kozlowski et al. 1967; Maroteaux and Spranger 1991; Taybi and Lachman 1996). A diagnosis of SMD rests on radiological abnormalities, and the diagnostic requisite comprises platyspondyly (flattening of the vertebral bodies) and metaphyseal dysplasia (generalized modifications of metaphyses of the tubular bones) (fig. 1). SMD is clinically and genetically heterogeneous, and the classification of SMD remains tentative. A few subtypes of SMD, such as the most common (Kozlowski type [Kozlowski et al. 1967]) and the second most common (corner fracture type), are well-defined distinctive entities (Taybi and Lachman 1996). However, there are a lot of rare subtypes and numerous unclassifiable cases still waiting for rational clarification (Taybi and Lachman 1996).

The genetic etiology of SMD is currently unknown; however, the type X collagen gene (*COL10A1*) is considered an excellent candidate, because type X collagen is a cartilage-specific short-chain collagen (Schmid and Linsenmayer 1985) that is expressed specifically in hypertrophic zones of the growth plate (Kirsch and von der Mark 1991). Derangement of type X collagen could lead to dysplasias of the spine and metaphyses which are formed as a result of enchondral ossification. Mutations of type X collagen have been found in patients with Schmid metaphyseal chondrodysplasia (Schmid MCD) (Warman et al. 1993; Kuivaniemi et al. 1997), an autosomal dominant condition characterized by dysplasias of metaphyses. Unlike SMD, Schmid MCD almost exclusively involves metaphyses of the long bones but not the spine (Lachman et al. 1988); however, the metaphyseal alterations of the long bones are very similar in both disorders. Furthermore, transgenic mice carrying deletions in the triple-helical domain of type X collagen have shown phenotypes of SMD (Jacenko et al. 1993).

To clarify this issue, we examined the entire coding region and flanking sequences of *COL10A1* in patients with SMD. We detected in one patient a heterozygous

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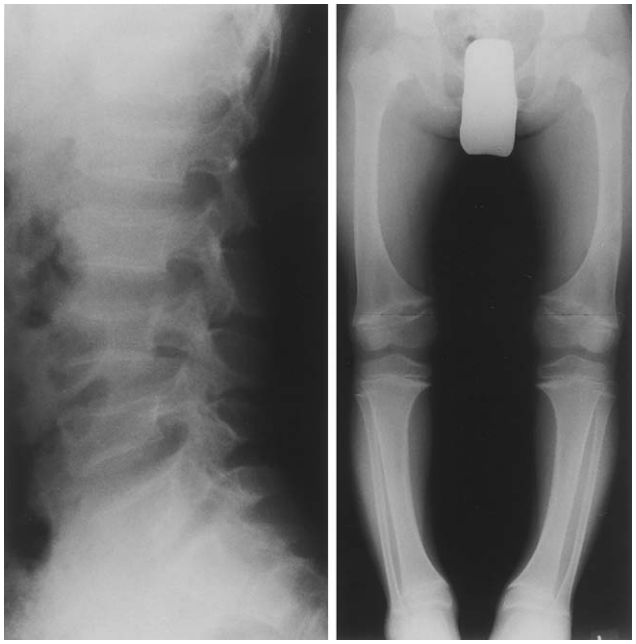

**Figure 1** Radiographs of a patient (the proband, III-5) with SMD at the age of 11 years. *Left,* Lateral radiograph of the lower spine, showing severe spinal dysplasia. *Right,* Radiograph of the legs, showing metaphyseal dysplasias with coxa vara.

missense mutation in the C-terminal globular domain of the gene, which segregated with the disease phenotype in his family. This is the first documented identification of a mutation in SMD.

## Patients, Material, and Methods

### *Patients and DNA Samples*

Patients were identified and followed up at special clinics in the Tokyo Metropolitan Kiyose Children's Hospital, the Dokkyo University Hospital, and the National Rehabilitation Center for Disabled Children. Diagnosis of SMD was made on the basis of clinical and radiographic examinations that showed short-trunked short stature and generalized dysplasias of the spine and metaphyses of the long tubular bones.

Five unrelated Japanese patients with SMD were included in this study. They were subclassified as follows: Kozlowski type, two cases; corner fracture type, one case; Japanese type, one case, and unspecified SMD, one case. The patient with Japanese-type SMD had a positive family history compatible with autosomal dominant inheritance; the detailed phenotypes of the family members have been reported elsewhere (Hasegawa et al. 1994). The other four SMD patients were sporadic.

Informed consent was obtained from patients and members of their families before blood samples were drawn. Genomic DNAs were extracted by standard procedures.

### *PCR and Direct Sequencing*

We used sets of primers described elsewhere (Ikegawa et al. 1997) to amplify the entire coding region of the *COL10A1* gene, with its flanking regions, by PCR. The PCRs were performed with the Takara ex*Taq* system (Takara Shuzo) according to the instructions of the manufacturer, in a total volume of 50 $\mu$l, with 50–100 ng of genomic DNA serving as a template. PCR products were purified by Ultrafree-MC (Millipore) and were sequenced directly by the ABI377 automated sequencer with the Prism Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (ABI).

### *RFLP Analysis*

The region containing nucleotide (NT) 1784 (NTs 1688–1962 [NTs are numbered starting from the initial base of the initiation codon as +1]) of the *COL10A1* gene (Thomas et al. 1991) was amplified by primers 5G (sense primer: 5′-CAGCAATAGGAACTCCCATACC-3′) and 3G (antisense primer: 5′-AAGCTGGAGCCA-CACCTG-3′). The PCR procedure was as follows: initial denaturation (94°C, 2 min) followed by 35 cycles of denaturation (94°C, 30 s), annealing (55°C, 30 s), and extension (72°C, 30 s), with a final extension step (72°C, 5 min). PCR products were digested for 10–12 h with 10–20 U of *Bst*NI /$\mu$g DNA (New England Biolabs) at 60°C, then electrophoresed on 3% NuSieve GTG agarose gels (FMC).

## Results and Discussion

The entire coding region of the *COL10A1* gene with flanking regions was examined for mutations. In the one patient with Japanese-type SMD (Hasegawa et al. 1994), we detected a G→A transversion at NT 1784 (fig. 2*A*) in one allele. The mutation occurred in the C-terminal globular domain of the gene and resulted in the substitution of a glutamic acid residue for a glycine at codon 595 (G595E).

Since G595E resulted in the loss of a *Bst*NI restriction-enzyme site, we performed PCR-RFLP analysis to examine the inheritance of the mutated 595E allele and its link with the disease in the patient's family. We found the mutation segregated with the disease phenotype (fig. 2*B*). The G595E mutation was absent in 50 normal individuals and other patients with SMD.

G595 of type X collagen is highly conserved. A sequence comparison of the C-terminal globular domains revealed that G595 is identical among chick, bovine, mouse, and human type X collagens; the residue is also identical in other short-chain collagen genes, human

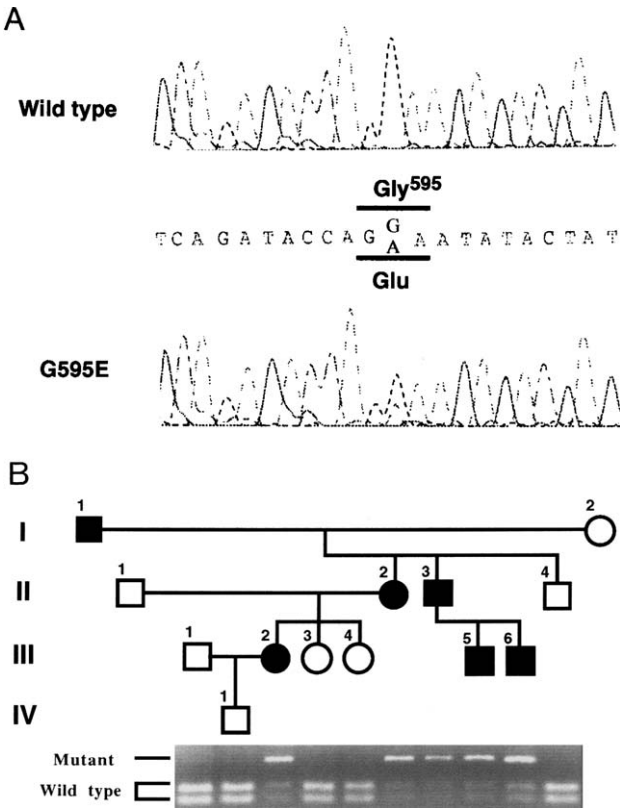


**Figure 2** Type X collagen (*COL10A1*) gene mutation in a Japanese family with SMD. *A,* Direct sequencing of PCR products demonstrating heterozygosity for G (wild-type allele) and A (mutant allele) at NT 1784. The G1784→A mutation resulted in the substitution of a glutamic acid residue for a glycine at codon 595 (G595E). *B,* Pedigree and RFLP analysis of the *COL10A1* gene in the family. Blackened symbols on the pedigree represent affected individuals; unblackened symbols represent unaffected individuals. The G595E mutation abolished a *Bst*NI site, yielding a mutant 210-bp band in heterozygous patients, in addition to the wild-type 116-bp and 94-bp bands. The mutation cosegregates with the disorder in this family.

*COL8A2* and rabbit *Col8a1,* and collagen-like molecules, the human C1qA-C complement chains (Brass et al. 1992). Thus, two lines of evidence lead to the conclusion that the G595E mutation causes the SMD phenotype in this family. This is the first mutation characterized in SMD.

Previous studies screened for the *COL10A1* mutations in patients with SMD, including the Kozlowski type and corner fracture type, and found no mutations (Warman et al. 1993; Wallis et al. 1996). In the present study, we also failed to identify *COL10A1* mutations in four of five SMD patients. These results suggest that *COL10A1* mutations in SMD may not be frequent. Whether other forms of SMD and SMD-like conditions are caused by *COL10A1* mutations remains to be determined.

Our observation presents in vivo evidence that type X collagen plays a critical role in the formation of the human spine as well as the long bones. In addition, our observation expands the range of pathological phenotypes that can be produced by aberration of type X collagen (type X collagenopathy). Significant phenotypic variability due to allelic heterogeneity that is caused by different mutations in the same gene is not uncommon in skeletal dysplasias. *FGFR3* (fibroblast growth-factor receptor 3) mutations produce a gradiation of phenotypes, from very mild hypochondroplasia through intermediate achondroplasia to severe thanatophoric dysplasia (Bonaventure et al. 1996). Similarly, mutations in the type II collagen gene cause a wide spectrum of manifestations including spondyloepiphyseal dysplasia congenita, hypochondrogenesis, Kniest disease, and Stickler syndrome (Spranger et al. 1994; Kulvaniemi et al. 1997). Mutations of *COL10A1* appear to lead to a phenotypic spectrum that includes Schmid MCD and SMD. The full extent as well as the pathological mechanism of type X collagenopathies must be clarified by additional studies.

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## Electronic-Database Information

Accession numbers and the URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for SMD types Strudwick, Kozlowski, Algerian, corner fracture, Richmond, and axial [MIMs 184250, 184252, 184253, 184255, 313420, and 602271, respectively])

## References

Bonaventure J, Rousseau F, Legeai-Mallet L, Le Merrer M, Munnich A, Maroteaux P (1996) Common mutations in the fibroblast growth factor receptor 3 (FGFR 3) gene account for achondroplasia, hypochondroplasia, and thanatophoric dwarfism. Am J Med Genet 63:148–154

Brass A, Kadler KE, Thomas JT, Grant ME, Boot-Handford RP (1992) The fibrillar collagens, collagen VIII, collagen X and the C1q complement proteins share a similar domain in their C-terminal non-collagenous regions. FEBS Lett 303: 126–128

Hasegawa T, Kozlowski K, Nishimura G, Hara H, Hasegawa Y, Aso T, Koto S, et al (1994) Japanese type of spondylometaphyseal dysplasia. Pediatr Radiol 24:194–197

Ikegawa S, Nakamura K, Nagano A, Haga N, Nakamura Y (1997) Mutations in the N-terminal globular domain of the type X collagen gene (COL10A1) in patients with Schmid metaphyseal chondrodysplasia. Hum Mutat 9:131–135

Jacenko O, LuValle PA, Olsen BR (1993) Spondylometaphy-

seal dysplasia in mice carrying a dominant negative mutation in a matrix protein specific for cartilage-to-bone transition. Nature 365:56–61

Kirsch T, von der Mark K (1991) Isolation of human type X collagen and immunolocalization in fetal human cartilage. Eur J Biochem 196:575–580

Kozlowski K, Maroteaux P, Spranger JW (1967) La dysostose spondylométaphysaire. Presse Med 75:2769–2774

Kuivaniemi H, Tromp G, Prockop DJ (1997) Mutations in fibrillar collagens (types I, II, III, and XI), fibril-associated collagen (type IX), and network-forming collagen (type X) cause a spectrum of diseases of bone, cartilage, and blood vessels. Hum Mutat 9:300–315

Lachman RS, Spranger J, Rimoin DL (1988) Metaphyseal chondrodysplasia, Schmid type: clinical and radiographic delineation with a review of the literature. Pediatr Radiol 18:93–102

Maroteaux P, Spranger J (1991) The spondylometaphyseal dysplasias: a tentative classification. Pediatr Radiol 21: 293–297

Schmid TM, Linsenmayer TF (1985) Immunohistochemical localization of short chain cartilage collagen (type X) in avian tissues. J Cell Biol 100:598–605

Spranger J, Winterpacht A, Zabel B (1994) The type II collagenopathies: a spectrum of chondrodysplasias. Eur J Pediatr 153:56–65

Taybi H, Lachman RS (1996) Skeletal dysplasias. In: Radiology of syndromes, metabolic disorders, and skeletal dysplasias, 4th ed. Mosby, St Louis, pp 928–937

Thomas JT, Cresswell CJ, Rash B, Nicolai H, Jones T, Solomon E, Grant ME, et al (1991) The human collagen X gene: complete primary translated sequence and chromosomal localization. Biochem J 280:617–623

Wallis GA, Rash B, Sykes B, Bonaventure J, Maroteaux P, Zabel B, Wynne-Davies R, et al (1996) Mutations within the gene encoding the alpha-1(X) chain of type X collagen (COL1A1) cause metaphyseal chondrodysplasia type Schmid but not several other forms of metaphyseal chondrodysplasia. J Med Genet 33:450–457

Warman ML, Abbott M, Apte SS, Hefferon T, McIntosh I, Cohn DH, Hecht JT, et al (1993) A type X collagen mutation causes Schmid metaphyseal chondrodysplasia. Nat Genet 5: 79–82