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BRIEF REPORT-CASE REPORT

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A novel type X collagen gene mutation (G595R) associated with Schmid-type metaphyseal chondrodysplasia

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Abstract Metaphyseal chondrodysplasia of the Schmid type (MCDS) is a skeletal dysplasia affecting the long bone metaphyses; it is characterized by short stature, bowlegs, and coxa vara. The spine is generally not involved. Here we report a novel missense mutation of the type X collagen gene in a sporadic case of MCDS. The mutation was a heterozygous single base-pair transition of G-to-A at nucleotide 1783, which predicted a substitution of glycine by arginine at codon 595 (G595R) in the carboxyl-terminal noncollagenous domain. Interestingly, another mutation of the same codon, in which glycine is substituted by glutamic acid (G595E), was previously reported to cause spondylometaphyseal dysplasia (SMD), another group of skeletal dysplasias with involvement of the spine in addition to the long tubular bones. The novel G595R mutation identified in the present study supports the concept of type X collagenopathy.

Key words Metaphyseal chondrodysplasia Schmid type (MCDS) · Mutation · Type X collagen gene (*COL10A1*) · Carboxyl-terminal noncollagenous (NC1) domain · Spondylometaphyseal dysplasia (SMD) · Type X collagenopathy

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Introduction

Metaphyseal chondrodysplasias are classified into several subtypes, including Jansen type, Schmid type, and McKusick type (International Working Group on Constitutional Diseases of Bone 1998). Among them, the Schmid type (MCDS; MIM 156500) is the most common and is characterized by short stature, bowlegs, and coxa vara. The radiological findings during childhood are diagnostic, featuring variable enlargement and irregularity of the metaphyses of the long tubular bones, with no involvement of the axial skeleton (Lachman et al. 1988). Since the first report by Warman et al. (1993), more than 20 mutations of the type X collagen gene (*COL10A1*) have been found in MCDS that do not occur in several related forms of chondrodysplasia (McIntosh et al. 1994; Bonaventure et al. 1995; Wallis et al. 1996; Chan et al. 1998 and references therein; Sawai et al. 1998). A recent report that the *COL10A1* mutation (G → A at nucleotide 1784, substituting glycine by glutamic acid at codon 595:G595E) causes spondylometaphyseal dysplasia (SMD), another group of skeletal dysplasias with involvement of the spine in addition to the long tubular bones, has introduced the concept of type X collagenopathy (Ikegawa et al. 1998). Here we report a novel mutation of the same codon (G → A at nucleotide 1783) leading to the substitution of glycine by arginine (G595R) in a sporadic case of MCDS.

Patient and methods

Patient

A 14-year-old Japanese girl was referred to us because of her short stature and bowlegs. Her height was 145 cm (−2.2 SD), her weight was 38.5 kg (−1.5 SD), and her arm span was 148 cm. Radiologic examination revealed bilateral coxa vara, irregular metaphyses of the long bones, and no evidence of spinal dysplasia (Fig. 1). She was diagnosed as

having MCDS on the basis of these clinical and radiological features. She had healthy parents and a healthy brother who showed no signs of osteochondrodysplasia. Detailed family history did not indicate inheritance of the disease. The patient and her family members gave informed consent before blood samples were collected.

Methods

DNA was extracted from peripheral blood leukocytes by a standard method. We used two sets of polymerase chain reaction (PCR) primers (primer H15: 5′-TCAATGGACCCACCGGGC-3′, primer H18: 5′-TGATGGCACTCCCTGAAGCCTG-3′, primer H17: 5′-GCCTGTGTCTGCTTTTACTG-3′, and primer H23: 5′-TTCAGCCTACCTCCATATGC-3′) to study the DNA sequence encoding the carboxyl-terminal noncollagenous (NC1) domain of COL10A1 (McIntosh et al. 1994; Matsui et al. 1996), while the coding region was analyzed using the PCR primers described by Sweetman et al. (1992). Amplification was performed with 2 U of TaKaRa *Ex Taq* (Takara, Shiga, Japan) in a total volume of 100 μl containing 1× reaction buffer, 100 μM of each dNTP, 25 pmol of each

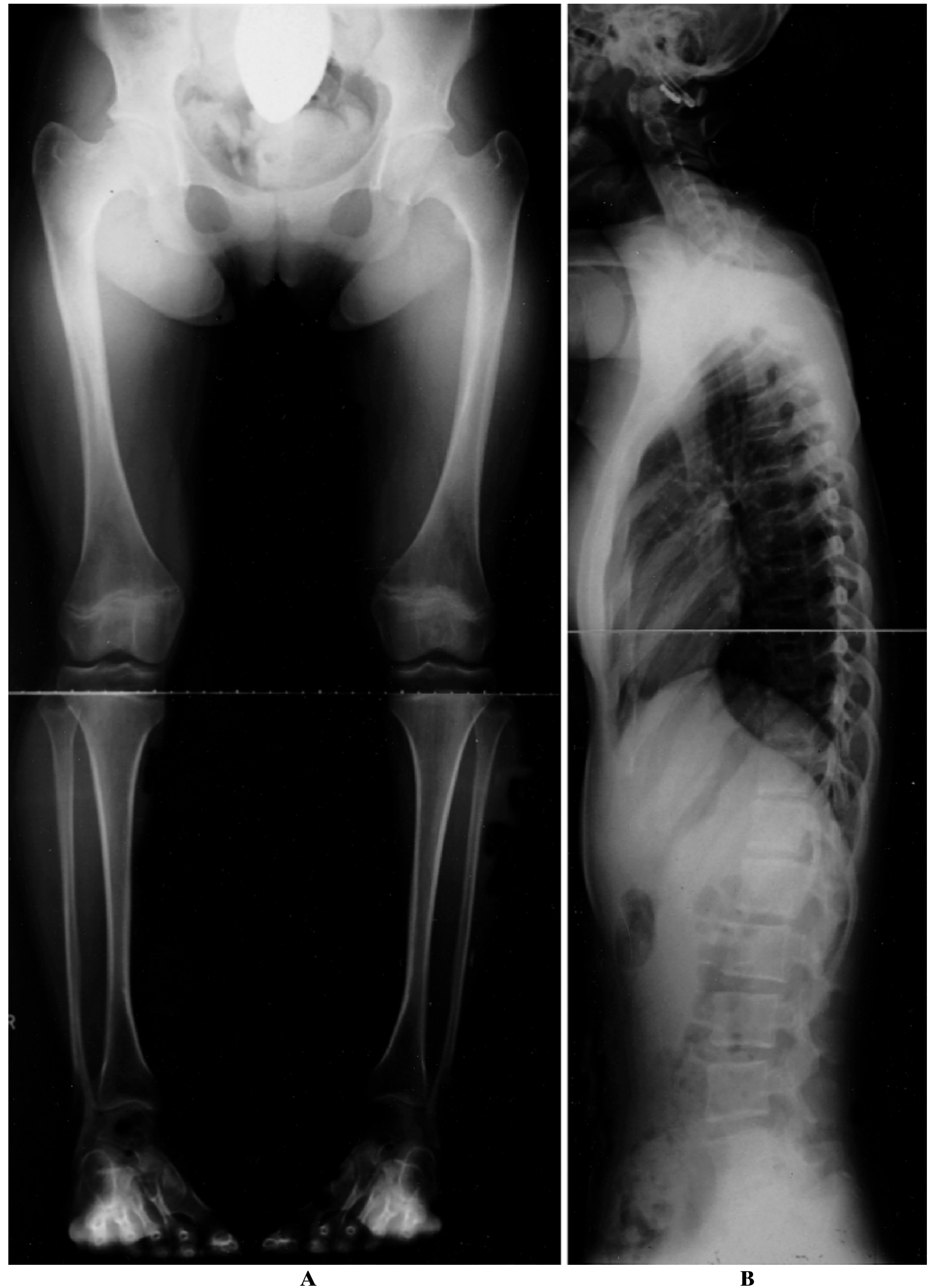

Fig. 1A,B. Radiographs of patient with metaphyseal chondrodysplasia of the Schmid type (MCDS) at the age of 14 years. **A** Radiograph of the legs, showing metaphyseal dysplasia of the long bones with mild bowlegs and bilateral coxa vara. **B** Lateral radiograph of the spine, showing no evidence of dysplasia

primer, and 100 ng of DNA. The PCR conditions were initial denaturation for 3 min at 95°C, 32 cycles of 30 s each at 95°C, 60°C, and 72°C, and final extension for 10 min at 72°C. The PCR products were purified and concentrated with a SUPREC-02 kit (Takara), and were directly sequenced using a BigDye Terminator Cycle Sequencing FS R/R Kit (Applied Biosystems, Foster City, CA, USA) and an Applied Biosystems 377 DNA sequencer according to the manufacturer's instructions. Primers used for sequencing were identical to those used for each PCR reaction.

Results

Direct DNA sequencing of the PCR products amplified with primers H15/H18 as well as H17/H23 revealed that the patient was heterozygous for a single base pair transition of G-to-A at nucleotide 1783 (sequence numbers according to Thomas et al. 1991) (Fig. 2A). In order to confirm this mutation, the PCR product obtained from the patient with primers H17/H23 was digested with *Hin*dIII/*Nsi*I (NEB, Beverly, MA, USA) and was subcloned into the *Hin*dIII/*Pst*I (NEB) cloning sites of pBluescript II SK$^+$ (Stratagene, La Jolla, CA, USA). The presence of a heterozygous mutation was confirmed by sequencing of the plasmid DNA from individual subclones (data not shown). This mutation predicted substitution of Gly at codon 595 by Arg (G595R) in the NC1 domain of COL10A1 (Fig. 2A). DNA sequence analysis of other family members demonstrated that the healthy brother and parents did not have this mutation (data not shown). Since the G595R mutation eliminated a *Bst*NI restriction enzyme recognition site, the PCR products amplified from the patient and her family members with primers H17/H23 were digested with *Bst*NI (NEB) and were electrophoresed on 3% agarose gel. The electrophoresis pattern segregated the patient from the other family members (Fig. 2B), suggesting the de-novo occurrence of this mutation. The G595R mutation was absent in 50 normal individuals.

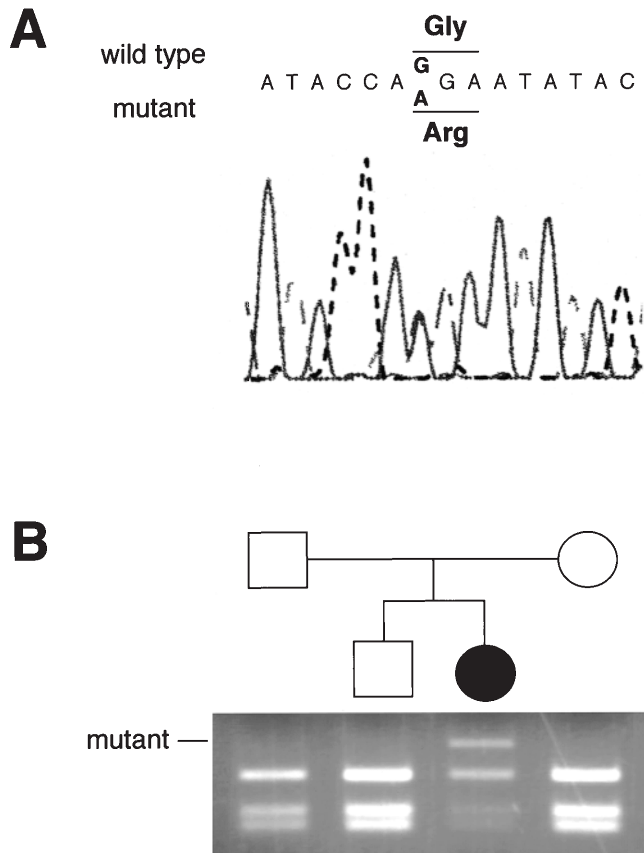

Fig. 2A,B. *COL10A1* mutation in Japanese patient with MCDS whose radiographs are shown in *Fig. 1*. **A** Direct DNA sequencing of polymerase chain reaction (PCR) product from the MCDS patient. The sequence shown is from nucleotide 1777 to 1791 of *COL10A1*, demonstrating a heterozygous G-to-A transition at nucleotide 1783 that predicts substitution of Gly at codon 595 by Arg (G595R). **B** Electrophoresis of the *Bst*NI digestion fragment of PCR products from the patient (*black circle*) and from her family members (*open symbols*). The heterozygous G595R mutation in the patient deleted a *Bst*NI site from one allele, producing a mutant 251-bp band (indicated), as well as shorter bands

Discussion

As is the case with the G595R mutation reported here, most of the *COL10A1* mutations reported to date have been identified within the coding region of the NC1 domain (Warman et al. 1993; Chan et al. 1998 and references therein; Sawai et al. 1998). The glycine at codon 595 of COL10A1 is interesting, not only because it is highly conserved among species but also because it is located within the 13-amino acid aromatic motif that is critical for intermolecular association (Brass et al. 1992; Marks et al. 1999; Chan et al. 1999). Therefore, the G595R mutation may interfere with the stable trimer formation of mutant and/or wild type alpha chains by altering the three-dimensional structure of the NC1 domain in vivo. Recently, Ikegawa et al. (1998) reported that a different mutation of the same codon (G595E) caused SMD, another type of skeletal dysplasia associated with spinal changes, conflicting with a previous report by Bonaventure et al. (1995) who identified the same mutation (G595E) in a family with MCDS. It has not been explained why the G595E mutation occurs in two different disorders, MCDS and SMD.

SMD was originally described by Kozlowski (1976) as an isolated skeletal dysplasia with major involvement of the spine. Since then, several subtypes of SMD with mild-to-moderate spinal involvement have been reported (Maroteaux and Spranger 1991; International Working Group on Constitutional Diseases of Bone 1998). Hasegawa et al. (1994) reported a Japanese type of SMD with variable spinal changes among the affected family members, and the G595E mutation mentioned above was identified in this family. In the past, Mizushima (1982) has independently described the existence of phenotypic overlap between SMDs and MCDS, suggesting that a certain type of SMD with mild or moderate spinal changes is

genetically identical to MCDS. In the Japanese family with SMD and the G595E mutation, two affected members had normal vertebral bodies and radiological findings were not distinguishable from those of MCDS (Hasegawa et al. 1994). We propose that such cases of SMD should be classified in the same category as MCDS, especially if the *COL10A1* mutation is confirmed. In conclusion, the present study provides additional information to support the concept of type X collagenopathy.

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