Early-Onset Osteoarthritis Due to Otospondylomegaepiphyseal Dysplasia in a Family with a Novel Splicing Mutation of the *COL11A2* Gene

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ABSTRACT ***Objective.*** To evaluate an approach to the clinical, radiographic, and molecular diagnosis of an underlying skeletal dysplasia in adults presenting with early-onset polyarticular osteoarthritis (OA).

Methods. We identified a family with 2 adults with polyarticular OA and a child with generalized arthralgia. General, musculoskeletal, ocular, and auditory evaluations were undertaken. Investigations included radiographs of symptomatic joints, analysis of serum inflammatory markers and joint fluid, and mutational analyses of the *COL11A2* gene.

Results. The 3 affected individuals had normal stature, mild mid-face hypoplasia, and hearing impairment, but normal eyes. Radiographs of the affected adults showed severe polyarticular OA but did not reveal diagnostic evidence of an underlying skeletal dysplasia. However, the child's radiographs showed enlarged epiphyses with an advanced bone age. The combination of skeletal, facial, and auditory features together with the absence of ocular features indicated that they had otospondylomegaepiphyseal dysplasia, also known as Stickler syndrome type III. The diagnosis was confirmed by identifying a mutation in the *COL11A2* gene that encodes the pre-pro-α2(XI) chain of type XI collagen that is involved in type II collagen fibrillogenesis.

Conclusion. Early-onset polyarticular OA may occur in adults without a known or obvious underlying skeletal dysplasia. This study provides an approach to the diagnosis of an underlying skeletal dysplasia in such individuals. (First Release Mar 15 2008; J Rheumatol 2008;35:920–6)

Key Indexing Terms:
OSTEOARTHRITIS SKELETAL DYSPLASIA COLLAGEN
GENETICS OTOSPONDYLOMEGAEPIPHYSEAL DYSPLASIA

Many studies have shown that genetic variants play a significant role in the etiology and pathogenesis of primary osteoarthritis (OA)[1]. Early-onset OA has been associated with allelic variants that result in skeletal dysplasias[2,3]. Major changes in chondrocyte morphology are frequent and are often accompanied by abnormalities in the amount, composition, assembly, and secretion of various components of the extracellular matrices of the articular and growth plate cartilages[4,5]. Such cartilage changes have been associated with allelic variants of the genes encoding type II, IX, and XI collagens; matrilin-3; and cartilage oligomeric matrix protein, as well as transport proteins and enzymes involved in the sulfation of proteoglycans[6].

Early-onset OA associated with a skeletal dysplasia usually manifests after closure of the growth plates and after complete formation of the secondary centers of ossification[3]. As a result, radiographs of the osteoarthritic joints may not reveal diagnostic information about the presence of or the properties of an underlying skeletal dysplasia. In this report we show that diagnosis of a mild, underlying skeletal dysplasia and its causative mutation can be achieved by carefully evaluating affected adults and children for extraskeletal and dysmorphic skeletal abnormalities. In this way, we determined that a man with early-onset OA of multiple joints, and members of his family who were also affected, had otospondylomegaepiphyseal dysplasia (OSMED; Mendelian Inheritance in Man no. 215150) due to an autosomal-dominant mutation of the *COL11A2* gene.

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MATERIALS AND METHODS

Clinical and radiographic examinations were performed in a family in which there were 2 adults with early-onset, polyarticular OA and a child with joint pains but without evidence of OA (Figure 1). Blood was obtained from case II:5 and buccal brush epithelial samples were obtained from family members for DNA analysis. These studies were undertaken with the approval of the Institutional Research Ethics Board and the family.

Total RNA was extracted from cultured lymphoblasts of case II:5 using an RNeasy Mini Kit (Qiagen). Complementary DNA (cDNA) was prepared for polymerase chain reactions (PCR), which yielded 9 fragments spanning the 66 exons of *COL11A2*[7]. Heteroduplex analysis of the fragments was performed as described[8]. PCR products for sequencing were separated on 1% agarose gels, excised, purified, and directly sequenced using a Dual CyDye Terminator Sequencing Kit (Amersham Biosciences) on an OpenGene System (Visible Genetics) automated sequencer. Genomic PCR and sequencing were used to confirm the abnormality noted on cDNA sequencing. Genomic DNA was also prepared and analyzed from other family members. Genbank sequences NM_080680, for the full-length cDNA sequence, and NC_000006, for the complete genomic DNA sequence, were used to design primers with the assistance of Oligo Primer Analysis Software (MBI Inc.). Nucleotide numbering used the A of the ATG translation initiation start site as nucleotide +1 of pre-pro-α2(XI) cDNA (NM_080680), while the amino acid residue numbering starts with the first methionine residue of the pre-pro-α2(XI) protein chain (NP_542411).

RESULTS

Clinical and radiographic findings. Case II:5 — A 37-year-old man was assessed because of early-onset OA of multiple joints. He presented elsewhere at the age of 9 years with recurrent foot, ankle, and knee pains. His pediatric radiographs had been destroyed. Initially, he was diagnosed as having growing pains and was later treated for atypical juvenile arthritis because of clinical evidence of synovitis. He had repeated intraarticular steroid injections and 2 radiation synovectomies of his knees, without benefit. At 17 years of age, arthroscopy of his left knee revealed severe patellofemoral OA. Subsequently, he underwent numerous arthroscopic procedures, culminating in bilateral total knee arthroplasties at the age of 33 years. He also had severe OA in his shoulders, wrists, metacarpophalangeal (MCP) joints, ankles, and metatarsophalangeal (MTP) joints (Figure 2). He had minimal osteophytic lipping of the acetabulum of each hip. Erythrocyte sedimentation rate (ESR) and complete blood cell count were normal. Antinuclear antibody (ANA) and rheumatoid factor (RF) assays were negative. No crystals were identified in synovial fluid aspirates from his knees.

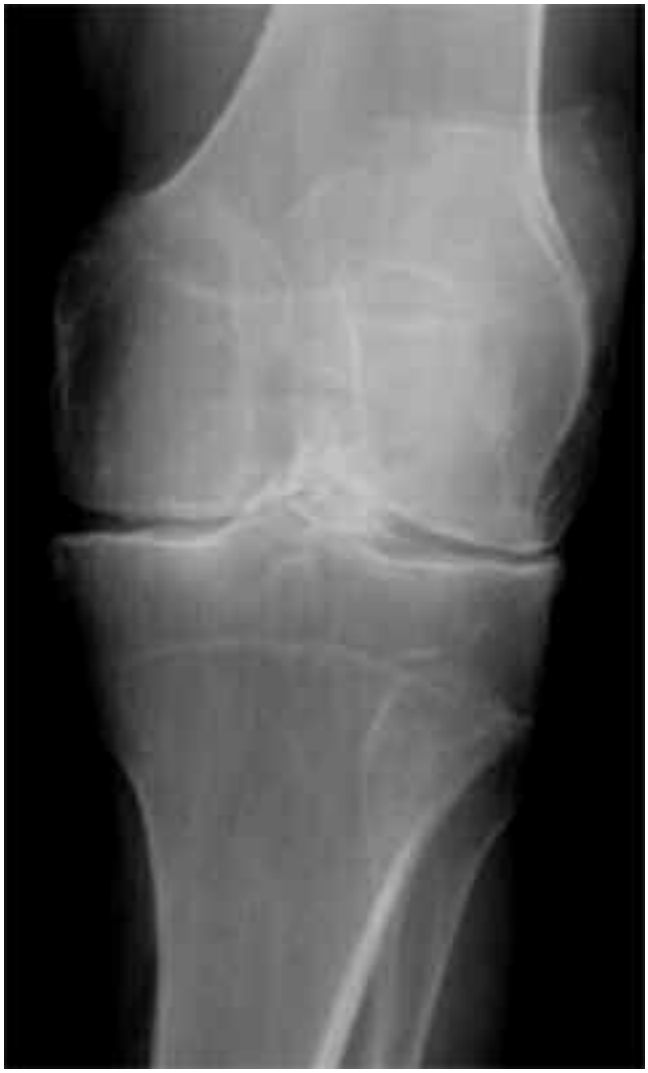

Figure 2. Case II:5: OA of the left knee at 33 years of age.

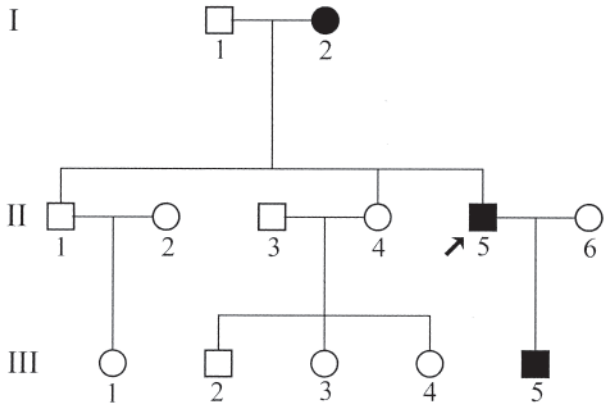

Figure 1. The family pedigree. Black symbols indicate clinically affected individuals. Arrow identifies the index case. White symbols indicate clinically unaffected individuals.

Clinical examination at age 37 years revealed mild shortening of his metacarpals and metatarsals, mild mid-face hypoplasia, and a depressed nasal bridge. His height was 175 cm (50th percentile). Apart from the shortening in his hands and feet he was normally proportioned. A detailed ophthalmological examination was normal. He had severe hearing impairment, which had progressed since it was first noted during his teenage years. Audiological examination demonstrated bilateral sensorineural hearing loss and he was advised to use bilateral hearing aids. There were no other known extraskeletal abnormalties.

Case III:5 — The youngest patient was a 6-year-old boy, son of case II:5 and grandson of case I:2. He was born pre-

maturely at 34 weeks of gestation (length 45 cm; 25th percentile) due to maternal renal disease. Dysmorphic features were not noted at birth. His longitudinal growth was normal and at 6 years of age his height was 122 cm (50th percentile). He had mildly delayed speech due to conductive hearing loss. An ophthalmological examination was normal.

He had recurrent, short-lived, acute episodes of pain and swelling of his knees and ankles that started at the age of 4 years. At age 6 years we observed that he had mild depression of his nasal bridge, mild mid-face hypoplasia, and enlarged knee, ankle and wrist joints but no signs of arthritis. The ESR and complete blood cell count were normal. His ANA and RF tests were also negative.

Radiographic examination at age 6 years revealed mild posterior scalloping of the lumbar vertebrae. The lumbar vertebral bodies were large and contained horizontal growth lines. The secondary ossification centers of his femora, tibiae, radii, metacarpals, and phalanges were abnormally large (Figure 3). At age 6 years, his hand and knee radiographs showed a bone age of about 10 years[9]. A magnetic resonance imaging (MRI) scan of his ankles confirmed the enlargement of the secondary ossification centers of the distal tibia and fibula (Figure 4). The scan did not show any signs of synovitis.

Case I:2 — The third affected member of the family was a 63-year-old woman, mother of case II:5 and grandmother of case III:5. As a child she was diagnosed as having growing pains. At age 29 years she had severe patellofemoral OA and underwent a right medial meniscectomy. At age 38 years, corrective surgery for bilateral hallux valgus with first MTP joint OA was performed. Intermittent pain in the temporomandibular joints, right shoulder, second and third MCP joints, proximal interphalangeal joints, and left ankle was present at that time. At age 43 years, she had persistent bilateral knee pain, and underwent right knee arthroplasty at the age of 48 and left knee arthroplasty age 53 years. She also had symptomatic OA of the hips.

She first noted high frequency hearing impairment at about 30 years of age, but did not have further investigations. She had no ocular or other complaints. Her height was 160 cm (30th percentile). She also had mild mid-face hypoplasia, depressed nasal bridge, and short hands and feet but otherwise had normal body proportions.

Mutational analysis. The phenotypic features of mild mid-face hypoplasia, depressed nasal bridge, enlarged epiphyses, early-onset OA, and hearing impairment, without ocular anomalies, suggested that the affected individuals had OSMED[10]. We carried out mutational analysis of *COL11A2* as mutations of this gene had been described in this skeletal dysplasia[11]. Heteroduplex analyses were undertaken on the 9 overlapping cDNA-PCR products that spanned the coding sequence of the 66 exons of *COL11A2*. Analysis of product 7 showed roughly equal amounts of the expected wild-type 724 base pair (bp) product and an abnormal product with a predicted size of 664 bp. DNA sequencing confirmed that the 724 bp product contained the wild-type sequence, while the 664 bp product lacked the 54 bp corresponding to exon 44 of *COL11A2* (Figure 5)[7]. The sequencing results showed that the abnormal PCR product was actually 670 bp in size. Genomic

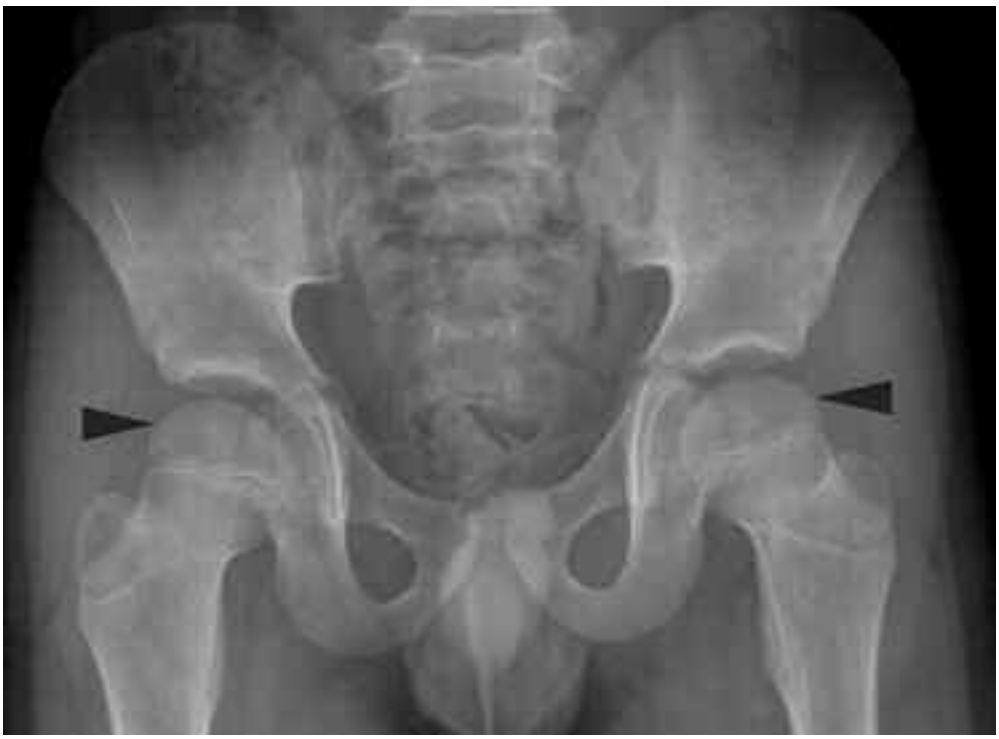

Figure 3. Case III:5: enlarged secondary centers of ossification of the proximal femora (arrowheads) at age 6 years.

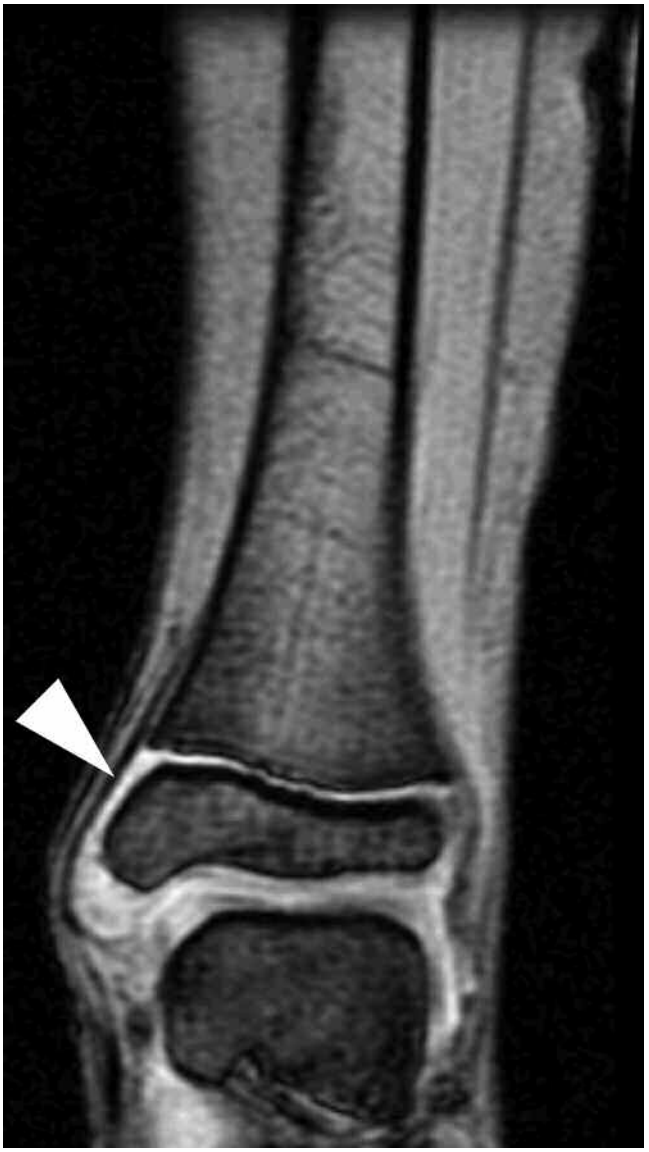

Figure 4. Case III:5: coronal MRI of the left ankle at age 6 years showing enlarged secondary center of ossification of the distal tibia (arrowhead). There were no signs of arthritis.

DNA sequencing of introns 43 and 44 as well as exon 44 showed that case II:5 was heterozygous for a T insertion at the +2 site of the splice-donor site of intervening sequence 44, designated IVS44+2insT (Figure 6). The latter change was confirmed in cases I:2 and III:5, but was not found in clinically unaffected members of the family. The nucleotide change was not found in any of the single-nucleotide polymorphism databases or in DNA from 100 normal subjects. Loss of the exon 44 encoded sequence from the cDNA was likely to be due to abnormal splicing arising from the nucleotide insertion in the splice-donor site of IVS44. The skipped sequence corresponded to G3259 to T3312 of α2(XI) cDNA and glycine-601 to histidine-618 of the main triple helical domain of the α2(XI) chain of type XI collagen[7].

DISCUSSION

Adults with early-onset OA of multiple joints due to an underlying skeletal dysplasia may present in several ways. First, an adult may present with a known underlying skeletal dysplasia diagnosed during childhood. Studies have shown that such individuals are likely to have moderately severe and severe forms rather than mild forms of bone dysplasias[6]. Second, an adult may present with an unrecognized or undiagnosed skeletal dysplasia. Careful phenotyping of affected individuals within such families, as in our study, may reveal features of diagnostic importance.

The 3 individuals in our study were of normal height but showed articular, facial, and auditory anomalies that were inherited in an autosomal-dominant manner. Radiographic ascertainment of the 2 adults provided no useful diagnostic information about the properties of the underlying skeletal dysplasia. However, radiographs of the 6-year-old child showed that the clinical enlargement of his joints was due to enlargement of his secondary ossification centers.

The phenotypes displayed by the 3 individuals were consistent with an autosomal-dominant form of otospondylomegaepiphyseal dysplasia. It is characterized by conductive and progressive sensorineural hearing loss, as well as skeletal abnormalities including mid-face hypoplasia, mild epiphyseal dysplasia with abnormally large joints, and mild platyspondyly[10]. The phenotype changes with age and the enlarged epiphyses present in childhood may become less recognizable in adults, as in this family. Most patients with OSMED have normal body lengths with short extremities, and develop premature OA in the second or third decade of life, consistent with our findings[10].

The heterozygous loss of exon 44 from α2(XI) mRNA as a result of a mutation in intron 44 has not previously been reported in OSMED or related phenotypes[12]. The splicing mutation was demonstrated using amplified cDNA prepared from the low basal amounts of α2(XI) mRNA produced from the *COL11A2* gene by transformed peripheral blood lymphocytes. This method of analysis was much simpler than undertaking mutational analysis of the 66 exons and introns of *COL11A2*[7]. Mutational analyses of low basal transcripts of genes encoding other proteins in the extracellular matrix of hyaline cartilage have also been successful using transformed lymphocytes and dermal fibroblasts[8]. The latter studies confirmed that splicing anomalies detected using low basal transcripts were qualitatively the same as those observed in the abundant transcripts prepared from chondrocytes.

Hyaline cartilage was not available for analysis from any of the individuals studied here. However, we expect that the loss of the exon 44 encoded sequence from the triple helical domain of α2(XI) chains would have had a dominant-negative effect on the assembly of type XI collagen and a detrimental effect on the formation of the heterotypic type II collagen-containing fibrils of hyaline cartilages and the inner ear[11]. Type XI collagen, normally present in low concentrations in

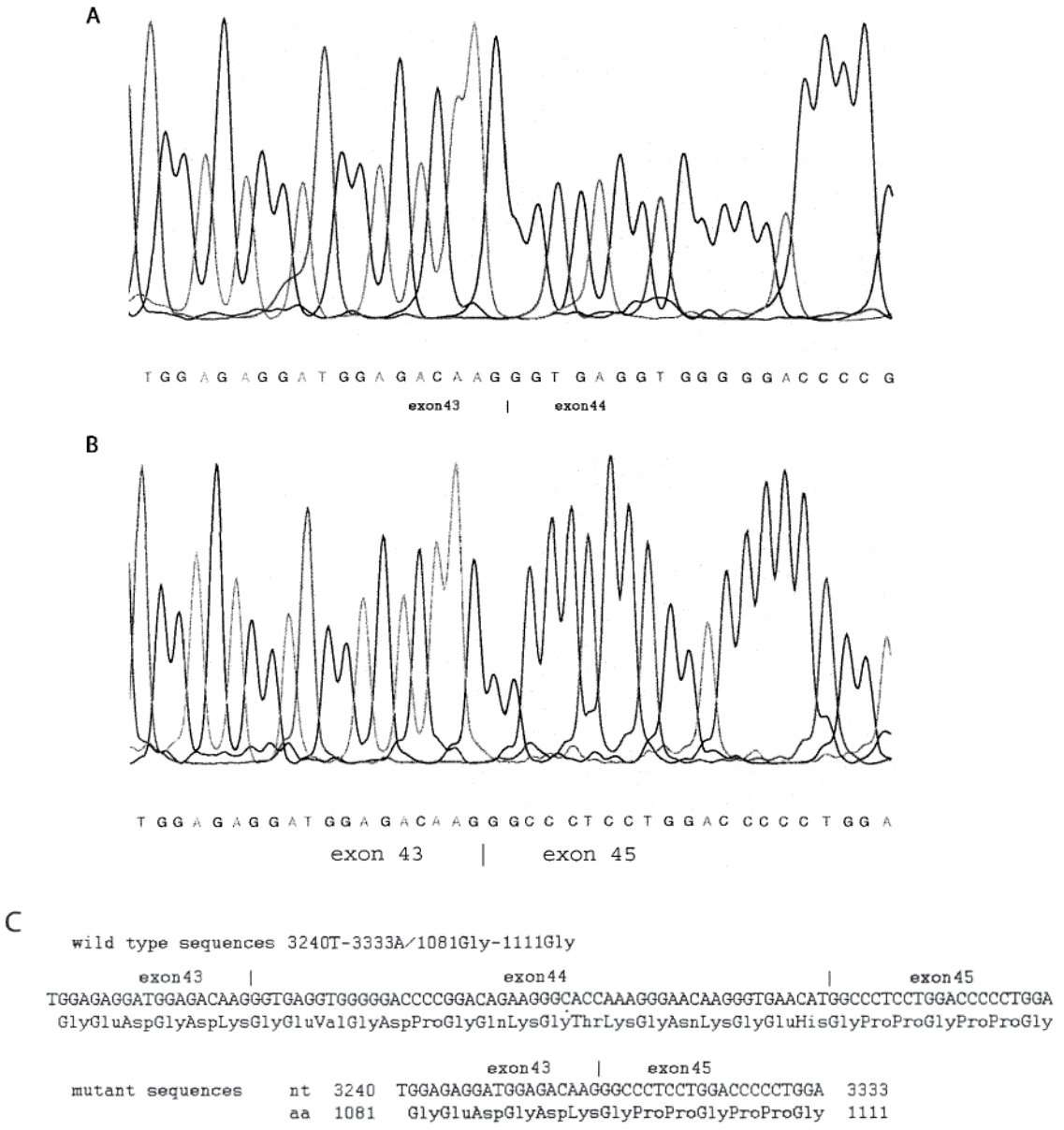

Figure 5. Sequences of PCR product 7 of α2(XI) cDNA from case II:5. A. Partial sequence of the wild-type 724 bp PCR product showing the junction of contiguous exon 43 and exon 44-encoded sequences. B. Partial sequence of the mutant 670 bp PCR product showing the loss of the exon 44-encoded sequence and the junction of the contiguous exon 43 and exon 45-encoded sequences. C. Partial cDNA and expected amino acid sequences from the wild-type and mutant PCR products. The mutant product lacked the 54 bp sequence normally encoded by exon 44 of *COL11A2*.

these tissues, plays a critical role in the regulation of growth of the collagen fibrils, which contain predominately type II and IX collagens[13].

Review of the reported mutations of *COL11A2* shows an emerging pattern of genotypes. Most patients who were heterozygous for their mutation had peptide deletions or amino acid substitutions within the translated α2(XI) chain[12]. The mutant collagen chains, as in this family, were likely to be incorporated into type XI collagen molecules, where they would be expected to exert a dominant-negative effect. In contrast, most autosomal-recessive cases were associated with homozygous or compound heterozygous loss-of-function mutations resulting from the introduction of premature translational stop codons into the α2(XI) mRNA sequence[12]. Individuals heterozygous for a stop codon mutation of *COL11A2* usually did not have any obvious phenotype[11]. An exception to this observation was a family in whom affected members were heterozygous for the autosomal-dominant inheritance of a stop codon mutation of *COL11A2*. In that case, the premature stop codon induced non-sense-associated

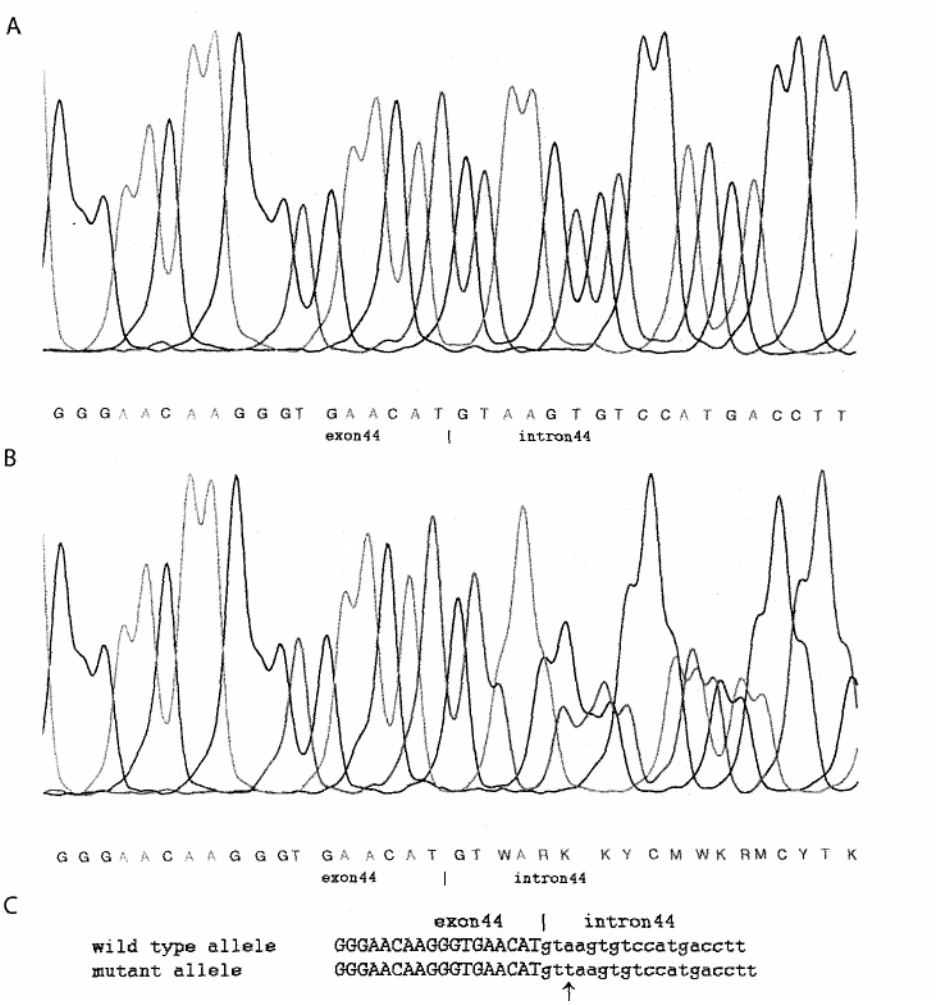

Figure 6. Sequences of genomic *COL11A2* DNA from case II:5. A. Control: partial sequence showing part of exon 44 and the splice-donor site of intron 44. B. Case II:5: partial sequence showing the normal sequence for exon 44 but a double sequence beyond the G^{+1} nucleotide of the splice-donor site of intron 44. The automated DNA sequencer labeled nucleotides as "W" when they could not be correctly assigned because of the double sequence. C. The wild-type and mutant splice-donor sequences for intron 44. The exonic sequence is in capital letters and the intron sequence is upper/lower case. The mutation consists of an insertion of t either before or after the t^{+2} position of the splice-donor site of intron 44.

splicing of the 54 bp exon 57-encoded sequence that contained the stop codon[12]. The mutant α2(XI) chains lacking 18 amino acids in the main triple helical domain would be expected to be incorporated into type XI collagen molecules and to exert a dominant-negative effect.

There is a continuing debate over the clinical overlap and differential diagnosis of the phenotypes produced by mutations of *COL11A2*[14]. The phenotypes include autosomal-dominant non-ocular Stickler syndrome (Stickler syndrome type III), autosomal-dominant and recessive forms of OSMED, Weissenbacher-Zweymuller syndrome, non-syndromic cleft palate, and non-syndromic hearing loss[11,15-19]. The phenotype of homozygous *Col11a2* knockout mice is also similar to that of non-ocular Stickler syndrome and OSMED[20]. It is likely that the non-syndromic cleft palate and hearing loss diagnoses will continue to be applied to cases in whom there are minimal or no detectable changes in the skeleton. However, the differential diagnoses of patients with skeletal, auditory, and palatal anomalies need to be simplified, as the phenotypes of non-ocular Stickler, OSMED, and Weissenbacher-Zweymuller syndromes are almost identical[6].

The absence of ocular anomalies distinguishes OSMED, also called Stickler syndrome type III, from Stickler syndrome types I and II[11]. In Stickler syndrome types I and II, the ocular anomalies can include myopia, vitreoretinal degeneration, cataracts, and retinal detachments[21]. The patterns of skeletal, ocular, and auditory anomalies provide important clues to the underlying molecular defects. The main candidate genes to consider in the 3 types of Stickler syndrome are those encoding the chains of type II, IX, and XI collagens[11,22,23]. The lat-

ter genes are each expressed in hyaline cartilages, the eye, and the ear, except for *COL11A2*, which is not expressed in the eye. In the vitreous humor, the α2(XI) chain of type XI collagen is replaced by the α2(V) chain of type V collagen in hybrid-type V/XI collagen molecules[13]. Stickler syndrome types I, II, and III are caused by mutations of *COL2A1*, *COL11A1*, and *COL11A2*, respectively[21]. An autosomal-recessive dysplasia resembling Stickler syndrome type I is produced by homozygous mutations of *COL9A1* that encode the α1(IX) chain of type IX collagen[23].

Establishing the diagnosis and molecular basis of an underlying skeletal dysplasia causing early-onset OA has potential clinical value. Confirmation of the mode of inheritance can be used for genetic counselling and for testing of individuals who wish to know if they bear the mutation. The phenotypic manifestations can also be used to plan surveillance and care. For example, affected individuals in the current family with OSMED require continuing surveillance of their joint health and hearing. However, in contrast to patients with Stickler syndromes types I and II, they do not need ocular surveillance beyond that needed for normal individuals.

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