Autosomal Dominant and Recessive Osteochondrodysplasias Associated with the COL11A2 Locus

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Summary

Identifying mutations that cause specific osteochondrodysplasias will provide novel insights into the function of genes that are essential for skeletal morphogenesis. We recently described linkage to markers near the COL11A2 locus, encoding the gene, which demonstrates that autosomal recessive and autosomal dominant forms of Stickler syndrome. We also show that an autosomal recessive disorder characterized by spondyloepiphyseal dysplasia and sensorineural hearing loss, similar to the otospondyloepiphyseal dysplasia (OSTMED) syndrome, is linked to the COL11A2 locus and is caused by a glycine to arginine substitution in COL11A2 gene, encoding the collagen XI, which will provide insights into the function of genes that are essential for skeletal morphogenesis.

Introduction

Molecular genetic analyses of osteochondrodysplasias, hereditary disorders affecting skeletal development in both mice and humans, promise to provide insights into the large number of genes essential for skeletal morphogenesis. With improved techniques for gene mapping, positional cloning, functional cloning, and mutation detection, the task of identifying the mutations causing these disorders is becoming less daunting. This is illustrated by the recent successes in identifying mutations in genes encoding structural, growth factor receptor, and sulfate transporter proteins as causes of distinct osteochondrodysplasias (Warman et al., 1993; Shiung et al., 1994; Reardon et al., 1994; Hästbacka et al., 1994). Adding to this is the accompanying paper by Li et al. (1995 [this issue of Cell]), which demonstrates that autosomal recessive chondrodysplasia (cho) in mice is due to a mutation in the gene, Col11a1, coding for one of the polypeptide subunits of the quantitatively minor fibrillar collagen XI in cartilage. These data suggest that the Col11a1 gene is essential for skeletal morphogenesis.

Here, we report that the COL11A2 locus, encoding the collagen XI, is associated with autosomal dominant and autosomal recessive human osteochondrodysplasias. We describe a mutation affecting a 5' splice site leading to in-frame exon skipping and causing an autosomal dominant form of Stickler syndrome. We also show that an autosomal recessive disorder characterized by spondyloepiphyseal dysplasia and sensorineural hearing loss, similar to the otospondyloepiphyseal dysplasia (OSTMED) syndrome, is linked to the COL11A2 locus and is caused by a glycine to arginine substitution in collagen XI, which will provide insights into the function of genes that are essential for skeletal morphogenesis.

Results

Identification of the COL11A2 Mutation in a Family with Stickler Syndrome

We recently described linkage to markers near the COL11A2 locus in a large Dutch kindred with a Stickler syndrome phenotype (Brunner et al., 1994). All 16 affected
individuals in the family had characteristic facial features of Stickler syndrome (Stickler et al., 1965) combined with hearing impairment. Several patients had cleft palate and mild arthropathy, but none had the ophthalmological signs usually associated with the Stickler syndrome. To identify the mutation causing the disorder in this family, we used reverse transcription–polymerase chain reaction (RT–PCR) from total RNA extracted from chondrocytes and Epstein–Barr virus-transformed (EBV-transformed) lymphoblasts from patients heterozygous for the defective allele and from unaffected individuals. Primers for nested PCR were designed to cover, in five overlapping fragments, the whole coding sequence (4953 bp) of the $a_2$(XI) collagen gene. Restriction enzymes were used to cut these genomic DNAs of all family members were PCR amplified, the micro DNAs of all family members were PCR amplified, the sense primer being end-labeled with $^{32}$P. After Nlalll digestion, these products were run on 5% denaturing sequencing gels. The G to A transition cosegregated with the disease (Figure 1). This change created a novel Nlalll site in the genomic sequence. To demonstrate that this sequence change was present in all the affected individuals, genomic DNAs of all family members were PCR amplified, the sense primer being end-labeled with $^{32}$P. After Nlalll digestion, these products were run on 5% denaturing sequencing gels. The G to A transition cosegregated with the disease (Figure 2).

**Linkage between an Autosomal Recessive Phenotype and Loci on Chromosome 6p**

A second Dutch kindred has been identified, in which three affected siblings have severe degenerative joint disease (osteoarthritis), which presents in early adulthood and affects predominantly the hips, knees, elbows, and shoulders (Figure 3). The spine is less severely affected, and adult height is only slightly below that of the unaffected siblings. There is increased lumbar lordosis and prominent interphalangeal joints. Short fifth metacarpals are found in all cases. The patients have distinct facial features: midface hypoplasia with a short upturned nose, prominent eyes, depressed nasal bridge, and prominent supraorbital...
Mutations in Osteochondrodysplasias

Figure 3. Severe Osteoarthritis at the Hip Joints of a Patient with Recessive Osteochondrodysplasia

Pelvic radiograph of patient 5 (aged 29 years) from the kindred with the autosomal recessive phenotype. Joint space narrowing, osteophyte formation, and osteosclerosis at the hips can be observed.

Figure 4. Homozygosity by Descent at the COL11A2 Locus and a Single Nucleotide Change in the Coding Sequence of COL11A2 in the Family with Recessive Osteochondrodysplasia

(A) Pedigree of the family with autosomal recessive osteochondrodysplasia (top). The genotype of each individual for 10 tested CA repeat markers from 6p21 is given. The haplotype associated with the disorder is in bold letters, and the haplotype for which the affected individuals are homozygous by descent is boxed. The result of the analysis of an intragenic MspI polymorphism (COL11A2) is shown in the photograph of an ethidium bromide-stained agarose gel (middle). MspI cleaves a 320 bp fragment into 230 bp and 90 bp (data not shown) fragments. The photograph of an ethidium bromide-stained agarose gel at the bottom of the figure shows the absence of an MspI site (note that this site is unrelated to the polymorphic site shown in the middle) in a genomic fragment due to the G to A transition in COL11A2.

When digested with MspI, the 1.2 kb genomic PCR product is digested into fragments of 800 bp (data not shown), 270 bp, and 150 bp in affected individuals. In unaffected children, the 270 bp fragment is cleaved into fragments of 200 bp and 70 bp (data not shown). The parents show the presence of all fragments.

(B) Schematic representation of the α2(XI) collagen mRNA showing the G to A transition in the codon of Gly-175 as counted from the amino terminus of the triple helical (COL) domain of the polypeptide chain. The mutation is located within a 45 bp-long exon of COL11A2. Genomic sequencing of the coding strand from a patient (lane 1), parents (lanes 2 and 3), and an unaffected child (lane 4) is shown (top right). The arrow points to the mutation site. The exon sequence and deduced amino acid sequence covering the mutation site are provided (top left). The G to A transition changes the glycine codon to an arginine codon. Closed symbols represent individuals with the disease phenotype.
Table 1. Comparison of Clinical Features in the Families with Autosomal Dominant and Autosomal Recessive Phenotypes Linked to COL11A2 with Those of the OSMED and Classical Stickler Syndrome

<table>
<thead>
<tr>
<th></th>
<th>Autosomal Recessive Kindred</th>
<th>OSMED Syndrome</th>
<th>Autosomal Dominant Kindred</th>
<th>Classical Stickler Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>High myopia and vitreoretinal degeneration</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Severe</td>
</tr>
<tr>
<td>Epiphyseal dysplasia and osteoarthritis</td>
<td>Severe</td>
<td>Mild</td>
<td>Mild</td>
<td>Mild</td>
</tr>
<tr>
<td>Vertebral involvement</td>
<td>Mild</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Absent/mild</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>Moderate/severe</td>
<td>Mild</td>
<td>Mild</td>
<td>Mild</td>
</tr>
<tr>
<td>Cleft palate (%)</td>
<td>Absent (0 of 3)</td>
<td>&gt;50</td>
<td>26 (4 of 16)</td>
<td>&lt;35</td>
</tr>
<tr>
<td>Midface hypoplasia and upturned nose</td>
<td>Yes</td>
<td>Yes</td>
<td>autosomal recessive</td>
<td>autosomal dominant</td>
</tr>
<tr>
<td>Inheritance pattern</td>
<td>autosomal recessive</td>
<td></td>
<td>autosomal recessive</td>
<td>autosomal recessive</td>
</tr>
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were obtained, however, when the consanguinity loop was taken into account. Since for most of these markers allele frequencies are not available from the Dutch population and the frequency of the abnormal allele is also unknown, the lod score calculations were performed with a range of parametric values. Conservative estimates of 0.002 for the abnormal allele and 0.005 for the marker haplotype yielded a lod score of 3.09 at \( \Theta = 0.0 \).

Incorporation of COL11A2 into the Human Linkage Map

Pairwise linkage analyses for COL11A2 and loci on human chromosome 6 were performed to place the COL11A2 gene into the human linkage map. As a marker for the COL11A2 gene, we used an intragenic SSCP. Zero recombination was found between COL11A2 and two loci, D6S291 (Z = 3.01) and D6S29 (Z = 7.65). The results of multipoint analysis are shown in Figure 5; the most favored order is [HSPA1-D6S273]-[COL11A2-D6S29-D6S291-D6S439]-GL01. This order is consistent with the recent consensus map of chromosome 6 (Volz et al., 1994) and the second-generation Genethon map (Gyapay et al., 1994). It should be noted that the relationship between D6S291 and COL11A2 is based on only 10 meioses within three Centre d'Études du Polymorphisme Humain (CEPH) pedigrees informative for both loci.

In the family with recessive osteochondrodysplasia, patient number 6 has a recombination between D6S273 and D6S291 (see Figure 4A). Since we did not observe any recombinations between the COL11A2 gene and the D6S291 marker in the three informative CEPH families, we wanted to confirm that the gene is within the inherited haplotype. We have recently identified a novel polymorphism within the COL11A2 gene that creates an Mspl restriction site. A 180 bp genomic fragment containing this polymorphism was amplified from all family members. This identified a G to A transition, converting a glycyl to an arginyl codon, within the triple-helical domain of \( \alpha_2(\text{XI}) \) collagen. This change in sequence eliminated an Mspl restriction site within the genomic sequence. To demonstrate that this sequence change was present in all the affected individuals, genomic DNAs of all family members were PCR amplified, and the products were electrophoresed through a 4% agarose gel after digestion with Mspl (see Figure 4A). The affected children showed the absence of the Mspl site. A similar analysis with DNA from 63 unrelated parents (corresponding to 126 chromosomes) of CEPH families showed the presence of this Mspl site in all samples (data not shown), thus excluding the possibility.
that the G to A sequence change represented a common polymorphism. Finally, cycle sequencing of these genomic PCR products demonstrated that affected children are homozygous for the arginyl codon, while unaffected children are homozygous for the glycyl codon; both parents are heterozygous for the sequence change (see Figure 4B).

Discussion

We present evidence that the COL11A2 gene is associated with both autosomal dominant and autosomal recessive human osteochondrodysplasias. These data suggest that collagen XI is essential for normal skeletal development, in agreement with the conclusions of Li et al. (1995) in their accompanying paper.

A Mutation in COL11A2 Causes Autosomal Dominant Stickler Syndrome without Eye Involvement

We have identified a G to A transition at a splice donor site within the COL11A2 gene, which cosegregates with an autosomal dominant Stickler syndrome phenotype in a large family. In vitro splicing experiments have previously demonstrated the importance of the five conserved nucleotides at the splice donor site for efficient and correct splicing of mRNA (Talorico and Berget, 1990), and similar mutations in \( \alpha(1)(I) \) and \( \alpha(2)(I) \) collagen genes have been implicated in other osteochondrodysplasias (reviewed by Kuivanen et al., 1991). The present mutation causes in-frame skipping of a 54 bp exon, encoding 18 amino acid residues within the triple helical domain of the \( \alpha(2)(XI) \) collagen molecule (Figure 1). Approximately 50% of the COL11A2 mRNA transcripts recoverable by RT-PCR from patient chondrocytes and EBV-transformed lymphoblasts contain the deleted exon (data not shown), suggesting that the mutation does not significantly affect mRNA stability. The mutated polypeptide chain may therefore be synthesized at a level comparable to that of the wild-type chain. Mutant \( \alpha(2)(XI) \) chains would be 18 amino acid residues shorter than wild-type chains, but would contain the intact sequence of the carboxy-terminal propeptide domain. In fibrillar procollagen molecules, including collagen XI, chain association during trimer assembly initiates at the carboxy-terminal propeptide domain (Dolz and Engel, 1990). Therefore, mutant \( \alpha(2)(XI) \) chains are likely to associate with \( \alpha(1)(XI) \) and \( \alpha(3)(XI) \) chains during trimer assembly. Once associated, however, the 18 amino acid deletion within the triple-helical domain is likely to interfere with normal triple helix formation. Whether the phenotypic effect of this mutation is due to the rapid degradation of abnormally folded heterotrimers causing a deficiency of collagen XI or whether it is a consequence of copolymerization of abnormal molecules with normal collagen II, IX, and XI molecules in cartilage collagen fibrils requires further study.

Mutations affecting another collagen gene, COL2A1, have previously been identified in several families affected by Stickler syndrome having eye involvement, but genetic heterogeneity has been observed in other families (reviewed by Vikkula et al., 1994). Snead et al. (1994) have recently extended this observation by linking COL2A1 in 20 additional families with eye involvement and excluding COL2A1 in four families lacking congenital vitreous anomaly. Our results suggest that COL11A2 is the likely candidate for these unlinked families.

Interestingly, although COL2A1 mutations can result in a spectrum of osteochondrodysplasia phenotypes (reviewed by Vikkula et al., 1994), only haploinsufficiency mutations (i.e., premature stop codons) have been identified in the COL2A1-linked Stickler syndrome families. Since type II collagen accounts for 90% of total cartilage collagen, it is not surprising that haploinsufficiency could have a phenotypic consequence. Whether haploinsufficiency mutations affecting collagen XI, which comprises less than 10% of total cartilage collagen, have a similar effect cannot yet be determined.

A COL11A2 Mutation Is Also the Cause of the Autosomal Recessive Phenotype

The three affected patients in the consanguineous family with autosomal recessive osteochondrodysplasia appear homozygous by descent for an interval defined by seven chromosome 6p21 CA repeat markers, within which we have mapped the COL11A2 gene (Figure 4A). Using conservative estimates for mutant allele frequency (0.002) and extended haplotype frequency (0.005), a lod score of 3.09 with the COL11A2 locus was obtained.

The phenotype of the affected members in this family, although more severe, resembles that of the family with the dominantly inherited COL11A2 splice site mutation (Table 1). It also shares similarities with the OSMED syndrome (Giedion et al., 1982), which itself shares radiographic features with Stickler syndrome (Spranger, 1985). The clinical similarity among these disorders and the evidence of linkage to the COL11A2 locus in our family strongly suggested that a mutation in COL11A2 was responsible for the autosomal recessive phenotype as well.

The COL11A2 mutation causing the autosomal recessive phenotype is likely to affect the stability of heterotrimers collagen XI molecules since it changes a glycine residue in a Gly-X-Y triplet to arginine. The location of the mutation within the triple-helical domain of \( \alpha(2)(XI) \) collagen suggests that it creates, like the mutation in the autosomal dominant Stickler syndrome, a mutant polypeptide capable of participating in trimer assembly. This raises the question of why heterozygous "carrier" parents for the glycinine arginine mutation are asymptomatic, while the heterozygotes for the exon-skipping mutation show a clinical abnormality. We suggest that a possible reason for this difference is that substituting arginine for glycine close to the amino terminus of collagen XI molecules may still allow incorporation of mutated molecules into cartilage fibrils and therefore may allow some residual function, while a large in-frame deletion close to the carboxyl end of the molecule causes a complete disruption of triple-helical folding and function. Supporting evidence for this comes from mice heterozygous for the cho mutation (a functional null allele in Col11a1) that are asymptomatic, suggesting that a reduction in the level of functional collagen XI may not be clinically apparent. In contrast, however, homozy-
gosity for the cho mutation, leading to complete deficiency of Col11a1, has profound phenotypic consequences (Li et al., 1995).

In the mice, homozygosity for a functional null mutation in Col11a1 results in a perinatal lethal chondrodysplasia. A partial loss of function would explain why the recessive mutation in Col11a2 is not likewise lethal. In addition, there is a difference in utilization of α1(XI) and α2(XI) chains within type XI collagen heterotrimeric molecules.

In mammalian vitreous, the COL5A2 gene product, α2(V), replaces α2(XI), forming a collagen V/XI hybrid molecule (Mayne et al., 1993). This most likely accounts for the lack of eye involvement associated with this syndrome, except for minor adjustments of annealing temperature and extension times, as needed.

The PCR products were sequenced by cycle sequencing. Sequencing primers were as follows: COL11A2-2, COL11A2-6, COL11A2-17, 9/13/22, 13/12/27, 14/15, and 1/6; for the second round, the PCR-nested primers were 23/33 (10/13-PCR used as a template). Amplification reactions and conditions were essentially the same as for the analysis of the dominant syndrome, except for minor adjustments of annealing temperature and extension times, as needed.

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uct was Nlalll digested and analyzed on a 5% denaturing polyacryl-

Linkage Analysis In the Family with Autosomal Recessive Osteochondrodysplasia

Genomic DNA was isolated from peripheral blood. To define the COL11A2 locus in 6p21.3 (Kimura et al., 1989), we used CA repeat polymorphisms from 10 loci that are in the same or adjoining chromosome subband. PCR amplification of genomic DNA was used to ana-

lyze the marker loci using [a-32P]dCTP in order to label the amplified DNA. Allelic bands were separated on a 6.6% denaturing polyacryl-

SSC and Mpl Mapl Polymorphisms within the COL11A2 Gene

The primer pair consisting of COL11A2-1 and COL11A2-2 was used to amplify an 898 bp genomic DNA fragment containing a 583 bp intron. Cycling conditions were 30 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 1 min, with a min 72°C final extension. This primer was used to incorporate the COL11A2 gene in the CEPH linkage map (Figure 5). Of 38 CEPH pedigrees, 12 were informative for the COL11A2 SSC polymorphism. Pairwise and multistage linkage analyses were performed between COL11A2 and loci on human chromosome 6 (CEPH Database, version 7.0) using version 5.10 of the LINKAGE programs supplied by Dr. J. Ott (Lathrop et al., 1984). Allele frequencies were determined by gancotyping 31 CEPH grandparents. Sex-specific recombination rates for males and females were set to be equal (θm = θf) for the pair-

Another polymorphism, detected during SSCP and heteroduplex analysis, created a novel Mapl restriction enzyme cutting site in the COL11A2 cDNA. Two primers were synthesized to amplify the region containing this polymorphism from genomic DNA: COL11A2-36 (antisense), 5'-TGATAGCCACACTGCGC (nucleotides 3438-3417) and COL11A2-37 (sense), 5'-TGTGGGACACGGTGGAGCAC (nucleotides 3308-3327). The PCR program used contained 30 cycles with 94°C for 30 s, 64°C for 30 s, and 72°C for 60 s, with a final exten-

SSP analysis of the full-length product identified a 3-allele polymor-

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