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Molecular Heterogeneity in the Mild Autosomal Dominant Forms of Osteogenesis Imperfecta

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SUMMARY

Mild osteogenesis imperfecta (OI type I and OI type IV) is characterized by postnatal onset of fractures, absence of skeletal deformity, presenile hearing loss with or without blue sclerae, and dentinogenesis imperfecta. Using one common DNA polymorphism associated with the proα2(I) human collagen gene, we found genetic heterogeneity in this disorder. In three families, the OI phenotype segregated independently of the DNA polymorphism, whereas in one family, the OI phenotype cosegregated with a DNA polymorphism in a manner suggesting linkage. Use of DNA polymorphisms associated with both type I procollagen genes should provide a tool to unravel the molecular heterogeneity of various heritable disorders of the connective tissue.

INTRODUCTION

Osteogenesis imperfecta (OI) is a highly heterogeneous group of heritable systemic disorders of the connective tissues [1]. Over the past several years, conclusive data have been developed correlating certain clinical phenotypes with specific

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structural defects of the proα chains of type I procollagen [2]. Recently, two lethal perinatal variants of OI (OI type II) [3] have been characterized at the molecular level as proα1(I) [4, 5] and proα2(I) collagen gene mutations (W. J. de Wet and F. Ramirez, personal communication, 1983). In the milder forms, biochemical studies have suggested alteration in synthesis of the proα1(I) chains (OI type I) [6], while linkage studies have pointed to defects in the proα2(I) collagen gene (OI type IV) [7].

To clarify these entities, we examined several families with mild variants of OI (clinically OI type I and OI type IV) to determine whether they are linked to the same gene.

The availability of specific DNA probes and their use in establishing restriction fragment length polymorphisms (RFLPs) have proved to be useful tools in the characterization of heterogeneous monogenic disorders [8–14]. Using recombinant DNA techniques, we isolated the proα1(I) and proα2(I) collagen genes [15, 16] and also identified an RFLP within the proα2(I) gene [7].

We report here on another RFLP associated with the proα2(I) gene and the use of the two molecular markers for linkage studies in six families with the mild form of autosomal dominant OI. Our data strongly suggest that several different gene defects are the cause in this form of OI.

MATERIALS AND METHODS

Subjects

Affected and nonaffected individuals from six families with mild dominant OI were evaluated (fig. 1). The clinical phenotypes of families A–D are shown in table 1. The

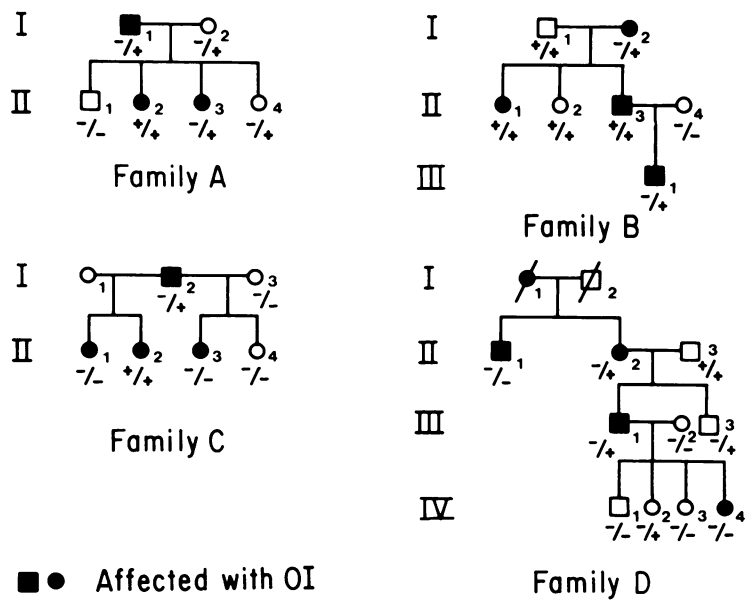

FIG. 1.—Pedigrees of four families with an autosomal dominant form of OI. *Symbols:* Individuals heterozygotes (−/+) and homozygotes for the absence (−/−) and presence (+/+) of the polymorphic *Eco*RI site.

TABLE 1

CLINICAL PHENOTYPES

	Onset	Short stature	Hearing loss	Dentinogenesis imperfecta	Scleral hue	Lod score at θ = .05
Family A	Postnatal	+	+	−	White	0.26
Family B	Postnatal	+	+	−	Blue	−0.72
Family C	Postnatal	−	+	−	Blue	−0.98
Family D	Postnatal	−	+	−	Blue	−2.60
Family E*	Postnatal	+	+	+	White	2.21

* Family reported in [7].

genotypes for the *Eco*RI and *Msp*I RFLPs were determined in all available individuals from the six families. Two families were not informative for linkage studies, due to lack of heterozygosity for both the *Eco*RI and the *Msp*I RFLPs in affected individuals, and they are not included in figure 1 and table 1. Families A–D (fig. 1) were not informative when tested for the *Msp*I RFLP because several of the affected individuals were homozygous for the marker.

The genotypes for the *Eco*RI and *Msp*I RFLP were also determined in two groups of randomly selected individuals with a negative family history for a heritable disorder of the connective tissue. The U.S. group consisted of 48 individuals studied for both the *Eco*RI and *Msp*I RFLPs, while the Norwegian group consisted of 46 individuals studied for the *Msp*I RFLP only.

Restriction Endonuclease Analysis of Genomic DNA

Nuclear DNA was isolated from the leukocytes contained in 10–15 ml of EDTA-anticoagulated blood. Ten to 15 μg of DNA were digested to completion under conditions recommended by the commercial supplier. Digested DNA and appropriate DNA size markers were separated by electrophoresis in 0.6 or 1.0% (w/v) agarose gels. The DNA fragments were transferred to nitrocellulose filters [17] and hybridized with the human proα2(I) probes for 24–48 hrs as described [7]. The filters were then washed for 10 min at 68°C with each of the following solutions: 2 × SSC, 1 × SSC, 0.5 × SSC, and 0.1 × SSC. The probes used in these experiments were labeled to a specific activity of $2–5 \times 10^8$ cpm/μg by nick-translation.

DNA Probes for the Human proα2(I) Gene

The genomic probes used in these experiments are shown in figure 2. The *Eco*RI RFLP specific probe consisted of 6.75 kilobases (kb) of genomic DNA extending downstream from the codon of amino acid residue 19 of the proα2(I) chain [15]. The *Msp*I RFLP specific probe consisted of 4.1 kb of genomic DNA containing coding sequences for the triple helical domain and the C-propeptide of the proα2(I) chain [15].

Nomenclature

The recommendations of the Seventh International Workshop on Human Gene Mapping were followed in naming the alleles generated by the presence or absence of the restriction endonucleases *Eco*RI and *Msp*I [18]. Thus, the allele generated by the presence of the *Eco*RI site is designated as *A1*, and the one generated by the absence of it as *A2*. Accordingly, we designated as *B1* and *B2* the two alleles in which the *Msp*I site was present or absent, respectively.

Linkage Analysis

Lod scores were calculated at various recombination fractions using the computer program LIPED [19]. A lod score of 3 is generally considered strong evidence for linkage, while a lod score of −2 is considered strong evidence against it.

RESULTS

Polymorphic Restriction Sites in the proα2(I) Gene

Nuclear DNA from 94 randomly selected individuals was cleaved with the restriction endonuclease *Msp*I. A polymorphic site was detected after hybridization with Hf-32, a proα2(I) cDNA probe [20]. Individuals homozygous for the presence of the polymorphic site (*B1,B1*) generated two fragments of 1.6 and 0.5 kb. DNA of individuals homozygous for the absence of the polymorphic site (*B2,B2*) generated only one fragment of 2.1 kb, whereas DNA from heterozygotes (*B1,B2*) generated three fragments of 2.1, 1.6, and 0.5 kb, respectively. In addition, three more fragments of 6.2, 4.4, and 0.8 kb were invariably observed in all individuals. Thus, we identified three different genotypes. Segregation analysis in two families showed that the polymorphic site segregated as an autosomal codominant trait (fig. 3). A total of 188 chromosomes from unrelated healthy individuals, consisting of the U.S. and Norwegian groups, were examined. No frequency differences were observed in the two groups. The allelic frequencies of the pooled population were .86 for the presence of the *Msp*I restriction site (*B1*) and .14 for the absence of the site (*B2*). The frequencies of the three genotypes generated by the *Msp*I restriction endonuclease were compatible with Hardy-Weinberg equilibrium.

We then proceeded to map the exact location of the polymorphic site within the proα2(I) gene. We used the various genomic subclones covered by the cDNA probe Hf-32. It was found that the *Msp*I RFLP was generated by the 4.1 kb *Eco*RI subclone of the genomic clone NJ-1 (fig. 2). DNA sequencing of this region of the gene indicated that the polymorphic site is in intron VI of the proα2(I) gene. This RFLP is most likely generated by a single base change since no size variations were observed in fragments generated by other restriction endonucleases that cleave in the same region of the gene.

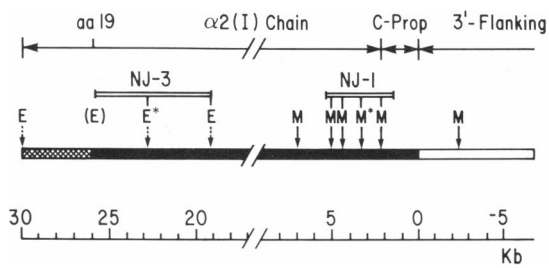

FIG. 2.—Schematic map of the proα2(I) gene of human type I procollagen and the DNA probes employed here. The 5′-boundary of the gene has not been precisely defined but is about 38 kb from the 3′-end [15]. *E* and *M* indicate *Eco*RI and *Msp*I sites, respectively. The two polymorphic sites studied here are indicated by *the asterisks*. (*E*) indicates an artificial *Eco*RI site in the genomic probe.

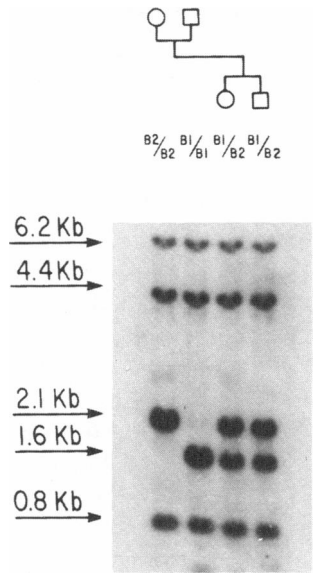

FIG. 3.—The *Msp*I RFLP segregates as an autosomal codominant trait as observed in this pedigree. The 0.5-kb band is not seen since DNA fragments of that size do not always transfer on nitrocellulose filters.

We previously reported an *Eco*RI RFLP associated with the proα2(I) human collagen gene [7]. Individuals homozygous for the presence of the *Eco*RI recognition site (*A1,A1*) generate two fragments of 9.5 and 3.5 kb in size; homozygotes for the absence of the *Eco*RI recognition site (*A2,A2*) generate one fragment of 13.0 kb; and heterozygotes (*A1,A2*) generate three fragments 13.0, 9.5, and 3.5 kb in size after hybridization with the *Eco*RI genomic subclone of NJ-3 (fig. 2). The allelic frequency for the presence of the *Eco*RI recognition site (*A1*) is .38, while the frequency for the absence of it (*A2*) is .62 [7]. We analyzed the genotypes of 48 individuals from the U.S. group for both markers in order to determine whether the two RFLPs were in linkage disequilibrium. Out of the nine expected combinations of genotypes, we observed six. A significant excess of individuals with the *A2A2B1B1* genotype was observed as well as a deficit of individuals with the *A1A2B1B1* genotype ($P < .01$) (table 2).

Linkage Analysis in Families with OI

In family A (fig. 1), the affected individual I-1 was heterozygous for the presence of the *Eco*RI polymorphic site. The same is true for his normal spouse (I-2). In generation II, the two homozygous offspring II-1 and II-2 give information about which alleles both parents contributed. The OI phenotype cosegregated with the *A2* allele. The lod score in this family was 0.26 at a recombination fraction of $\hat{\theta} = .05$. In families B, C, and D (fig. 1), the OI phenotype segregated independently of the *Eco*RI restriction polymorphic marker. The cumulative lod score from

these three families at a recombination fraction of $\hat{\theta} = .05$ was -4.3 (table 1), a value that excluded tight linkage. Family E (table 1) was previously reported [7]. The lod score obtained at a recombination fraction of $\hat{\theta} = .05$ was 2.21.

DISCUSSION

Osteogenesis imperfecta is a group of genetic disorders characterized by bone fragility and other systemic manifestations of the connective tissue. These disorders can be classified in at least four different groups based on the clinical phenotype and mode of inheritance [3]. One variety (OI type II) presents with multiple in utero fractures leading to death at birth or soon after. Another variety of intermediate severity (OI type III) with multiple fractures, short stature, and skeletal deformity has been distinguished by some investigators. Autosomal dominant OI (OI type I and OI type IV) is characterized by postnatal onset of fractures, mild or absent skeletal deformity, and presenile hearing loss. It can be further divided in subgroups based on the presence or absence of blue sclerae and dentinogenesis imperfecta [21, 22]. The apparent clinical heterogeneity of the autosomal dominant OI could be further clarified with genetic linkage studies.

A high-frequency RFLP has been found within the proα2(I) human collagen gene [7]. We used that *Eco*RI RFLP to study four families with mild dominant forms of OI. Our data ruled out linkage in three families (B, C, and D) (fig. 1). Linkage of the OI phenotype to the proα2(I) collagen gene RFLP is compatible in family A (fig. 1) as in the previously reported family E (table 1). The data from all five families support the idea that mild autosomal dominant OI is heterogeneous at the gene level. The affected individuals in the three families where linkage was excluded had blue sclerae, while in families A and E, the scleral hue was white. These data confirm the suggestion made by Sillence et al. [3] that scleral hue can be used to segregate biochemically distinguishable forms of OI. It is probably an oversimplification to say that blue sclerae are found only in proα1(I) collagen gene defects [6] because at least one patient with blue sclerae and a deletion in one allelic product of the proα2(I) collagen gene has been described [23].

The availability of more RFLPs associated with the genes for type I procollagen will increase the number of families with dominant OI syndromes that can be studied. Here, we report on another proα2(I) gene RFLP. This second marker is

TABLE 2

OBSERVED NO. INDIVIDUALS WITH DIFFERENT GENOTYPES FOR THE TWO RFLPS ASSOCIATED WITH THE proα2(I) COLLAGEN GENE

	A1A1	*A1A2*	*A2A2*	Total
B1B1	3 (6.89)	10 (11.49)	23 (17.62)	36
B1B2	5 (1.92)	5 (3.19)	0 (4.89)	10
B2B2	1 (0.19)	0 (0.32)	0 (0.49)	1
Total	9	15	23	47

NOTE: $\chi^2 = 19.14$; $P < .01$. Expected no. if the two markers were in linkage equilibrium is in parentheses.

also transmitted as an autosomal codominant trait. The two markers, *Eco*RI RFLP and *Msp*I RFLP may be in linkage disequilibrium. We observed six of the nine different combinations of genotypes for both markers after studying 48 randomly selected individuals. A larger sample size will be needed in order to establish if the two markers are in linkage equilibrium or disequilibrium.

When additional high-frequency RFLPs associated with type I procollagen genes as well as other collagen genes become available, they should be useful for systematically defining the molecular basis of a variety of monogenic disorders of the extracellular matrix and make prenatal diagnosis feasible in some.

NOTE ADDED IN PROOF: In the course of this work, we learned that the *Msp*I RFLP was also observed by Dr. Chris Mathew, University of Stellenbosch, South Africa.

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