

Clinical variability of osteogenesis imperfecta linked to *COL1A2* and associated with a structural defect in the type I collagen molecule

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SUMMARY We report a family in which dominant osteogenesis imperfecta segregates with a *COL1A2* haplotype and is associated with a structural defect in the helical region of the type I procollagen molecule. All affected subjects had short stature, dentinogenesis imperfecta, and myopia; however, great differences were observed in the number of fractures and in the degree of bone deformity. Identical biochemical changes were found in the type I collagen molecules synthesised by fibroblasts of subjects with severe or minimal bone fragility. These results confirm that mutations in the triple helical region of $\alpha 2(I)$ chains produce a milder phenotype than analogous mutations in the $\alpha 1(I)$ chains, but indicate that, in addition to defects in the type I collagen molecule, other factors may modulate the degree of bone involvement in osteogenesis imperfecta.

In recent years, there have been significant advances in our understanding of the genetic basis of osteogenesis imperfecta (OI). Most cases are caused by mutations at one of the two loci (*COL1A1* and *COL1A2*) which code for the $\alpha 1$ and $\alpha 2$ chains of type I collagen,¹⁻³ although a severe form of OI unlinked to the collagen genes is known to exist.⁴ Since these mutations affect the synthesis, structure, and secretion of type I collagen, the predominant protein in bone tissue, the causal relationship between type I collagen defects and bone fragility seems straightforward. However, while this is certainly true for the more severe cases, it may be less so for the milder cases, since in a few instances the phenotypic expression of defects in type I collagen has been found to be very variable.⁵⁻⁷ Here, we describe a family with dominant osteogenesis imperfecta associated with a structural defect in the type I procollagen molecule. While all affected members had short stature and dentinogenesis imperfecta, the degree of bone fragility showed marked variability.

Methods

BIOCHEMICAL STUDIES

Skin biopsies were obtained from subjects IV.1,

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III.2, I.3, II.3, and II.4 (fig 1), and fibroblast cultures were prepared by standard methods. Cultures from controls without connective tissue disease were studied in parallel. Radiolabelling of the fibroblast cultures with (2,3-³H)proline in the presence of ascorbate, harvesting of procollagens from medium and cell layer, and purification of collagens with pepsin were performed as described previously.⁸ The thermal stability of the pepsin

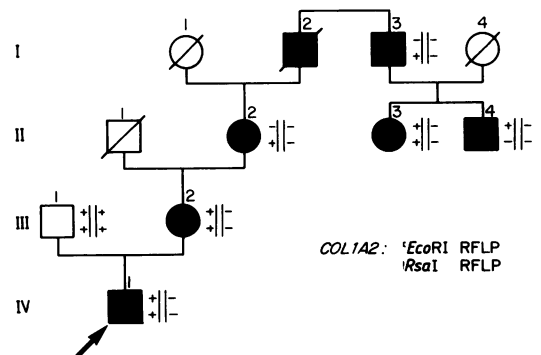


FIG 1 Partial pedigree of the family with osteogenesis imperfecta described in the text. Black symbols denote affected subjects. The *COL1A2* haplotypes are also shown (see fig 2).

purified collagens was determined using trypsin as a probe for triple helical conformation.⁹ Separation of the collagens by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and two dimensional mapping of cyanogen bromide derived peptides (CB peptides) was performed as described previously.⁸

GENETIC STUDIES

Extraction of genomic DNA from peripheral blood leucocytes, digestion of DNA with restriction enzymes, electrophoresis of fragments in agarose gels, Southern blotting of fragments onto nylon membranes, and hybridisation of membranes were performed using standard techniques.¹⁰ The membranes were probed with either Hp-2010, a plasmid clone containing the full length cDNA for the pro α 2(I) chain, which recognises several intragenic RFLPs¹¹ (courtesy of Dr Helena Kuivaniemi, Philadelphia), or with a *Bam*HI-*Xho*I genomic subclone from the 5' end of the *COL1A1* gene, which recognises an intragenic *Rsa*I RFLP² (courtesy

of Dr F Ramirez, New York). The probes were labelled with ³²P by nick translation.

Results

CASE REPORTS (FIG 2)

IV.1, the proband, was referred to us at two months of age because of a fracture of the left femur. At the age of 19 months, he had suffered four femoral fractures. He was short (below the 3rd centile for age) and his femora and tibiae were bowed laterally and anteriorly. His face was triangular and his sclerae were blue.

III.2 was the 28 year old mother of the proband. At the age of two years, she suffered a fracture of the ulna after a fall, her only fracture. Her adult height was 152 cm, her sclerae were light blue, and she was shortsighted; she did not have a triangular face. She had lost several teeth and those remaining were yellowish in colour, suggesting dentinogenesis imperfecta. Wormian bones were seen on radiological examination of the skull. She worked as a

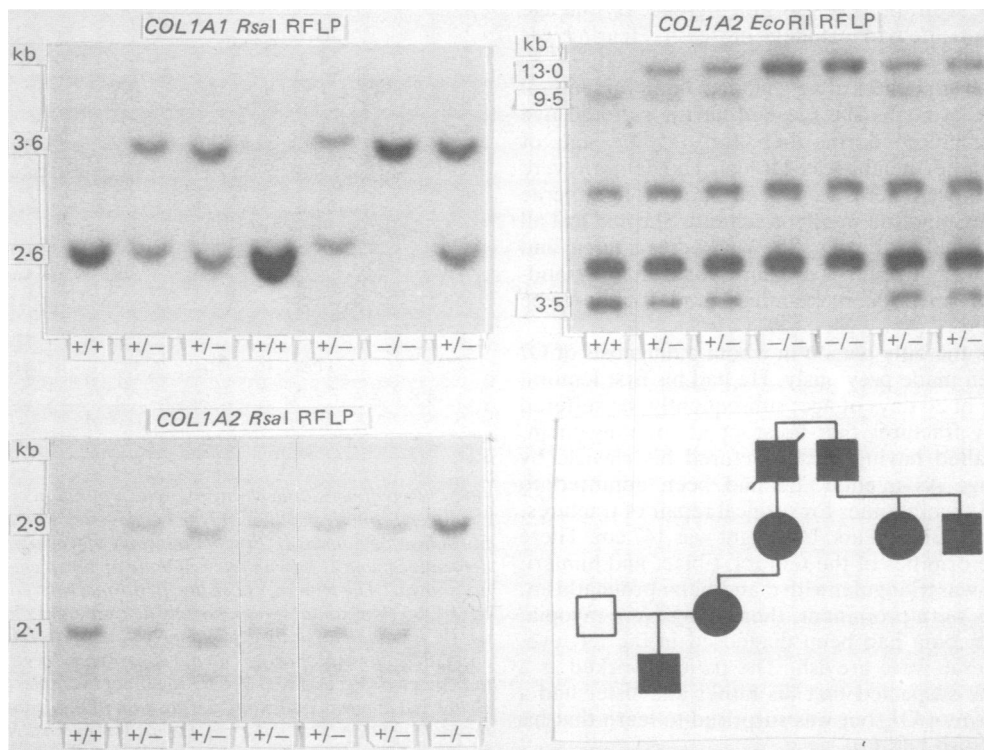


FIG 2 Southern blots showing the segregation of a *COL1A1* RFLP and two *COL1A2* RFLPs in the OI family described. There is discordant segregation between OI and the *COL1A1* *Rsa*I RFLP, and cosegregation between OI and the *Eco*RI and *Rsa*I '—' alleles.

housewife, had had no major medical problems, and did not know she was affected with OI.

II.2 was the grandmother of the proband. At the age of 66 years, she was 145 cm tall and suffered from backaches; however, she had never had a fracture. The face was not suggestive of OI. Her sclerae were only slightly bluish but there was severe myopia. Mild hypoacusis had developed in recent years. She recalled having lost most of her teeth in her twenties. She had worked lifelong as a mailwoman, did not have major medical problems, and did not know she had osteogenesis imperfecta.

Subject I.2 (dead) was small (about 145 cm) and was said to resemble his affected brother I.3. He had been a shoemaker.

I.3, the brother of I.2, was a mason. His height at the time of military service was 159 cm; he had suffered five fractures during his life (femora, clavicle, and radius). When we saw him aged 82 years, he was confined to a wheelchair because of severe osteoporosis. The femora were slightly bowed laterally. His face was triangular and there was mandibular prognathism. His eyes were very prominent, the sclerae were greyish, and an arcus senilis could be seen; he was also shortsighted. By the age of 30 years, he had lost all of his teeth. Hearing was only mildly impaired.

II.3, the daughter of I.3, had had bilateral femoral fractures at birth. She recalled having suffered five other fractures during her life. At the age of 48 years, her height was 146 cm. Her femora were slightly bowed, her eyes were prominent, her sclerae were grey, and she was shortsighted. She had lost all her teeth in her thirties. She worked as a nurse and complained of backaches. Her daughter and granddaughter have been examined and found to be normal.

II.4 is the only person in whom a diagnosis of OI had been made previously. He had his first femoral fracture at 20 days of age; subsequently, he suffered so many fractures that he stopped counting them. He recalled having once fractured his clavicle by coughing. As a child, he had been admitted to hospital several times for surgical repair of fractures. At the age of 42 years, his height was 147 cm. There were deformities of the femora, tibiae, and humeri, the face was triangular with mandibular prognathism, the eyes were prominent, there was severe myopia, and glaucoma had been diagnosed in the left eye. The sclerae were greyish. The patient worked as a clerk; he suspected that his father and sister had a mild form of OI, but was surprised to learn that his cousins also had OI.

SEGREGATION ANALYSIS

Two informative RFLPs (*EcoRI* and *RsaI*) at the

COLIA2 locus were detected with Hp-2010 and were used to construct haplotypes. One haplotype was found to segregate with the disease; no recombination was observed in three fully and two partially informative meioses. In contrast, discordant segregation was found between the *RsaI* RFLP within the *COLIA1* gene and the disease. These findings, together with the biochemical and clinical data, indicate that the mutation responsible for OI in this family was in a *COLIA2* allele.

BIOCHEMICAL ANALYSIS

The population of slow migrating $\alpha 1(I)$ and $\alpha 2(I)$ chains previously found in fibroblasts from some family members¹² was found to be present in fibroblast cultures from all biopsied members of this family, thus confirming the existence of a common collagen defect. The slow migrating chains were not observed after labelling of the cells at 32°C, rather than at 37°C, as previously reported,¹² indicating

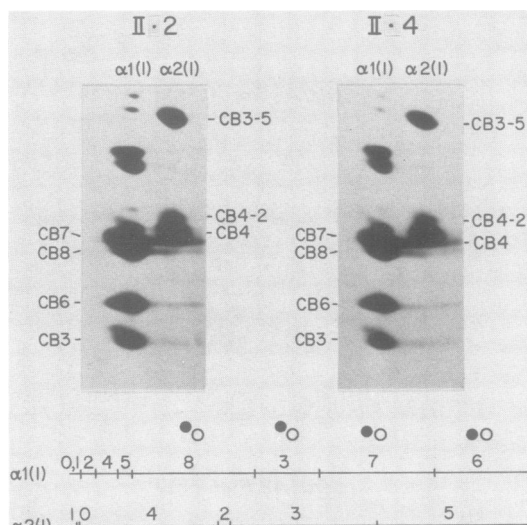


FIG 3 Two dimensional electrophoretic separation of cyanogen bromide peptides of type I collagen synthesised by fibroblasts of subjects II.2 (with no history of fractures or deformities) and II.4 (with marked bone involvement) (IV.1 not shown.) The pattern, consisting of normal migration of $\alpha 1(I)$ CB6 (horizontal spots), slightly delayed migration of $\alpha 1(I)$ CB7, and markedly delayed migration of $\alpha 1(I)$ CB3 and CB8 (diagonal spots), is identical in both subjects and indicates a defect in the $\alpha 1(I)$ CB7 region of the molecule. Below is a diagram indicating the position of the cyanogen bromide peptides within the $\alpha 1(I)$ and $\alpha 2(I)$ chains. Note that the $\alpha 1(I)$ peptides are used to localise the defect within the molecule because of their easier electrophoretic separation, in spite of the mutation being in the $\alpha 2(I)$ chains.

that the increased molecular weight was the result of increased post-translational modification and not of a peptidyl insertion.

To elucidate the relationship between the clinical changes and the defect in the type I collagen molecule, cells from one mildly affected subject (II.2) and from two severely affected subjects (II.4 and IV.1) were chosen for further studies. Two dimensional analysis of the CB peptides of type I collagen (fig 3) showed that, in all three cell lines, the defect mapped to the $\alpha 1(I)CB7$ region of the collagen molecule, as had been previously found in collagen from IV.1.¹² The melting temperature of the type I collagen molecules was also found to be identical in all three cell strains: 39.5°C for the overmodified molecules and 41°C for their normal counterpart. Thus, type I collagen from severe and mild cases appeared to contain the same mutation, which caused identical changes in structure and stability of the molecule.

Discussion

In the family described, osteogenesis imperfecta is transmitted as an autosomal dominant trait. In the more severely affected subjects (I.3, II.3, II.4, and IV.1) the phenotype can be classified as OI type IV after Silience *et al.*¹³ However, while some clinical features were invariably observed in affected family members (short stature, dentinogenesis imperfecta with premature loss of teeth, and, apparently, myopia), the degree of bone involvement was very variable. Thus, the extraosseous manifestations of osteogenesis imperfecta were, paradoxically, more accurate indicators of the disease than bone fragility itself.

Osteogenesis imperfecta is associated, in this family, with a structural alteration in the $\alpha 1(I)CB7$ region of the type I procollagen molecule, which causes a delay in the folding of pro α chains into the triple helical conformation and lowers the stability of the molecule.¹² The most likely explanation for these findings is a substitution of a glycine residue in some of the pro α chains of the type I procollagen molecule^{1,3}; however, the biochemical data do not indicate whether the substitution has occurred in pro $\alpha 1(I)$ or pro $\alpha 2(I)$ chains. Since both the clinical and the biochemical changes are milder than those produced by glycine substitutions in the CB7 region of the pro $\alpha 1(I)$ chains,³ a substitution in the pro $\alpha 2(I)$ chains seems more likely. In agreement with this, cosegregation of the disease with a COL1A2 haplotype, and discordant segregation with a COL1A1 RFLP, were observed, indicating that the mutation was in a COL1A2 allele. These findings confirm recent results which suggested that OI type IV is

often caused by mutations within the triple helix coding region of the pro $\alpha 2(I)$ gene, COL1A2.¹⁴⁻¹⁷

In dominant osteogenesis imperfecta, variability in clinical expression between affected members of the same family is a well known phenomenon.¹⁸ However, the biochemical and genetic basis for this variability has rarely been investigated. De Vries and De Wet⁵ described a family in which dominant osteogenesis imperfecta was caused by a glycine-cysteine substitution in the $\alpha 1(I)$ chains of type I collagen ($\alpha 1(I)$)¹⁷⁵ GLY→CYS(W De Wet, Potchefstrom, 1988, personal communication); the phenotype was very variable. Sippola *et al.*⁶ found shortened $\alpha 2(I)$ chains in the fibroblasts of a boy with osteogenesis imperfecta and signs of the Ehlers-Danlos syndrome; subsequently, the same alteration was found in his asymptomatic mother.⁷ Byers *et al.*¹ found the same molecular defect in a newborn with lethal OI and in his father, affected by OI type IV. In the attempt to find a biochemical correlate with the variability in phenotype, we have not only mapped the structural defect within the type I collagen molecule in different family members, but also tested the stability of the molecule to look for the presence of other, unidentified mutations with an aggravating or protective effect. The results showed that the type I collagen from mildly and from severely affected subjects contained the same defect and had the same thermal stability and thus could not be distinguished by these biochemical criteria. Although our studies do not completely rule out the presence of other subtle mutations in the molecule which we were not able to detect, the data suggest that polymorphisms in other components of the bone matrix may have an aggravating or compensating effect, which is superimposed on the defective type I collagen to modulate the phenotype.

These results have implications for clinical practice. Specifically, a negative history for bone fractures and deformities does not rule out osteogenesis imperfecta; some of the other clinical signs, like dentinogenesis imperfecta, short stature, and, at least in this family, myopia, may suggest the diagnosis. Biochemical and genetic studies may yield valuable information and should be performed whenever possible. Moreover, the variability in clinical expression makes it difficult to give an accurate prognosis for an infant and appropriate genetic counselling to the parents.

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References

- ¹ Byers PH, Tsipouras P, Bonadio JF, Starman BJ, Schwartz RC. Perinatal lethal osteogenesis imperfecta (OI type II): a biochemically heterogeneous disorder usually due to new mutations in the genes for type I collagen. *Am J Hum Genet* 1988;**42**:237-48.
- ² Sykes B, Ogilvie D, Wordworth P, Anderson J, Jones N. Osteogenesis imperfecta is linked to both type I collagen structural genes. *Lancet* 1986;**i**:69-72.
- ³ Steinmann B, Superti-Furga A, Royce PM. Imperfect collagenesis in osteogenesis imperfecta. *Ann NY Acad Sci* 1988;**543**:47-61.
- ⁴ Aitchison K, Ogilvie D, Honeyman M, Thompson E, Sykes B. Homozygous osteogenesis imperfecta unlinked to collagen I genes. *Hum Genet* 1988;**78**:233-6.
- ⁵ De Vries N, De Wet W. Cysteine in $\alpha 1$ chains of human type I collagen produces a clinically heterogeneous form of osteogenesis imperfecta. In: Sen A, Thornhill T, eds. *Development and diseases of cartilage and bone matrix*. UCLA Symp Mol Cell Biol. New York: Alan R Liss, 1987;**46**:55-64.
- ⁶ Sippola M, Kaffe S, Prockop DJ. A heterozygous defect for structurally altered pro $\alpha 2$ chain of type I procollagen in a mild variant of osteogenesis imperfecta. *J Biol Chem* 1984;**259**:14094-100.
- ⁷ Kuivaniemi H, Sabol C, Tromp G, Sippola-Thiele M, Prockop DJ. A 19-base pair deletion in the pro $\alpha 2$ (I) gene of type I procollagen that causes in-frame splicing from exon 10 to exon 12 in a proband with atypical osteogenesis imperfecta and in his asymptomatic mother. *J Biol Chem* 1988;**263**:11407-13.
- ⁸ Superti-Furga A, Gugler E, Gitzelmann R, Steinmann B. Ehlers-Danlos syndrome type IV: a multi-exon deletion in one of the two *COL3A1* alleles affecting structure, stability and processing of type III procollagen. *J Biol Chem* 1988;**263**:6226-32.
- ⁹ Superti-Furga A, Steinmann B. Impaired secretion of type III procollagen in Ehlers-Danlos syndrome type IV: correction of the defect by incubation at reduced temperature and demonstration of subtle alterations in the triple-helical region of the molecule. *Biochem Biophys Res Commun* 1988;**150**:140-7.
- ¹⁰ Old JM. Fetal DNA analysis. In: Davies KE, ed. *Human genetic diseases—a practical approach*. Oxford: IRL Press, 1986:1-17.
- ¹¹ Kuivaniemi H, Tromp G, Chu ML, Prockop DJ. Structure of a full-length cDNA clone for the prepro $\alpha 2$ (I) chain of human type I procollagen. *Biochem J* 1988;**252**:633-40.
- ¹² Superti-Furga A, Royce PM, Pistone FM, Romano C, Steinmann B. Delayed triple-helix formation of abnormal type I collagen is corrected by reduced temperature—studies on a family with variable expression of osteogenesis imperfecta. *Ann NY Acad Sci* 1988;**543**:85-92.
- ¹³ Silience DO, Senn AS, Danks DM. Genetic heterogeneity in osteogenesis imperfecta. *J Med Genet* 1979;**16**:101-16.
- ¹⁴ Wenstrup RJ, Tsipouras P, Byers PH. Osteogenesis imperfecta type IV: biochemical confirmation of genetic linkage to the pro $\alpha 2$ (I) gene of type I collagen. *J Clin Invest* 1986;**78**:1449-55.
- ¹⁵ Wenstrup RJ, Cohn DH, Cohen T, Byers PH. Arginine for glycine substitution in the triple-helical domain of the products of one $\alpha 2$ (I) collagen allele (COL1A2) produces the osteogenesis imperfecta type IV phenotype. *J Biol Chem* 1988;**263**:7734-40.
- ¹⁶ Falk CT, Schwartz RC, Ramirez F, Tsipouras P. Use of molecular haplotypes specific for the human pro $\alpha 2$ (I) collagen gene in linkage analysis of the mild autosomal dominant form of osteogenesis imperfecta. *Am J Hum Genet* 1986;**38**:269-79.
- ¹⁷ Cohn DH, Byers PH. Osteogenesis imperfecta and other inherited disorders of the structure and synthesis of type I collagen: models for the analysis of mutations that result in inherited chondrodysplasias. *Pathol Immunopathol Res* 1988;**7**:132-8.
- ¹⁸ Smith R, Francis MJO, Houghton GR. *The brittle bone syndrome*. London: Butterworths, 1983;111-25.

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