Segregation analysis of dominant osteogenesis imperfecta in Italy

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Abstract

We have performed linkage analysis in seven Italian families, in which mild osteogenesis imperfecta (OI) segregated as a dominant trait, by means of six DNA restriction fragment length polymorphisms (RFLPs) of type I collagen genes. OI type I was linked to the $\alpha 1(I)$ gene (COL1A1) in two families, and to the $\alpha 2(I)$ gene (COL1A2) in one family. OI type IV segregated with COL1A2 in two families. In two OI type I families, the molecular genetic data were insufficient for exclusion of one gene. Four DNA polymorphisms were particularly informative for cosegregation analysis of OI in Italian kindreds.

New data at the biochemical, genetic, and molecular levels have indicated in recent years the association of osteogenesis imperfecta (OI) with various mutations in the genes (COL1A1 and COL1A2) which encode for type I procollagen.^{1,2} The striking clinical heterogeneity³ of brittle bone disease is emphasised by the molecular data which have been accumulating in the last three years; each mutation studied so far is a different one. They occur sporadically in the severest cases, while they are transmitted in an autosomal dominant fashion in milder cases. Mutations which cause structural abnormalities of pro $\alpha 1(I)$ and pro $\alpha 2(I)$ chains are more commonly found in severe variants of OI and can often be shown by the biochemical analysis of collagen I.⁴

In milder OI variants (OI type I and type IV according to Sillence *et al.*³), which can be dominantly inherited, analysis and identification of the mutations are far less accessible since they often do not produce

evident alterations in the collagen chains. Such mutations may, for instance, decrease the relative rate of synthesis of pro $\alpha 1$ chains and result in lower than normal levels of structurally unaltered collagen type I molecules in OI type I.^{5,6} Segregation analysis with RFLPs in affected families can be of great use in these cases, in order to localise the mutation in either gene.^{7,8} Thus, prenatal diagnosis of OI may become feasible.^{9,10}

In a recent study, we determined COL1A1 and COL1A2 RFLP haplotype frequencies in a large sample of random, normal, Italian subjects.¹¹ As a result of this analysis we indicated which RFLPs might be more informative for the identification of the affected gene in familial type I collagen diseases in this population.

Here, we report our genetic studies on some Italian families with dominantly inherited forms of OI. Our data agree with the above indications, and show the usefulness of COL1A1 and COL1A2 RFLP haplotype determinations in segregation analysis in affected families in the Italian population. They also confirm the different associations reported between disease and affected locus in type I and type IV forms of osteogenesis imperfecta.

Patients

Seven pedigrees in which OI segregated as a dominant trait were considered: a total of 53 subjects was examined, 28 of whom were diagnosed as affected by OI. All subjects who participated in the genotypic characterisation were seen by at least one of the authors and a detailed clinical history was recorded for each of them. Classification was according to Sillence *et al.*³ In all families, parental relationships were checked for variable numbers of tandem repeat (VNTR) DNA polymorphisms with probes YNH24 and EFD64.2¹² in *MspI* and *RsaI* DNA digests respectively, as described previously.¹³

Methods

DNA was prepared from peripheral blood, digested with the appropriate restriction endonuclease, electrophoresed on agarose gels, transferred to Hybond C filters, and hybridised with probes which

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Received for publication 28 December 1989.

Accepted for publication 8 January 1990.

had been labelled with ^{32}P by multiprimer directed synthesis. Hybridisation conditions were as previously described.¹¹

Segregation of COL1A1 and COL1A2 was analysed in the pedigrees by means of three restriction site polymorphisms for each locus, as previously described.¹¹ The following RFLPs were used: FG2/*Msp*I, 2FC6/*Rsa*I, and NST70/*Rsa*I for COL1A1, and NJ3/*Eco*RI, Hf32/*Rsa*I, and Hf32/*Msp*I for COL1A2. Table 1 shows the eight possible RFLP haplotype combinations at the COL1A1 and COL1A2 loci; these haplotype numbers are used in the pedigree representations. Lod score analysis was performed with the M-LINK program.¹⁴ In some cases, fibroblasts were grown by standard methods from skin biopsies obtained from affected subjects and

healthy relatives. Electrophoresis of collagens was performed by sodium dodecylsulphate-polyacrylamide gel electrophoresis.⁴ Total RNA was purified from approximately 10^8 fibroblasts by the guanidinium isothiocyanate method.¹⁵ Northern blot analysis was performed by standard methods using *pro α 1(I)* and *pro α 2(I)* full length cDNA probes, kindly provided by Dr F Ramirez.

Results

Families were chosen from among a wider number of Italian OI patients, as they clearly showed a dominant pattern of inheritance of the disease and were large enough to allow segregation analysis to be performed. Linkage to either collagen type I gene was investigated by segregation analysis of COL1A1 and COL1A2 RFLPs. The results obtained are shown in detail in the figure; under each pedigree symbol, COL1A1 and COL1A2 RFLP haplotypes are shown. In five families (1, 2, 3, 5, and 6), the affected locus was identified by showing discordance at one locus. In two families (4 and 7), association of the disease with either collagen type I gene locus was not possible as neither could be excluded. An additional COL1A2 *Stu*I RFLP¹⁶ was used in the analysis of the two pedigrees which were not informative for the other six polymorphisms: no additional information was gained.

From these data, lod scores at zero recombination frequency were calculated for the seven families, and are reported in table 2. The low values are the result of the small number of meioses available for each family.

Table 3 summarises the affected locus and the diagnostic features common to each family, notwithstanding a remarkable variability in clinical severity among the affected members.

Electrophoretic analysis of collagens obtained from skin fibroblasts of at least one or more affected members from families 1, 2, 3, and 7 showed no structural alteration. Preliminary data on collagen type I mRNA levels in family 1 indicate a decrease in *pro α 1(I)* mRNAs compared to normal controls (subject III.6).

Table 1 RFLP haplotypes at collagen type I loci.

	COL1A1			COL1A2		
	<i>Msp</i> I FG2	<i>Rsa</i> I 2FC6	<i>Rsa</i> I NST70	<i>Eco</i> RI NJ3	<i>Rsa</i> I Hf32	<i>Msp</i> I Hf32
1	+	+	+	+	+	+
2	+	+	—	+	+	—
3	+	—	+	+	—	+
4	+	—	—	+	—	—
5	—	+	+	—	+	+
6	—	+	—	—	+	—
7	—	—	+	—	—	+
8	—	—	—	—	—	—

+ and — indicate the presence or absence of the corresponding restriction site.

Table 2 OI linkage to collagen type I loci.

Pedigree No	COL1A1	COL1A2
1	0.6	—
2	—	0.0
3	—	1.2
4	0.6	0.0
5	—	0.0
6	1.2	—
7	0.3	0.0

Lod scores were calculated for the two genes at zero recombination frequency. Discordance is indicated by a dash.

Table 3 Clinical and genetic features of OI pedigrees.

No	Sclerae	No of fractures*	Presenile hearing loss	Dentinogenesis imperfecta	Deformity	Joint laxity	Type ³	Concordant locus
1	Blue	>10	Yes	No	Moderate	No	IA	COL1A1 (h 2)
2	White	>10	No	No	Moderate	No	IVA	COL1A2 (h 5)
3	Blue	5–10	Yes	No	No	Yes	IA	COL1A2 (h 5)
4	Blue	<5	Yes	No	No	No	IA	COL1A1 & COL1A2
5	White	>10	No	Yes	Moderate	No	IVB	COL1A2 (h 1)
6	Blue	<5	No	No	Moderate	No	IA	COL1A1 (h 6)
7	Blue	>10	No	No	Moderate	Yes	IA	COL1A1 & COL1A2

*=number of fractures reported at the time of observation.
h=haplotype (see table 1 and the figure).

OI pedigrees. Numbers under symbols indicate COL1A1 (upper row) and COL1A2 (lower row) haplotypes (see table 1). RFLPs were determined on DNA samples from each family member indicated.

Discussion

The data reported in this paper show that, using RFLP cosegregation analysis, the affected locus could be identified in five out of seven families affected by dominantly inherited forms of osteogenesis imperfecta.

In our recent study of COL1A1 and COL1A2 haplotype frequencies in the Italian population,¹¹ we emphasised the potential usefulness of four restriction

site dimorphisms (FG2/*Msp*I and 2FC6/*Rsa*I for COL1A1 and NJ3/*Eco*RI and Hf32/*Rsa*I for COL1A2) for linkage analysis, owing to their high PIC values in our population. Indeed, their combined use was sufficient to gain the necessary information in this study, while the other RFLPs used (NST70/*Rsa*I for COL1A1, Hf32/*Msp*I and 4.1 kb *Stu*I for COL1A2) turned out to be of little interest for diagnostic

purposes, owing to their low PIC values. Our data show that it was possible to determine the affected gene even when no collagen alteration was readily detectable. Prenatal or preclinical diagnosis can be made available to the families in which the affected gene has been identified.

It is interesting to note that the number of subjects typed is not a sufficient previous indicator of the possibility of identifying the affected locus by segregation analysis. Three subjects were enough in family 5, while four were not sufficient in family 7, neither were the seven typed members distributed over four generations in family 4. For familial cases of OI where segregation analysis is not informative, new probes, as well as biochemical or other molecular evidence, might help to identify the affected locus. It is also worth noting that new dominant mutations seem to have occurred recently in five (2, 4, 5, 6, and 7) out of seven families with mild OI.

New mutations in mild dominantly inherited OI were described in 12 out of 71 families examined by Silience *et al*³ and in the majority of severe (type II and type III) OI cases.^{17, 18} An effect of paternal age on the frequency of OI mutations has been described.¹⁹ Mean paternal age at birth of the first OI case in the five families described here was 30 years (range 27 to 35 years), which was similar to normal control values.

In OI type IV pedigrees (families 2 and 5), the disease segregated with COL1A2. In OI type I cases, the disease segregated with COL1A1 in two pedigrees (families 1 and 6), and with COL1A2 in one family (3). Our observations are in agreement with the reported data, which ascribe OI type IV to mutations occurring at COL1A2,^{20, 21} while OI type I has been shown to be linked to both loci.⁸ While a severe autosomal recessive form of OI unlinked to collagen type I genes has been reported,^{22, 23} all dominant OI pedigrees studied so far have shown linkage with collagen type I loci. At present, the hypothesis of another locus involved in dominant OI can therefore be discarded.

We are indebted to the participating family members, and to the many colleagues who contributed by bringing OI families to our attention and collecting blood samples and skin biopsies, among them Drs F Antoniazzi, R Aldegheri, F Bertoldo, and C Esposito. We thank the Centre for the Study of OI of Valeggio s/Mincio (Verona) for collaboration, and Drs R Tenni, M Valli, and G Cetta for the biochemical analysis of collagens. We also thank Ms R Galavotti for excellent technical assistance and Drs F Ramirez, B Sykes, and Y Nakamura, for kindly providing their DNA probes. LC was the recipient of a fellowship from the Italian OI Association. The work was supported by grants from the Italian Ministry of University and Scientific and Technologic Research

(MURST), and by the National Research Council "Progetto Finalizzato Biotecnologie" and "Progetto Finalizzato Ingegneria Genetica".

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