Exclusion of the $\alpha 1(\text{II})$ cartilage collagen gene as the mutant locus in type IA osteogenesis imperfecta

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SUMMARY Using two restriction site polymorphisms within the structural gene coding for human type II collagen we have examined the segregation of this gene in three pedigrees with dominantly inherited osteogenesis imperfecta (Sillence type IA). We have demonstrated that the gene does not segregate with clinical expression of the disease and cannot, therefore, contain the mutation responsible for osteogenesis imperfecta in these families.

The inherited connective tissue disorders are a clinically and genetically heterogeneous assembly of disorders presenting with mechanical failure of collagen rich tissues such as bone, skin, ligaments, tendons, and blood vessel walls. The most frequently encountered clinical groups are osteogenesis imperfecta, in which bones have an increased susceptibility to fracture, Marfan's syndrome, and Ehlers-Danlos syndrome, but within these categories there is also a great deal of variation. Since the extracellular protein collagen is the major mechanical component of the affected tissues, it has reasonably been supposed that abnormalities in collagen may be responsible. Owing in part to the nature of the protein, specific biochemical evidence to support such a hypothesis has been difficult to establish. Nevertheless, in vivo and in vitro differences in collagen stability, chain length, or metabolism have been reported in individual cases or unrelated groups. In a few rare recessive forms of the Ehlers-Danlos syndrome the effects on collagen are due to deficiencies in enzymes which modify procollagen chains after translation. This explanation does not cover the majority of cases and it is likely that some causal mutations lie within or close to structural genes coding for collagen. By taking advantage of DNA sequence polymorphisms revealed by restriction endonuclease digestion, it is usually possible to test this hypothesis for a given collagen gene.

Here we report results using the human pro $\alpha 1(\text{II})$

collagen gene (COL2A1). The gene is contained within cosmid cosH.col.1 and is located on chromosome 12.^{1 2} Preliminary nucleotide sequence failed to identify the gene and it was initially referred to as an $\alpha 1$ like collagen gene. More extensive nucleotide sequence and comparison with collagen gene sequences from chicks have now identified the gene as encoding the pro $\alpha 1(\text{II})$ chain.³ This is the subunit of type II collagen which is the major collagenous component of both hyaline and articular cartilage. As such this gene is a candidate for the mutant locus in any disorder where abnormalities of cartilage or bone are suspected.

Materials and methods

DNA was prepared from frozen whole blood⁴; 10 μg aliquots were digested with restriction enzymes and the fragments separated in agarose under standard conditions. Nitrocellulose blots were hybridised at 42°C with nick translated probe in 3 $\times$ SSC, 50% formamide, 5% dextran sulphate, 1 $\times$ Denhardt's, 20 $\mu\text{g ml}^{-1}$ denatured salmon sperm DNA, and, when using the whole cosmid, 20 $\mu\text{g ml}^{-1}$ denatured human placenta DNA to compete with repetitive sequences in the probe. Filters were washed twice in 2 $\times$ SSC at room temperature, then twice in 0.1 $\times$ SSC, 0.1% SDS at 65°C, air dried, and exposed to Fuji RX film.

Results

Two restriction site polymorphisms were used in this

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analysis. The variation revealed by HindIII digestion has been described in detail elsewhere.⁵ Another polymorphism was detected by PvuII digestion when a 3.3 kb fragment was cleaved into two fragments of 1.7 and 1.6 kb when the site was present (+ phenotype). When the whole cosmid was used as a probe, the 1.6 kb fragment was masked by a coincident non-variable fragment (fig 1a). The

variant site was located to the 9.3 kb EcoRI fragment of cosH.col.1 by using separated EcoRI fragments of the cosmid as hybridisation probes. When this fragment, subcloned into pAT 153, was used as a probe the pattern was simplified, although the non-variable 1.6 kb fragment was still detected (fig 1b). The HindIII and PvuII variable sites were separated by 1.5 kb (fig 2) and the notation of the

FIG 1 *PvuII* fragments in three normal subjects with different genotypes revealed by hybridisation of the filter with the whole cosmid cosH.col.1 (a) and rehybridisation with subcloned EcoRI fragment 1 (b). Fragment sizes are shown in kilobases.

FIG 2 Partial restriction maps of whole cosmid cosH.col.1 (upper) and 9.3 kb EcoRI fragment (lower). R=EcoRI, H=HindIII, B=BamHI, P=PvuII. Variant sites are starred and their distribution in the four haplotypes is given below.

TABLE 1 Comparison of observed and expected distribution of haplotypes in 36 unrelated subjects. Expected values are in brackets.

	HindIII	
	+	-
	A 30 (18.8)	B 11 (22.2)
PvuII		
+		
-	C 3 (14.2)	D 28 (16.8)

four possible haplotypes is shown. In the English population examined the frequency of the variants were PvuII: $+=0.55$, $- =0.45$ ($n=72$); HindIII: $+=0.54$, $- =0.46$ ($n=126$); and when considered separately their individual distributions were close to Hardy-Weinberg expectations. However, when the information from separate sites was combined to define haplotypes in 36 unrelated subjects, significant association of phenotypes was evident (table 1). χ^2 was 23.8 with Yates's correction for this series ($p<0.001$). Haplotype C was particularly uncom-

mon. One effect of this disequilibrium was to reduce heterozygote frequency at this locus from 0.75 to 0.65.

Clinical details for the three pedigrees are shown in table 2. Judged by clinical criteria all three families are examples of osteogenesis imperfecta type IA.^{6,7} The pedigrees were examined for independent segregation of the COL2A1 and osteogenesis imperfecta genes. Since the maximum physical distance from the polymorphic markers to the limits of the gene is 25 kb the probability of crossing over between these markers and any hypothetical mutation within the gene is effectively zero. Thus, any examples of independent segregation exclude the gene as the mutant locus in that pedigree. Inspection of the pedigrees yields the following examples.

PEDIGREE a (FIG 3)

(1) III.3 carries the gene for osteogenesis imperfecta in this pedigree (OIa⁻). Her COL2A1 genotype is AB. Of her two children, IV.1 receives A and IV.2 receives B yet neither inherits the OIa⁻ gene.

TABLE 2 Clinical details of affected members of three pedigrees. These are consistent with classification as osteogenesis imperfecta type IA.

	Age	Age at first fracture	Total fractures	Teeth	Sclerae	Deafness	Height (cm)
Pedigree a							
II.1	63	4 y	5	N	Blue	Yes from age 30	157
II.5	56	5 y	10	N	Blue	No	178
III.3	37	4 y	6	Caries +	Blue	Yes from age 26	153
III.4	35	3 y	22	Caries +	Blue	Yes from age 30	163
III.6	24	4 y	4	Caries +	Blue	No	151
III.10	24	13 mth	25	N	Blue	Yes from age 20	178
III.11	21	5 y	7	N	Blue	No	185
IV.4	2	—	0	N	Blue	No	86
IV.5	3	10 mth	2	N	Blue	No	84
Pedigree b							
II.1	55	19 y	5	Not DI, pyorrhoecia	Blue	Yes from age 21	164
II.5	50	1 y	33	N	Blue	No	164
II.6	49	2 y	15	Caries +	Blue	Yes from age 13	155
III.3	29	9 mth	21	N	Blue	No	170
III.5	26	4 y	2	N	Blue	No	158
III.8	26	Birth	76	Fragile not discoloured	Blue	No	138
III.9	16	Birth	60	Caries +	Blue	No	142
Pedigree c							
III.2	45	Not known	12	N-caries	Blue	Yes	157
III.4	54	12 mth	16	N	Blue	Yes	148
III.5	52	2 y	25	N-caries	Blue	No	148
III.8	45	9 mth	17	N	Blue	No	155
IV.1	22	22 mth	11	N-caries	Blue	No	157
IV.4	24	13	5	N-caries	Blue	No	nd
IV.7	12	18 mth	3	N	Blue	No	nd
IV.9	17	7 mth	3	N	Blue	No	109

FIG 3 Three pedigrees with dominantly inherited osteogenesis imperfecta showing COL2A1 haplotypes. Solid symbols represent affected subjects.

(2) II.5 carries the OIa^- gene and has a COL2A1 genotype AB. Both III.9 and III.11 receive a paternal A haplotype but only III.11 inherits the OIa^- gene.

These examples of discordant segregation assume complete penetrance of the OIa^- gene. Careful clinical assessment of the three normal subjects did not reveal any features of the disease and so it is unlikely that any had inherited the OIa^- gene.

PEDIGREE b (FIG 3)

(1) Both II.6 (BD) and III.3 (AA) carry the mutant gene for this pedigree (OIb^-) yet they do not share a common COL2A1 haplotype.

(2) The three affected subjects in generation II, II.1 (AD), II.5 (AB), and II.6 (BD), do not share a common haplotype.

(3) II.6 carries the OIb^- gene and has COL2A1 genotype BD. Two of his children, III.8 (AD) and III.9 (AB), inherited the OIb^- gene but received different paternal haplotypes. Two of his children, III.7 (AB) and III.9 (AB), have inherited the same paternal B haplotype yet only III.9 received the OIb^- gene.

PEDIGREE c (FIG 3)

IV.7 (DD) carries the OIc^- gene yet does not share a common haplotype with several other affected subjects in the pedigree, for example, III.2 (AA) and III.8 (AA).

The examples of discordant segregation in pedigrees b and c involve clearly affected subjects (except

III.7; pedigree b) and unlike pedigree a are not dependent on the complete penetrance of the OI^- genes.

Discussion

Collagen structural genes are reasonable candidates for the mutant loci in several inherited connective tissue diseases. We have shown that one of them, the gene encoding pro $\alpha 1(II)$, segregates independently of the disease phenotype in three pedigrees of typical type IA osteogenesis imperfecta and is thereby excluded as the mutant locus in these pedigrees. Deletions near the 3' end of this gene have been suggested as a causal mutation in four cases of type II OI (perinatal lethal form)⁸ and in a father and son with Ehlers-Danlos syndrome.⁹ However, apparently identical deletions also occur as normal variants of the gene.¹⁰ Segregation analysis using an EcoRI site polymorphism in the gene encoding pro $\alpha 2(I)$ chains has established linkage of this gene ($Iod=2.41$ at $\theta=0.00$) to the disease phenotype in one pedigree with type IV OI.¹¹

It should soon be possible to establish or exclude all of the major collagen genes as the mutant loci in most inherited connective tissue disorders.

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