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Clinical and genetical aspects of autosomal dominantly inherited osteogenesis imperfecta tarda

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ABSTRACT Osteogenesis imperfecta (OI) tarda dominant type is caused by mutations in the type I collagen genes, COL1A1 and COL1A2. The essence of our haplotype analysis of osteogenesis imperfecta (OI) was to get information about the value of 8 short tandem repeat (STR) markers for the segregation of COL1A1 and COL1A2 genes on the 12 OI pedigrees and to delimit the place of mutation to one locus. The molecular genetic analysis supported the linkage to COL1A1 gene in 6 families, and in 4 families with type I B, and in one family with type III B the linkage to COL1A2 gene was supported. One patient had type IV A, where linkage to COL1A1 gene had been excluded, and haplotype analysis for COL1A2 was non conclusive in six families. As both genes consist of more than 50 exons, the haplotype analysis is very important before direct mutation screening. To achieve the maximum theoretical LOD scores for the haplotype analysis, more STR markers are needed as in many cases our markers were non informative.

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KEY WORDS

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molecular genetics
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Osteogenesis imperfecta (OI) is characterised by brittle bones, pathological fractures, blue sclera, dentinogenesis imperfecta (with or without) and caused by mutations in the type I collagen genes, COL1A1 and COL1A2.

All types of osteogenesis imperfecta are caused by structural or quantitative defects in type I collagen, the primary component of the extracellular matrix of bone and skin. In about 10% of clinically indistinguishable cases, no biochemical defect of collagen protein can be demonstrated. It is not clear whether these cases represent limitations in biochemical detection or genetic heterogeneity of the disorder.

OI is an autosomal dominant disorder that occurs in all racial and ethnic groups. The incidence of detectable OI in infancy is about 1 in 20,000. There is a similar incidence of the mild form, type I OI.

Pathology: The collagen structural mutations cause OI bone to be globally abnormal. The bone matrix contains abnormal type I collagen and relatively increased levels of types III and V collagen. In addition, several non collagenous proteins of bone matrix are found in reduced amounts. The hydroxyapatite crystals deposited on this matrix are poorly aligned with the long axis of fibres.

Pathogenesis: Type I collagen is a heterotrimer, composed of two α -1(I)-chains and one α 2(I)-chain. The chains are synthesized as pro collagen molecules with short globular extensions on both ends of the central helical domain. The

helical domain is composed of uninterrupted repeats of the sequence Gly-X-Y, where Gly is glycine, X is mostly proline, and Y is mostly hydroxyproline. The presence of glycine at every third residue is crucial to helix formation because its small side chain can be accommodated in the spatial constraints of the interior of the helical trimer. Concomitant with helix assembly and formation, the chains are glycosylated at lysine residues.

The collagen structural defects are of two types: 85% are point mutations causing substitutions of glycine residues by other amino acids, 12% are single exon splicing defects. The clinically mild type I OI has a quantitative defect with mutations that cause one α 1(I) allele to be functionally void. These patients make a reduced amount of normal collagen. The relationship between genotype and phenotype remains elusive for the structural mutations. Lethal and nonlethal mutations occur with about equal frequency on both chains. For α 2(I) mutations, lethal and nonlethal mutations occur in alternating regions along the chain. For mutations on the α 1(I)-chain, no model adequately predicts the phenotype. A minority of OI cases with apparent recessive inheritance are due to parental mosaicism and are also dominant.

Pace et al. (2002), identified a mutation in the carboxyl-terminal propeptide coding region of one COL1A1 allele in an infant who died with an OI phenotype that differed from the usual lethal form and had regions of increased bone density.

Linkage studies were performed for genetic markers from candidate intervals known to contain genes responsible for

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dentinogenesis imperfecta (DGI) on chromosomes 4q, 7q, and 17q. Conclusive evidence for linkage of DGI was obtained for genetic markers on chromosome 17q21-q22 (DLX-3, $Z(\text{max}) = 5.34$, $\theta = 0.00$; Pallos et al. 2001). This was the first report of joint pain associated with a COL1A1 mutation and DGI. The mild skeletal features and reduced penetrance of the non-dental findings illustrate the importance of genetic evaluations for families with a history of DGI.

McKenna et al. (2002) determined whether milder abnormalities in COL1A1 expression might account for the development of otosclerosis in the 7 clinical cases that did not reveal evidence of zero expression by the gel technique. Of the same 2 cases of otosclerosis that demonstrated evidence of zero expression by gel electrophoresis, both were found to have significant differences in COL1A1 mRNA expression by the Taqman analysis. The remaining 7 cases revealed equal expression of the two COL1A1 alleles similar to that seen in controls. These results suggest, that mutations in COL1A1 which are similar to those in type I osteogenesis imperfecta may account for a small percentage of cases of otosclerosis, and that the majority of cases of clinical otosclerosis are related to other genetic abnormalities that have yet to be identified.

In summary, there are two known loci (COL1A: at chromosome 17, 17q21.31-q22; COL1A2: at chromosome 7, 7q22.1) of osteogenesis imperfecta type I; 70-80% of these patients carry mutations in one or both of these genes.

The aim of our study was to analyse the segregation of the autosomal dominant osteogenesis imperfecta type I in some Hungarian families and to evaluate the usefulness of short tandem repeat markers (STR) for the linkage analyses of certain loci of collagen gene.

Materials and Methods

In our retrospective study we investigated 18 families with autosomal dominant inherited osteogenesis imperfecta tarda (OIT) type I, III and IV from the Pediatric Department Szeged and 12 families from the Semmelweis University

Department of Orthopedics, Budapest. Diagnostic criteria for OIT were pathological bone fractures, blue sclera, dentinogenesis imperfecta, scoliosis (w/wo). Type II (lethal) did not occur.

We chose 4-4 short tandem repeat (STR) markers (Table 1) for the haplotype analysis of the two loci (COL1A1 and COL1A2). The markers were D17S788, D17S790, D17S943, D17S1795 for COL1A1, and D7S657, D7S527, D7S2482, D7S1820 for COL1A2. The touch down PCR amplifications were carried out by the following program: initial denaturation at 94°C for 5 min followed by 10 cycles denaturation at 94°C for 30 sec, annealing at 61°C for 30 sec (-0.5°C per cycle) and extension at 72°C for 30 sec continued for 25 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec and extension at 72°C for 30 sec followed by a final extension at 72°C for 10 min for all markers except for D17S943 where we had the same program but the annealing temperatures were 5°C higher, respectively. The PCR products were run on 8% polyacrylamide (PA) gel and visualized by ethidium bromide with an AlphaImager, AlphaEase 5.5 gel documentation system (Fig. 1).

Results

Twelve OIT families, 3-7 family members per family, including 2-4 members affected by autosomal dominant inheritance (AD) per family, had been genotyped by STR markers for the COL1A1 and COL1A2 loci. The clinical and genetical data of our OIT patients have been summarized in Table 2. Eight out of twelve OIT families had type I, among them 5 families had type I B, the molecular genetic analysis supported the linkage to COL1A1 gene in 6 families, and in 4 families with type I B and in one family with type III. B the linkage to COL1A2 gene was supported.

One patient had type IV A, where linkage to COL1A1 gene had been excluded, and haplotype analysis for COL1A2 was non conclusive. The K. brother's had type IV B with COL1A1 mutation.

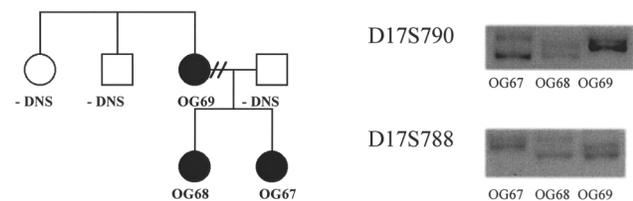


Figure 1. Haplotype analysis of the family of M. Sz. The amplified PCR products were run on 8% PA gel. We could exclude the COL1A1 locus with several markers as affected children inherited different alleles from the affected mother. The STR markers of COL1A2 locus (data not shown) did not disagree with the segregation to COL1A2.

Table 1. STR markers for the haplotype analysis of COL1A1 and COL1A2 loci

D17S788F	5' CTA GGC AGC CAC TAC CAA AT
D17S788R	5' CAG CAT CTT TGC TAT AAG CAT C
D17S790F	5' AGG AAA AAT GAG TGG ACC AT
D17S790R	5' AGC TGG GTT ATT GTT TTT CC
D17S943F	5' GGT GGA GGG AGT AGA AAG AA
D17S943R	5' TAT GGC TGT CTC ATT CCA AC
D17S1795F	5' AGT GCC AGA GATATA CCG TG
D17S1795R	5' GTC TGC AAG GCA AGT TGT C
D7S657F	5' TCA ATC ACC ACT TAC ATG CA
D7S657R	5' AGA GAA GTG GTG TCA CTT GG
D7S527F	5' TGT TTC TTC AAG GTA GTC
D7S527R	5' TAA CAG AGG CAT GAA AAC CA
D7S2482F	5' TTA ATC CCA CAG GAA TGA ATG
D7S2482R	5' TGG CTC TAG AAT TCT GAA TGG
D7S1820F	5' TGA ATG ACT TTG GTG AGT ATG C
D7S1820R	5' ACC TCA AGC AGA ACA CTT GC

Table 2. Clinical and genetic data of patients with osteogenesis imperfecta tarda.

Name	Gen-der	Age (y)	Fracture	Onset of fr. (y)	Blue sclera	Dentino-genesis imp.	Scoliosis	Affected family members	Sillence type	Molecular genetic data supported	Molecular genetic data excluded
Ms. N. H.	F	19	8X	1.5	4+	neg.	pos.	sister, father, paternal grandmother	IV.		COL1A1, COL1A2 non informative
Mrs. G. Sz.	F	20	8-10X		3+	neg.	neg.	mother, sister	IV.A		
Ms. S.	F	22	5X	8	2+	pos.	neg.	mother, brother	I. B		
R.S.	M	25	2X	10	1+	pos.	neg.	mother, sister	I. B		
Ms. A. Cs.	F	13	4-5X	6	2+	pos.	neg.	mother incomp.	I. B	COL1A2	COL1A1 non informative
Ms. A.P.	F	3	2X	1.5	1+	pos.	neg.	father (4-5 fra.), grandmother 14 fra.	I. B	COL1A2	COL1A1 non informative
Ms. D.J.	F	6	3X	3y	1+	pos.	neg.	brother, father	I. B	COL1A1	COL1A2 non informative
Mr. R.K.	M	28	12X	4y	2+	pos.	neg.	brother, paternal uncle	IV.B	COL1A1	COL1A2 non informative
Mr. N. K.	M	32	20X	10m	2+	pos.	neg.	father (cox arthrosis), paternal grandmother	IV.B	COL1A1	COL1A2 non informative
Mr. Zs. F.	M	26	4X	2.5y	1+	pos.	neg.	mother incomp.oi.	I. B	COL1A2 (2A)	COL1A1 (1A)
Mr. A.F.	M	56	5X	14y	2+	pos.	neg.	mother	I. B	COL1A2 (2A)	COL1A1 (1A)
N.R.	M	8	2-3X	15m	1+	pos.	neg.	new mutation ?	I. B		
K. T.	M	12	2-3X	i.ut.	1+	pos.	operated		III.		
Ms. K. É.	F	22	>10X	i.ut.	4+	pos.	operated	intrauterin fractures	III. B	COL1A2	COL1A1
Mrs. B.	F	44	8-10X		1+	pos.	neg.	father, brother	I.	COL1A1	COL1A2 non informative
Mr. L.O.	M	39						sister, father, paternal grandmother	I.	COL1A1	COL1A2.
I. K.	M	7						sister, mother inc.OG..	I.	COL1A1	COL1A2 non informative
Ms. M. K.	F	14	5-6X	3y	2+	pos.	neg.		I.	COL1A1	COL1A2 non informative

A = absence of dentinogenesis imperfecta

B = presence of dentinogenesis imperfecta

* = the fetus and the Mr. A. P. inherited the same COL1A2 allele, probably he has been an affected person for OI. No interruption.

One female patient had type III B with intrauterine fractures and very severe short state (115 cm) and clinical prognosis with multiplex extra uterine fractures. She had been operated several times, and developed kyphoscoliosis. She completed her secondary school with excellent marks, and she is unable to walk. The affection of COL1A2 has been supported and COL1A1 gene has been excluded.

Thus the molecular genetic data linkage to COL1A1 gene was confirmed in 6 families, and in 3 families this gene was excluded. The linkage to COL1A2 was confirmed in 3 families—with type I B (n=2) and type III (n=1)—and in one family the locus was excluded.

The STR markers for COL1A2 gene were non informative in 6 families.

Discussion

Because of the low number of patients in several of our families, we were able to carry out the haplotype analysis in 12 families only.

Tsipouras et al. (1984) performed linkage analysis of the four RFLPs of the COL1A2 in families with mild forms of osteogenesis imperfecta and in a family with Marfans syndrome. In 2 of the OI positive families lod scores were found. In three other families the osteogenesis imperfecta phenotype segregated independently of the polymorphisms. Accumulative lod score from these 3 families was -4.3 at recombination fraction 0.05, and close linkage is excluded. The data from these 5 families with OI support the hypothesis that the mild dominant osteogenesis imperfecta is heterogeneous at the gene level. Skyes et al. (1986) found a polymorphism in the COL1A1 gene, and they have used this together with the polymorphisms at the COL1A2 gene in linkage studies in 11 families with dominant OI. In each family the OI gene was inherited with one or the other collagen locus. OI phenotype IV seemed to segregate with the COL1A2 gene, which is in agreement with the COL1A2 gene in four families and with COL1A1 in one family (Borresen 1986).

Comparison of phenotypic features with the concordant collagen locus showed that in four pedigrees with OI Sillence type I segregated with COL1A1, while two pedigrees with OI Sillence type I and OI type IV segregated with COL1A2.

In the five of our OI type I families the linkage to COL1A1 gene have been supported by the haplotype analysis. COL1A1 locus dimorphisms A/MspI, B/RsaI and F/MnII, showed PIC values of 0.327, 0.191 and 0.366, respectively, giving a combined PIC of 0.656 at the locus, while COL1A2 locus dimorphisms C/EcoRI, D/MspI and E/RsaI RFLPs had PIC values of 0.357, 0.168 and 0.331, respectively, giving a combined PIC of 0.655 at the locus (Benuslene and Kucinkas 2000).

The cell surface expression and functional properties of TGF- β receptors I, II and III on osteoblasts were compared

to healthy controls. The human osteoblastic cells from investigated patients with OI all have an elevated number of cell surface receptors for TGF- β , without any evidence for a transcriptional regulation of TGF- β receptor II. On the functional level, there is some evidence for an impaired adaptive behavior of receptor presentation, whereas receptor affinity is unchanged (Gebken et al. 2000).

Apart from the affected bone density, the other main manifestation of OI is the dentinogenesis imperfecta and the otosclerosis.

Recurrent mutations in the COL1A2 gene (1121 G>T, Gly 238 cys) in patients with osteogenesis imperfecta type III were reported by Trummer et al. (2001). A new recurrent point mutation in the COL1A2 gene was found in a patient with type III OI. A G-to-T transversion in nucleotide position 1121 leads to an amino acid substitution Gly238Cys. This was the first report on the most N-terminal cysteine substitution in COL1A2 reported to date. Until now, at this position, only serine substitutions were observed five times in unrelated patients showing a highly variable expression of OI. Our patient (Ms. K. É.) with type III is a candidate for COL1A2 gene mutation because her STR data were supportive for COL1A2 gene affection.

Prenatal diagnosis of a novel COL1A1 mutation in osteogenesis imperfecta type I carried through full term pregnancy was published by Ries et al. (2000). The father has OI type I, with a novel mutation in the COL1A1 gene: a C to T change at position c3076 (c.3076C—>T) leading to a change of arginine at codon 848 to a stop codon (R848X). Prenatal diagnosis by chorionic villous sampling (CVS) was performed during the fourth pregnancy, and revealed that the fetus is a carrier of the same COL1A1 mutation. The parents elected to carry the pregnancy to term, and a male child with mild OI was born.

The only serious attempts to show linkage in OI have shown that the disease is linked to type I collagen genes in all studied families in which it segregates as a clear mendelian dominant trait. For prenatal diagnosis the probability that a new family is linked can be taken as greater than 0.95. Some phenotype correlations, notably between the OI type IV phenotype and linkage to COL1A2 and between presenile hearing loss in OI type I and linkage to COL1A1, can be used to improve risk estimates substantially in families where there are no segregation data to distinguish whether COL1A1 or COL1A2 is the mutant locus (Sykes 1993).

The segregation of COL1A1 and COL1A2, the two genes which encode the chains of type I collagen, was analyzed in 38 dominant OI pedigrees by using polymorphic markers within or close to the genes. This was done in order to estimate the consistency of linkage of OI genes to these two loci. None of the 38 pedigrees showed evidence of recombination between the OI gene and both collagen loci, suggesting that the frequency of unlinked loci in the population must be low. From these results, approximate 95%

confidence limits for the proportion of families linked to the type I collagen genes can be set between .91 and 1.00. This is high enough to base prenatal diagnosis of dominantly inherited OI on linkage to these genes even in families which are too small for the linkage to be independently confirmed to high levels of significance. When phenotypic features were compared with the concordant collagen locus, all eight pedigrees with Sillence OI type IV segregated with COL1A2. On the other hand, Sillence OI type I segregated with both COL1A1 (17 pedigrees) and COL1A2 (7 pedigrees). The concordant locus was uncertain in the remaining six OI type I pedigrees. Of several other features, the presence or absence of presenile hearing loss was the best predictor of the mutant locus in OI type I families, with 13 of the 17 COL1A1 segregants and none of the 7 COL1A2 segregants showing this feature (Sykes et al. 1990).

The essence of our haplotype analysis of OI was to get information about the value of 8 STR markers for the segregation on the pedigrees and to delimit the place of mutation to one locus. As both genes consist of more than 50 exons the haplotype analysis is very important before direct mutation screening. From our study we can conclude that the analysis of AD OI families is problematic as in most cases the number of affected people and informative members in the families are few. To achieve the maximum theoretical LOD scores for the haplotype analysis more STR markers are needed as in many cases our markers were non informative. However by the data of HGP there are limited numbers of available STR markers with high allele frequencies in 3-4 cM proximity of COL1A1 and COL1A2 loci.

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