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Strategy for prenatal diagnosis of *osteogenesis imperfecta* by linkage analysis to the type I collagen loci COL1A1 and COL1A2

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SUMMARY

To improve prenatal diagnosis of osteogenesis imperfecta (OI) in Lithuania, possibilities of indirect molecular genetic diagnosis were investigated in 11 families with dominant OI. Segregation of polymorphic DNA markers closely linked to COL1A1 and COL1A2 genes with OI phenotype was investigated. Polymorphic DNA markers applied were individual haplotypes constructed using a set of restriction enzyme sites within or close to the genes. 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. Out of six remaining pedigrees with OI Sillence type I, three were concordant at both loci, two pedigrees were discordant at the locus COL1A2 and non-informative at the locus COL1A1 and one pedigree was concordant at the locus COL1A1 and non-informative at the locus COL1A2.

Informativity of DNA markers applied was also investigated in the Lithuanian OI families. The frequencies of six restriction enzyme site dimorphisms in type I collagen loci were estimated and polymorphism information content (PIC) values were calculated for each restriction site and for a combination of three sites. COL1A1 locus dimorphisms A/MspI, B/RsaI and F/MnlI, 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.

INTRODUCTION

Osteogenesis imperfecta (OI) is a clinically, genetically, biochemically, and radiologically heterogeneous group of inherited connective-tissue disorders characterised by bone fragility and other evidence of connective tissue involvement [1,2]. This definition embraces a very wide range of phenotype, from intrauterine or perinatally lethal forms through an almost continuous spectrum to a barely noticeable increase in fracture tendency. Four OI types are recognised according to Sillence et al. [1] on the basis of clinical features that include stature, bone deformity, osteopenia, scleral hue, dental problems, and hearing loss. OI type II is lethal in the perinatal or neonatal period. OI type III is very severely deforming and, although patients can survive into their teens and beyond, they only rarely have children. OI types I and IV are less severe. The clinical heterogeneity apparent in OI is explained largely by different mutations in two non-syntenic genes, COL1A1 (17q 21.31–22.15) and COL1A2 (7q 21.3–22.1), which encode (respectively) the proα1(I) and proα2(I) chains of type I procollagen [2–4]. Although rare autosomal recessive forms of OI due to mutations in these genes and possibly in non-collagen genes exist, the vast majority of individuals with OI are heterozygous for dominantly acting mutations [5]. These abnormalities fall into two general classes: one in which there is too little type I procollagen synthesised but the product is normal (OI type I) and one

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in which abnormal type I procollagen molecules are made (OI types II, III and IV). Until recently almost 200 mutations in the two genes coding for type I procollagen have been detected in probands with OI [2]. The disease is sufficiently severe for there to be a demand for prenatal diagnosis from parents at risk of having an affected child.

Prenatal diagnosis is of concern in families at risk for recurrence of OI: (a) family is affected with an autosomal dominant form of OI, (b) the parent is only individual in the family affected with an autosomal dominant form of OI, (c) child with OI is born to normal parents (due to parental germline+somatic mosaicism, germline mosaicism or mutation *de novo* in a single gamete or very early in embryogenesis). The strategy of prenatal diagnostic investigation used for a particular pregnancy depends on the nature of diagnostic studies performed earlier and the type of abnormality identified.

Four techniques are used at present for prenatal diagnosis [6,7]. High resolution ultrasound scan of fetal limb length and morphology can reliably identify fetuses with OI type II by 14–16 weeks' gestation, even in the absence of a previously affected sib, and can identify some fetuses with the progressive deforming variety of OI (OI type III) by 18–20 weeks' gestation. Nevertheless, in the absence of fracture or significant bowing, ultrasound cannot be used to identify fetuses with the milder forms of OI, even in the presence of a positive family history. Some progress has been made in diagnosing OI from analysis of the collagen synthesised by cultured chorionic villi fibroblasts. If cultured fibroblasts from an affected parent or child have been studied and an abnormal type I procollagen molecule has been identified, then mesenchymal cells cultured from chorionic villi biopsies taken at about 10–11 weeks' gestation, which synthesise collagens comparable to dermal fibroblasts, can be used for diagnostic studies. Because OI represents a 'private' mutation in most families, mutational analysis of DNA at COL1A1 and COL1A2 loci is the most straightforward method in identifying the molecular basis of the disease. It enables prenatal diagnosis as early as 10 weeks' gestation when ultrasound- or protein-based testing for OI is not yet possible and allows best accomplished prenatal diagnosis of OI type I. Direct identification of a disease-causing mutation by analysing DNA from CVS or amniotic fluid cells can be used only in pregnancies where the molecular defect has been previously identified. The fourth method, which this paper addresses, works by indirect identification of the mutant collagen I allele in the affected members of an OI family and thus permits prenatal diagnosis by haplotype analysis of fetal DNA. This type of molecular examination can be used in pregnancies at risk for all types of OI.

Concordant segregation of the dominantly inherited OI phenotype and a collagen structural gene was first reported in 1983 [8] when no recombinants between the disease and COL1A2 were found in eight informative meioses within a single family when an EcoRI RFLP was used at the locus. When markers at COL1A1 were tested in addition to those at COL1A2, there were no examples of discordance at both loci in 11 families [9]. This indicated that the extent of genetic locus heterogeneity might be limited to these two collagen genes. An international multicentre study of 38 dominant OI families has shown that the disease can be linked to either gene [3]. 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 0.91 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. Thus, at present, accurate prenatal diagnosis of OI in any family depends on information from not one but both type I collagen loci. The first step must be to distinguish whether the disease in that family is linked to either COL1A1 gene or COL1A2 gene, by analysing the segregation patterns of the two loci markers and the disease until one locus is seen to be discordant. Prenatal diagnosis then depends on being able to distinguish, in the usual way, the mutant from the normal allele in the fetus at risk. Because the DNA marker sequence used for gene tracking is not the sequence leading to the disease, there is always the possibility of recombination between the disease and the marker, which should be included in the calculation of OI recurrence risk in the family. The recombination fraction between DNA markers within or close to the COL1A1 and COL1A2 genes and the OI causing mutation, presumably either somewhere in the collagen I gene, was estimated as 0.0005 [6].

The aim of the study was to establish DNA markers for mutant COL1 loci and alleles in Lithuanian OI

Table 1. Possible haplotypes at the COL1 loci based on three dimorphic restriction site marker systems used (see Fig. 1)

Haplotype / Locus	Dimorphic sites		
COL1A1	F	A	B
COL1A2	C	D	E
Haplotype 1	+*	+	+
2	+	+	-
3	+	-**	+
4	+	-	-
5	-	+	+
6	-	+	-
7	-	-	+
8	-	-	-

* restriction enzyme site is present

** restriction enzyme site is absent

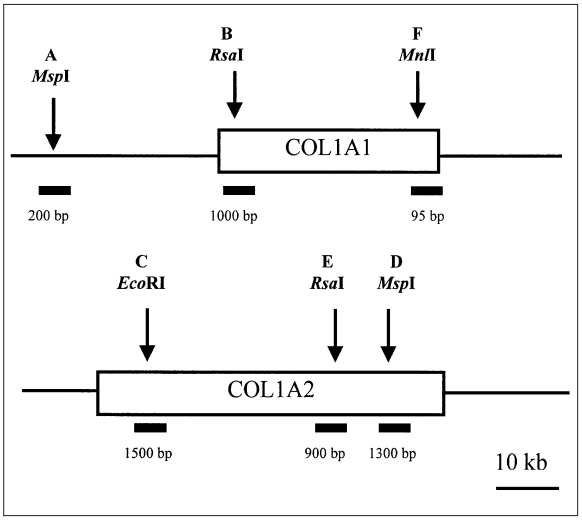

Figure 1. Physical map of dimorphic restriction enzyme sites at the COL1A1 and COL1A2 loci. Black blocks (drawn not to scale) represent PCR-amplified DNA fragments carrying a restriction site. Fragment size is shown under a relevant block.

families and, on this basis, to optimise the strategy for prenatal diagnosis of OI in Lithuania.

MATERIAL AND METHODS

A total of 92 individuals from 11 Lithuanian families were analysed for the presence of clinical OI features and for a linkage phase of OI phenotype and a COL1 gene allele.

Clinical OI diagnosis was based on the examination of physical phenotype (stature, scleral hue, limb deformity, muscular hypotonia, hypermobility of joints), evaluation of dentinogenesis and long bone X-ray data. Audiography was performed to estimate hearing loss.

DNA was isolated from peripheral blood by salting or phenol-chloroform extraction. DNA fragments at COL1A1 and COL1A2 loci were PCR amplified and then digested with restriction enzymes MnlI, MspI, RsaI and EcoRI. Primer pairs that flanked six restriction enzyme sites in two COL1 loci (see Fig. 1) were used for the PGR amplification of relevant DNA fragments [10,11].

PCR, restriction enzyme digestion and electrophoresis conditions were used as described by Baker et al, 1991 [10] and Willing et al, 1992 [11].

PCR conditions: 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.2 mM each primer, 0.1–1 μg genomic DNA, 1.5 units Ampli Tag in total volume 50 μl.1 drop mineral oil. Cycling conditions: Site A: 94°C×3.0 min then 35 cycles of 55°C×2.0 min, 72°C×1.0 min, 94°C×1.5 min. Site B: 93(C×3.0 min then 30 cycles of 60°C×1.5 min, 70°C×1.0 min, 93°C×0.5 min. Site C: 94°C×3.0 min then 30 cycles of 60°C×2.0 min, 72°C×1.0 min, 93°C×0.5 min. Site D: 94°C×3.0 min then 30 cycles of 55°C×1.5 min, 70°C×1.0 min, 93°C×0.5 min. Site E: 93°C×3.0 min then 30 cycles of 64°C×2.0 min, 93°C×0.5 min. Site F: 94°C×3.0 min then 35 cycles of 58°C×1.4 min, 72°C×1.0 min, 94°C×1.3 min. 8μl aliquots of PCR product were digested with 10 units of the appropriate restriction enzyme and 1 μl of 10×enzyme buffer for 2 hours at 37°C then run on gels containing either 6% polyacrylamide (site A and site F) or 1.5% agarose (other sites) and stained with ethidium bromide.

Two restriction site marker systems, each comprising a set of three dimorphic sites, were used for each gene analysed to distinguish OI-linked COL1 alleles (Fig. 1):

- F, A and B for COL1A1 locus,
- C, D and E for COL1A2 locus.

Haplotypes were constructed on the basis of the segregation of the presence/absence of a restriction site (Table 1).

Statistical analysis

Information provided by fully informative non-recombinant meioses at the concordant locus was calculated using Bayes's formula.

Polymorphism information content (PIC) for a marker was calculated by Botstein et al. [12].

PIC of a marker is given by:

$$\mathrm{PIC} = 1 - \left(\sum_{i=1}^{n} p_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 p_j^2$$

where p_i is the frequency of the ith allele.

The markers were considered as highly informative with PIC>0.5, reasonably informative with 0.5>PIC>0.25 and slightly informative with PIC<0.25 [12].

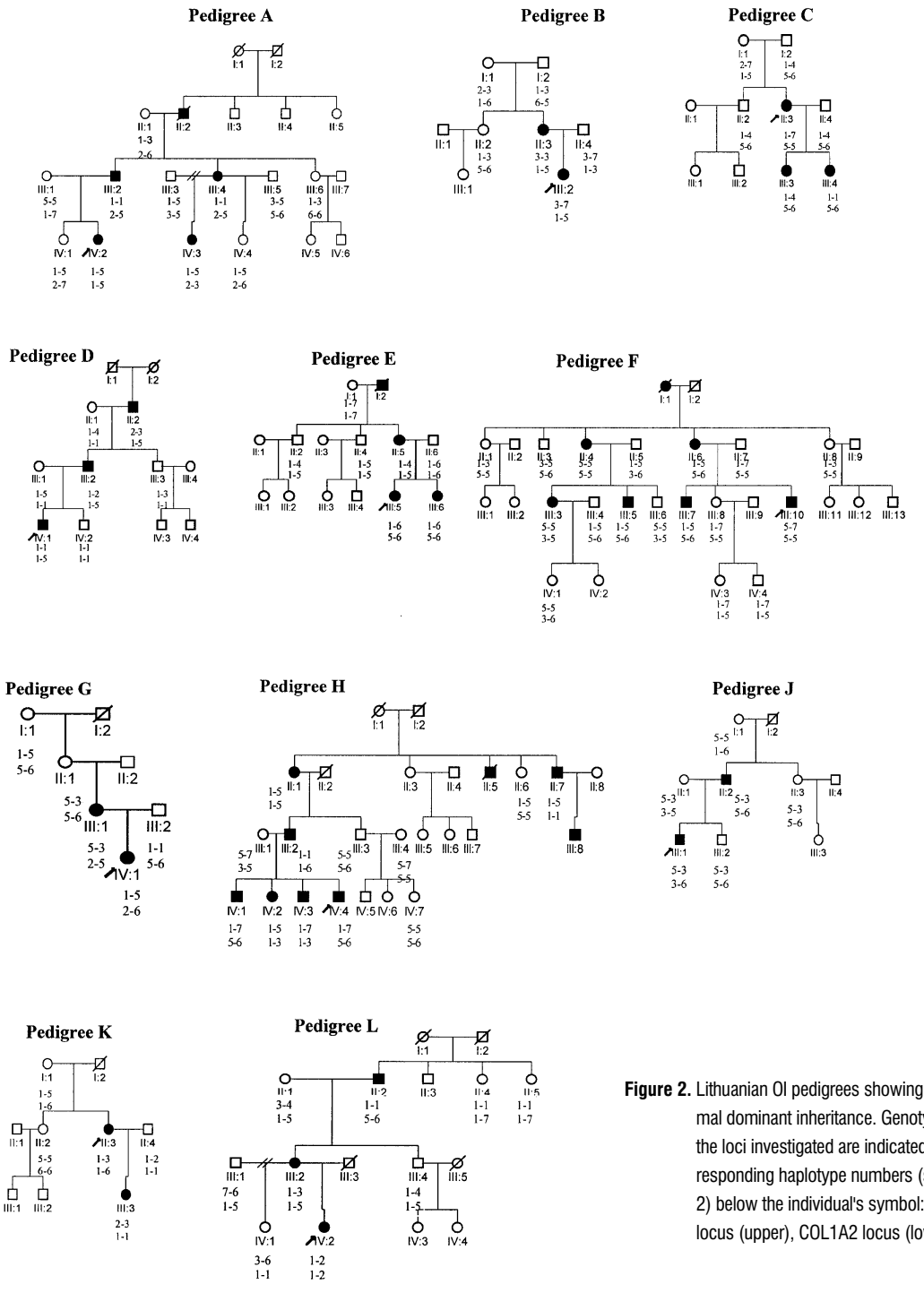

Figure 2. Lithuanian OI pedigrees showing autosomal dominant inheritance. Genotypes at the loci investigated are indicated as corresponding haplotype numbers (see Table 2) below the individual's symbol: COL1A1 locus (upper), COL1A2 locus (lower).

RESULTS AND DISCUSSION

A total of 92 individuals from 11 Lithuanian pedigrees (Fig. 2, A–L) with familial cases of OI were analysed. In all pedigrees investigated in this study OI was inherited as a dominant trait. To reveal a DNA marker segregating with OI in a definite family, pedigree members were tested for the presence or absence of six restriction enzyme sites in the COL1A1 and COL1A2 loci (Fig. 1). The haplotypes at each locus were constructed from the combination of restriction site dimorphisms segregating with OI.

Pedigree A (Fig. 2) is a large OI Sillence type IA family. The proband, IV: 2, was an eight years old girl with dark blue sclerae and normal teeth. She had had four fractures of long bones from the age of two years. Proband's cousin, IV: 3, had similar OI symptoms (seven fractures since 3.5 years of age). Proband's father, III: 2, and aunt, III: 4, were typical of the OI type IA patients in having fractures (up to 10) without limb deformity, with dark blue sclerae, normal stature, normal teeth and arcus senilis. Progressive hearing loss was observed since 30 years of age.

The family study showed that mutant OI linked allele was transmitted to affected individuals, III: 2, and III: 4, and their affected offspring, IV: 2 and IV: 3, from the affected proband's grandfather, II: 2 (deceased). His genotype at the locus must had been haplotypes 5–6 as follows from the genotypes of his children and spouse. Although haplotype 5 allele at this locus seems to be a likely candidate for the linkage with OI in this pedigree, it was not transmitted to the affected proband's cousin, IV: 3. Thus it is reasonable to assume that segregation of OI phenotype with the COL1A2 gene is discordant in this pedigree and, subsequently, the probability that OI is caused by a mutation in the COL1A1 gene can be estimated as 0.95. As affected proband's father, III: 2, and aunt, III: 4, were homozygous for all restriction sites tested at the COL1A1 locus, there were no informative meioses to support the latter assumption. DNA markers used might appear to be informative if relevant genotypes at the 5th generation were available. Otherwise, an additional marker closely linked to the COL1A1 locus is necessary in this pedigree. Bearing in mind a 0.0005 probability of a recombination at the COL1A2 locus leading to a false conclusion regarding the discordance of this locus, a final risk that the disease is linked to the COL1A1 gene in this pedigree can be estimated as 0.9495.

Pedigree B (Fig. 2) is a small Sillence type IB family. The proband, III: 2, was a four years old girl with mid blue sclerae and two fractures since the first year of age. Her mother, II: 3, had had several long bone fractures up to the age of 12. She had normal stature, weak and yellow teeth, dark blue sclerae, progressive mixed deafness which had begun in her thirties and symptoms of dentinogenesis imperfecta (weak yellow teeth).

Family structure did not allow identification of a disease-related allele on the basis of the restriction site dimorphisms tested. Affected proband's mother, II: 3, appeared to be homozygous for all COL1A1 sites thus disabling all assumptions regarding the linkage of OI phenotype to this locus. What concerns the COL1A2 locus, the only conclusion to be drawn is that haplotype 1 allele is not linked to OI phenotype in this family.

Pedigree C (Fig. 2) is a small Sillence type IA family. Phenotypes of all affected individuals in this pedigree (II: 3, III: 3 and III: 4) were typical of this type OI patients: long bone fractures without limb deformity and blue sclerae. Proband, II: 3, was 30 years old woman with normal stature. She had had four fractures since three years of age, the last one in 1998. Her affected daughters, III: 3 and III: 4, each had had two fractures. Their stature was normal for their age.

Segregation of OI phenotype with a COL1A1 gene allele was concordant in this family and haplotype 1 allele cosegregated with the disease. Presence of a two informative non-recombinant meioses increased the final probability of the OI linkage to one of the COL1 loci from 0.95 to 0.975. The COL1A2 locus appeared to be uninformative in this family because the proband, II: 3, was homozygous for haplotype 5. Anyway, the COL1A2 gene haplotype 6 allele is apparently not linked to OI in this case. Additional informative DNA marker is essential for diagnostic analysis of the COL1A2 locus in this family.

Pedigree D (Fig. 2) is a Sillence type IA family. The proband, IV: 1, was a five years old boy with blue sclerae and three fractures since birth without deformity. His father, III: 2, was born with a fracture and had suffered more than 15 since then, while the proband's grandfather, II: 2, had had >90 fractures. Proband's father and grandfather had blue sclerae and progressive mixed deafness since in their thirties.

Molecular genetic investigation showed COL1A1 locus to be discordant in this family because none

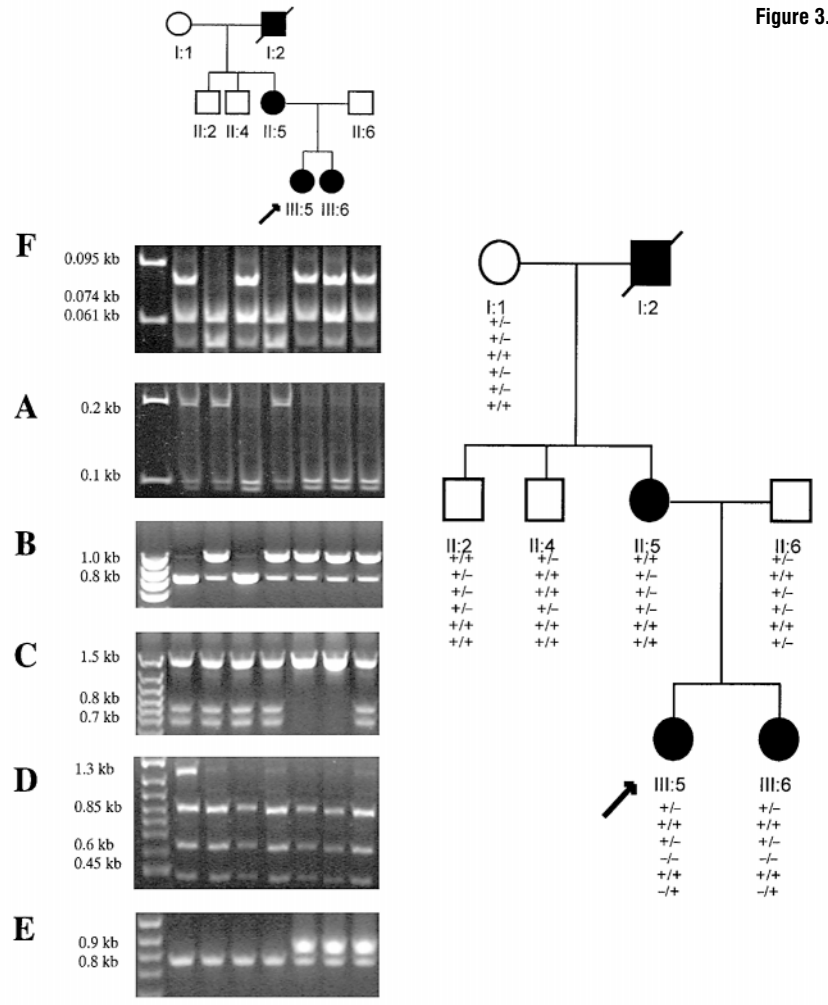

Figure 3. Molecular genetic analysis in the OI family E (fragment of the pedigree E; see Fig. 2, E for full pedigree). Gel electrophoresis (8% PAAG: F, A; 1.5% agarose: B, C, D, E) of PCR products digested with relevant restriction enzymes (see Fig. 1)

of the haplotypes were found to cosegregated with OI. What regards COL1A2 locus, linkage phase of the haplotype 5 allele and OI phenotype was confirmed by four informative meioses: (1) affected proband's father, III: 2, inherited haplotype 5 allele from his affected father, II: 2, while (2) unaffected sibs, III: 3, inherited paternal haplotype 1 allele; (3) proband IV: 1 inherited paternal haplotype 5 allele, while (4) his unaffected brother, IV: 2, inherited paternal haplotype 1 allele. Thus probability of OI linkage to COL1 loci in this pedigree increases from 0.95 to 0.99375. After subtracting the 0.0005 probability that discordance of the COL1A1 is caused by a crossover at this locus, final probability that OI is linked to COL1A2 locus haplotype 5 in this family can be estimated as 0.993.

Pedigree E (Fig. 2) is a Sillence type IV family. The proband, III: 5, was a 16 years old girl. Both proband's, III: 5, and her mother's, II: 5, stature was short, they both had light blue/grey sclerae and less than 10 fractures. The proband had moderately deformed arms. Her mother had weak and yellow teeth.

The first known individual with OI in this pedigree was proband's grandfather, I: 2 (already deceased). Although his DNA was not available for the analysis, his wife's and children's genotypes allowed reconstruction of the I: 2 genotype at the COL1A1 locus as haplotypes 4–5. Both haplotypes at the COL1A1 locus were transmitted to the unaffected sons, II: 2 and II: 4, indicating discordance of this locus in the family. Such assumption was supported by the genotypes of affected grandchildren, III: 5 and III: 6, who did not inherit either haplotype 4 or 5 at theCOL1A1 locus. As for the COL1A2 locus, the only haplotype possibly linked to OI in this pedigree was haplotype 5 transmitted from affected father, I: 2, to affected daughter, II: 5. Normal phenotype of her brothers, II: 2 and II: 4, carrying the same paternal haplotype 5 allele

points to a most likely homozygosity (genotype 5–5) of their father, I: 2, at this locus. This assumption was supported by the fact that affected granddaughters, III: 5 and III: 6, had haplotype 5 allele transmitted from affected grandfather, I: 2, via affected mother, II: 5. Thus concordance of the COL1A2 locus in this pedigree was confirmed by two informative meioses and the probability of the linkage phase of the haplotype 5 and OI phenotype was estimated as 0.987.

Results of the molecular genetic analysis in the OI family E are shown in Fig. 3.

Pedigree F (Fig. 2) is a large OI Sillence type IA family. The proband, III: 10, was typical of other affected family members in being of normal height, having dark blue sclerae, normal teeth, and fractures less than 30 without limb deformity. His brother, III: 7, mother, II: 6, and aunt, II: 4, suffered from progressive mixed deafness which had begun in their thirties.

Pedigree analysis shows that OI linked allele of proband's grandmother, I: 1, must have been transmitted to all affected individuals in the family. At COL1A2 locus, only haplotype 5 allele might be linked to OI according to the genotype of the proband, III: 10. Nevertheless, this gene appears to be discordant in this pedigree because unaffected proband's sister, III: 8, inherited haplotype 5 allele from her affected mother, II: 6, while affected proband's brother, III: 7, did not.

Haplotype 5 allele at the COL1A1 locus appears to cosegregate with OI in this pedigree: it was identified in all affected individuals and absent in all unaffected individuals tested except the proband's uncle, II: 3, who most likely had inherited it from his unaffected father, I: 2. As affected proband's aunt, II: 4, and her affected daughter, III: 3, were both homozygous for this haplotype, this branch of the pedigree appeared to be uninformative regarding linkage of the above-stated allele and OI phenotype. On the other hand, genotypes at COL1A1 locus of the affected proband's mother, II: 6 and her unaffected spouse, II: 7, allowed identification of each allele transmitted to their offspring. In this branch of the pedigree there were three informative meioses confirming the linkage phase of the COL1A1 haplotype 5 allele and OI. Bearing in mind two informative meioses in unaffected pedigree branches originating from the proband's aunts, II: 1, and II: 8, total number of informative meioses confirming linkage phase of this haplotype and OI in this family was 5. Thus the probability of such linkage is 0. 998 in the pedigree F.

Pedigree G (Fig. 2) is a small OI Sillence type IA family in which both affected individuals proband, IV: 1, and his mother, III: 1, had normal stature, blue sclerae, and few fractures.

Maternal origin of the haplotype 5 allele at the COL1A1 locus and haplotype 2 allele at the COL1A2 locus in the proband's, IV: 1, DNA is evident, thus linkage of one of these loci and OI phenotype is most likely in this family. Although data available appeared to be insufficient for the identification of a definite disease linked allele, prenatal diagnosis for subsequent pregnancies in this family is possible to some extent: probability of OI cosegregation with the combination of COL1A1 haplotype 3 and COL1A2 haplotype 5 is just 0.05.

Table 2. Molecular genetic and phenotype features of the Lithuanian OI families investigated

Pedigree	Mutant locus	OI-linked haplotype	Sillence type	Scleral hue	Dentino-genesis imperfecta	Hearing loss	Fractures at birth	Total fracture number	Deformity
A	COL1A1	?	IA	dark blue	-	+	-	<10	-
B	U	?	IB	dark blue	+	+	-	<10	-
C	COL1A1	?	IA	mid blue	-	-	-	<10	-
D	COL1A2	5	IA	mid blue	-	+	+	>50	-
E	COL1A2	5	IV	light blue/grey	+	-	-	<10	-
F	COL1A1	5	IA	dark blue	-	+	-	20-50	+ (mild)
G	U	?	IA	mid blue	-	-	-	<10	-
H	COL1A1	1	IB	mid blue	+	+	-	20-50	-
J	COL1A1	?	IB	mid blue	+	+	-	50	+ (mild)
K	U	?	IB	dark blue	+	+	-	<10	+ (mild)
L	COL1A1	1	IB	dark blue	+	+	-	20-50	-

U - mutant locus is unknown: markers at both COL1 loci are non-informative, ? - not identified, + - feature is present, (-)- feature is absent

Pedigree H (Fig. 2) is a large OI Sillence type IB family. The proband, IV: 4, was a severely affected teenager. He was unable to walk due to a severe osteoporosis and muscular atrophy of legs. He had had more than 50 fractures. Proband's relatives were not severely affected and had had less than 10 long bone fractures up to the age of 14 and only two or three since then. Father, III: 2, and grandmother, II: 1, had weak and yellow teeth and hearing loss.

Although genotypes at the COL1A2 locus identified in the 2nd generation individuals imply possible linkage phase of OI phenotype and haplotype 1 allele in this pedigree, such assumption should be rejected due to the absence of such allele in the 4th generation affected individuals, IV: 1 and IV: 4. Thus the COL1A2 locus appears to be discordant in this pedigree. At the COL1A1 locus, haplotype 1 allele is the only one apparently linked to OI in this family. Such assumption is supported by a single informative non-recombinant meiosis, because the proband's father, III: 2, appeared to be homozygous for this haplotype. Thus final probability of the linkage phase of the COL1A1 locus haplotype 1 allele and OI phenotype in this family is 0.974.

Pedigree J (Fig. 2) is an OI Sillence type IB nuclear family with two affected individuals. The proband, III: 1, was a 12 years old boy with blue sclerae and 9 fractures since birth. His father, II: 2, was severely affected, had weak and yellow teeth, progressive mixed deafness and had had at least 90 fractures with moderate limb deformity.

The COL1A2 gene appeared to be discordant in this family because paternal haplotype 6 allele, although coinherited with the OI phenotype by the proband, III: 1, was also inherited by the unaffected sibs, III: 2. Thus the probability of OI being caused by a mutation in the COL1A1 gene in this family is 0.949. Haplotype analysis at the COL1A1 locus did not allow the identification of a mutant OI-related allele in this nuclear family because both parents, II: 1 and II: 2, and both children, III: 1 and III: 2, were heterozygous for the haplotypes 5 and 3 at this locus.

Pedigree K (Fig. 2) is a small Sillence type IB family. The proband, II: 3was a 33 years old woman with dark blue sclerae, weak and yellow opalescent teeth, progressive hearing loss and four fractures since 1.5 years of age without limb deformity. The proband's two years old daughter, III: 3, had clinical manifestation of OI restricted to dark blue sclerae.

Family structure in this case did not allow identification of a mutant OI linked allele. The only judgement to be made is that OI in this family might be linked to the COL1A1 haplotype 3 or COL1A2 haplotype 1 inherited from the affected individual.

Pedigree L (Fig. 2) is a Sillence type IB family. The proband, IV: 2, was a seven years old girl with blue sclerae and four fractures since birth. Her mother, III: 2, and grandfather, II: 2, had dark blue sclerae, hearing loss and fracture number up to 30 without limb deformity. Their stature was normal, teeth were yellow and weak.

Pedigree structure and molecular genetic data available did not allow the identification of a mutant COL1 allele linked to OI. The COL1A2 locus appeared to be discordant in this case (i.e. none of the haplotypes at this locus identified in affected individuals were co-transmitted with the OI phenotype). Thus there is a 0.95 probability for the COL1A1 gene to carry a mutation leading to OI phenotype in this family. What regards the latter locus, only haplotype 1 allele apparently maintained the linkage phase with the disease, i.e. was present in all three affected individuals (II: 2, III: 2 and IV: 2). Although the proband's grandfather, II: 2, was homozygous for this haplotype, there were two informative non-recombinant meioses supporting this assumption and thus increasing the final probability of OI linkage to the COL1A1 locus to 0.987 (after the subtraction of the 0.0005 probability of a crossover at the COL1A2 locus).

Clinical and molecular features of OI in all families investigated in this study are summarised in Table 2. Analysis of DNA markers segregation with OI phenotype in each family enabled identification of both discordant and concordant COL1 loci in eight out of 11 Lithuanian OI pedigrees: OI Sillence type I segregated with COL1A1 in the pedigrees F, H and L, while OI Sillence type I and IV segregated with COL1A2 in the pedigrees D and E, respectively. The case of OI type IV (Fig. 2, pedigree E) apparently linked to the COL1A2 locus is in accordance with the observation that this form of the disease is most often caused by a mutation in the gene coding for the pro(2(I) polypeptide chain [1,2,8].

DNA marker (a combination of three restriction enzyme site dimorphisms) for a mutant disease-linked allele was established in five Lithuanian OI pedigrees (see Fig. 2 and Table 2), namely D, E, F, H and L. Likelihood for such linkage phase was estimated high enough to enable DNA testing-

Table 3. COL1 loci allele frequencies and PIC values in the sample of 214* chromosomes of Lithuanian Caucasian.

Locus	Site	Allele with a restriction site		Allele without a restriction site		PIC	Combined PIC at the locus
		n	frequency	n	frequency		
COL1A1	F	150	0.59	64	0.41	0.366	0.656
	A	127	0.71	87	0.29	0.327	
	B	152	0.88	62	0.12	0.191	
COL1A2	C	188	0.37	26	0.63	0.357	0.655
	D	79	0.90	135	0.10	0.168	
	E	192	0.70	22	0.30	0.331	

* 214 chromosomes analysed comprise chromosomes of 92 individuals tested in this study and 15 individuals from other OI families not included in this study.

based prenatal diagnosis of OI for the nuclear families in these pedigrees. Some nuclear families in these five pedigrees appeared to be non-informative due to the insufficient heterozygosity of their members for the DNA markers used.

Molecular genetic prenatal diagnosis is available for the 3rd and 4th generation families in the pedigrees D and L and for the 2nd and 3rd generation families in the pedigree E. In the pedigree F, affected individual III: 3 appeared to be homozygous for the COL1A1 haplotype 5, which is a marker for the OI-linked allele in this pedigree. Thus DNA-based prenatal diagnosis is not yet available for the corresponding nuclear family. Prenatal diagnosis for the families of other affected 3rd generation individuals (III: 5, III: 7, III: 10) would be available, provided genotypes of their spouses were informative.

In the pedigree H, affected individual III: 2 was homozygous for the COL1A1 haplotype 1 shown to be a marker for the OI-linked allele in this pedigree, thus being non-informative for prenatal diagnosis in further pregnancies in the family. Nevertheless, prenatal diagnosis of OI will be available for all his offspring (IV: 1, IV: 2, IV: 3 and IV: 4) provided the genotypes of their future spouses are informative.

Out of six remaining pedigrees investigated, three (Fig. 2, pedigrees B, G, K) appeared to be concordant at both COL1 loci due to an insufficient number of informative non-recombinant meioses. Thus prenatal diagnosis cannot be offered at present for the families in these pedigrees. If more informative meioses become available, one locus will eventually become discordant thus enabling the identification a mutant OI-linked locus.

Although genetic information available appeared to be insufficient to establish OI phenotype linkage with a DNA marker in the pedigree G, the data obtained can be useful for future pregnancies in this case because the COL1A1 haplotype 3 allele and COL1A1 haplotype 3 allele were shown to be unlinked with OI. Thus if both these alleles were transmitted from the affected mother, III: 1, to a prospective 4th generation child, the risk for the latter to inherit OI would be just 0.05.

In two pedigrees (Fig. 2, pedigrees A, J) the COL1A2 locus was found to be discordant and the COL1A1 locus was non-informative. Pedigree A, although relatively big, appeared to provide insufficient information for a comprehensive linkage analysis. Thus molecular genetic prenatal diagnosis of OI is unavailable in both families of the affected 3rd generation individuals, III: 2 and III: 4. Genotypes in the 5th generation might provide data enabling further prenatal diagnosis in the prospective families of the affected 4th generation individuals, IV: 2 and IV: 3. In pedigree J prenatal diagnosis of OI is not available for the OI family (2nd and 3rd generations) because all four its members appeared to have identical genotypes at the COL1A1 locus.

One family (Fig. 2, pedigree C), although concordant at the COL1A1 locus, was non-informative at the COL1A2 locus. Thus tracking a mutant COL1 allele and, subsequently, DNA-based prenatal diagnosis of OI was impossible in this family.

Additional informative DNA markers are necessary to enable DNA-based prenatal diagnosis of OI in the pedigrees A, B, C, G, J and K.

It is to be stressed for all OI families applying for DNA-based prenatal diagnosis that the results of indirect identification of a mutant OI-linked allele are not absolute: likelihood of an established linkage phase of a DNA marker and OI phenotype is always less (although slightly) than 1.

To estimate the usefulness of each dimorphic restriction site tested for OI linkage analysis in the Lithuanian OI families, PIC values [12] for each site were calculated after the initial estimation of corresponding allele frequencies in the Lithuanian population sample investigated (Table 3).

The distribution of genotypes in the Lithuanian population sample investigated appeared to be at Hardy–Weinberg equilibrium at all sites tested.

PIC values show that individual dimorphic markers used (i.e. presence or absence of a restriction site) are reasonably informative (sites F and A for the COL1A1 locus, C and E for the COL1A2 locus) or just slightly informative (site B for the COL1A1 locus and site D for the COL1A2 locus) in the Lithuanian population sample investigated. Combination of three restriction sites significantly increases polymorphism of the restriction enzyme-based DNA markers allowing eight different haplotypes at each COL1 locus (see Table 1). In the Lithuanian population sample tested, seven and six different haplotypes out of eight theoretically possible were identified at the COL1A1 and COL1A2 loci, respectively. Thus combined PIC at each COL1 locus appeared to be highly informative.

CONCLUSIONS

1. Combination of three restriction enzyme sites within or close to both COL1 loci provided a marker system informative enough (PIC values were 0.656 at the COL1A1 locus and 0.655 at the COL1A1 locus) for the identification of a linkage phase with OI phenotype in Lithuanian OI families.

2. DNA marker for a mutant COL1 locus was identified in five out of 11 Lithuanian OI pedigrees. Thus prenatal diagnosis of OI based on indirect identification of a mutant COL1 allele is available in the corresponding families.

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