Screening for mutations in patients with osteogenesis imperfecta and estimation of clinical manifestation

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Osteogenesis imperfecta (OI) is a heritable connective tissue disorder caused in >90% cases by dominant mutations in the genes COL1A1 and COL1A2, which encode the proα1(I) and proα2(I) chains of type I procollagen, respectively. Lithuanian OI database comprises 147 case records covering the period 1980–2001. Clinical and genealogical analysis of OI cases/families from Lithuania available for examination revealed 18 familial cases of OI type I and 22 sporadic cases of OI type II (3 cases), OI type III (8 cases) and OI type I (7 cases). As a result, 10 mutations were identified in 12 unrelated patients (eight were novel). Out of them, eight (E500X, R183X, c.2165-2166insCTCTCTAG, c.1787delT, c.1786-1787insC, IVS19+1G>A, IVS20-2A>G, IVS22-1G>T) were null mutations, leading to a mild OI phenotype. A relation between the location and nature of glycine substitution and OI severity was observed for expressed point mutations (G79R, G481A). OI was differently manifested in related patients with the identical genotype and ranged from a mild to severe phenotype. This finding suggests that the phenotypic expression of the disease may be influenced by other factors, genetic or epigenetic, which may play a role in the process of bone formation.

Keywords: osteogenesis imperfecta, collagen, mutations

INTRODUCTION

Osteogenesis imperfecta (OI) is a heritable connective tissue disorder caused in >90% cases by dominant mutations in the genes COL1A1 and COL1A2, which encode the proα1(I) and proα2(I) chains of type I procollagen, respectively [7]. Until recently, more than 300 mutations in these genes have been registered in the Human Collagen Mutations Database [12]. The mutations vary in type and location and result in a disrupted collagen type I architecture by either undersynthesis of non-mutated collagen (null alleles) or by producing abnormal collagen (structural mutations: missense mutations, exon skipping, etc.).

The clinical classification of OI consists of four broad categories based primarily on the degree of bone fragility and other clinical features (stature, bone deformity, osteopenia, scleral hue, dental problems, and hearing loss). The mildest form of OI (type I in the Sillence classification [9]) is caused almost entirely by mutations in COL1A1 that result in null alleles and haploinsufficiency of normal type I collagen. The clinically severe forms of OI (types II, III, and IV) encompass a broad phenotypic range, from lethal through progressive deforming to moderate. They are caused by structural defects in either the α1(I) or α2(I) collagen chains resulting mostly from a Gly substitution within the Gly–X–Y amino acid repeat located within the triple helical domain of the polypeptide. The severity of the OI phenotype is strongly associated with the polypeptide chain type, mutation site, flanking sequences, and amino acid substituting Gly.

The aim of the present study was to evaluate clinical manifestations of OI on the molecular genetic basis of disease in OI patients/families residing in Lithuania.

PATIENTS AND METHODS

Lithuanian OI database comprises 147 case records covering the period 1980–2001. Out of them, 18 familial and 22 sporadic cases were available for detailed clinical examination and molecular genetic testing.

Clinical and genealogical analysis. The OI diagnosis and classification were based on the Sillence

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Table 1. **Phenotypic features of OI families from Lithuania in the case of identified mutation in the COL1A1 gene**

Pedigree	Identified mutation	OI Sillence type	Scleral color	Dentino-genesis imperfecta	Hearing loss	Fractures at birth	Fracture number	Deformity
OI-2	c.2032G>T (E500X)	IB	mid blue	+	+	–	<10	–
OI-3	c.769G>A (G79R)	IA	mid blue	–	–	–	< 10	–
OI-14	c.2046-2166 ins CTCTCTAG	IB	dark blue	–	+	+	> 80	–
OI-15	IVS19+1G>A	I	dark blue	–	–	–	< 10	–
OI-8	c.1668 del T	IB	dark blue	+	+	–	> 50	–
OI-11	c.1667-1668insC	IB	dark blue	+	+	–	< 10	–
OI-1	IVS20-2A>G	IA	dark blue	–	+	–	10	–
OI-23	c.769G>A (G79R)	IA	dark blue	–	–	–	10–20	+
OI-36	c.769G>A (G79R)	III	dark blue	–	+	–	>50	+
OI-37	IVS22-1G>T	IB	dark blue	+	–	–	10–20	–
OI-26	c.1857(G481A)	III	dark blue	+	–	+	5	+
OI-30	c.1081(R183X)	IB	dark blue	+	+	–	<5	–

Note: feature: – absent, + present.

OI classification [9]. The OI cases were classified as familial or sporadic on the basis of genealogical analysis.

Molecular genetic testing. Eighteen familial and 22 sporadic OI cases were available for molecular genetic testing. DNA was isolated from peripheral blood by salting or phenol-chloroform extraction.

Linkage analysis. A total of 100 individuals from 13 OI families were analysed. Three dimorphic restriction site marker systems were used for each gene analysed to distinguish OI-linked alleles. DNA fragments at COL1A1 and COL1A2 loci were PCR-amplified and then digested with restriction enzymes *Mnl*I, *Msp*I, *Rsa*I and *EcoR*I. PCR, restiction enzyme digestion and electrophoresis conditions were used as described by Baker et al. [2], 1991 and Willing et al., 1990 [11].

***DNA heteroduplex analysis*.** Thirty-eight probands with OI were screened for mutations in 42 exons of the COL1A1 gene, using PCR of a relevant exon followed by conformation sensitive gel electrophoresis to detect DNA heteroduplex formation. PCR and electrophoresis conditions were used as described by Korkko et al., 1998 [7] and Ganguly et al., 1995 [6]. The gels were examined in a UV-image analyser (BIO-RAD Gel Doc 1000 Gel Documentation System).

***Direct DNA sequencing*.** PCR products showing DNA heteroduplex formation were sequenced using ABI PRISM™ 310 Sequencer and Big Dye Terminator Sequencing protocol (Perkin Elmer Applied Biosystems).

The mutations reported according to the new guidelines have nucleotides numbered from the first base of the start codon [1].

RESULTS

Clinical and genealogical analysis of OI cases/families from Lithuania available for examination revealed 18 familial cases of OI type I and 22 sporadic cases of OI type II (3 cases), OI type III (8 cases) and OI type I (7 cases).

Linkage analysis in 13 OI pedigrees allowed discrimination between the OI-linked COL1A1 and COL1A2 loci in five families. In three pedigrees OI type I phenotypes segregated with COL1A1 locus markers and in two pedigrees OI type I and type IV phenotypes segregated with COL1A2 locus markers. In the remaining eight pedigrees, the available data appeared to be insufficient to identify a definite OI-linked COL1A locus. As COL1A1 gene mutations had been shown to cause the majority of mild OI (type I) cases, this gene was tested for the presence of OI-related mutations in 38 probands (out of them, 27 cases of OI type I, 3 cases of OI type II, 8 cases of OI type III).

Mutations in eight COL1A1 gene exons showing DNA heteroduplex formation were identified by subsequent direct automated DNA sequencing. As a result, 10 mutations were identified in 12 unrelated patients: missense (4), nonsense (2), frameshift (3), and splice site mutations (3). The data on these mutations and their clinical manifestation are summarised in Table 1. Out of them, eight mutations have not yet been registered in the Human Collagen Mutations Database [12].

DISCUSSION

The great majority (about 85%) of mutations for patients with OI types II, III and IV are **structural mutations**, *i.e.* point mutations that cause the sub-

stitution of glycine (Gly) residues occurring in every third position along the polypeptide chains of type I collagen and thus disrupt higher order collagen formation [4]. The severity of the clinical OI phenotype strongly correlates with the polypeptide chain type (coded by a definite gene), mutation site within the gene, nucleotide sequences flanking a mutation, and an amino acid substituting Gly.

Two single nucleotide substitutions leading to a substituted Gly (G79R, G481A) in the COL1A1 gene were found in the present study in the probands from the families OI-3, OI-23, OI-36 and OI-26 (Table 1). G79R mutation was found in three unrelated patients (two of Polish and one of Lithuanian origin). The clinical manifestation of the identical G79R genotype appeared to be different in these cases (see Table 1): two patients (OI-3, OI-23) had mild OI (type I), while the other one (OI-36) had a severe form of the disease (OI type III). Two cases of this mutation were reported in other populations [5, 8]. A mild OI phenotype was reported for one proband of non-Ashkenazi Jew of mixed Kurdish-Iraqi origin [5]. However, no information (familial backgroud or phenotypic features) was provided on the other patient. Phenotypic variability has already been reported in several instances of OI in both related and unrelated probands with the same collagen I mutation [4]. In general, the severity of the OI phenotype is strongly associated with the specific site of mutation. Different Gly substitutions at the N-terminal half of the protein most often result in a less severe phenotype than substitutions at the C-terminal half. Three missense mutations other than G79R were identified in COL1A1 gene exon 11 in patients with OI type I [12], **conforming to this general rule**. However, one mutation in this exon was found to result in OI III phenotype [3]. It still remains highly speculative why identical mutations in a classical monogenic autosomal dominant disorder lead to such a phenotypic variability. A variety of aspects can be discussed, such as modifier genes or epigenetic factors, but none has been proven as yet.

The majority of other missense mutations leading to OI are “private”. There are some sites in the collagen I loci suggestive of mutational hot spots rather than coincidental occurrence. One such point is codon 238 in the COL1A2 gene, where a Ser substitution has been reported five times and a Cys substitution once [10]. The results of the present study imply the presence of such mutational hot spot in the COL1A1 gene codon 79. These two putative mutational hot spots are in a CpG dinucleotide.

While structural mutations most often lead to severe OI types, **null alleles** resulting from a premature translation termination codon or aberrant RNA splicing merely decrease the production of normal type I collagen, thus leading to a mild OI type. Eight mutations (E500X, R183X, c.2165-2166insCTCTCTAG, c.1787delT, c.1786-1787insC, IVS19+1G>A, IVS20-2A>G, IVS22-1G>T) identified in mild OI type I patients from Lithuania were null mutations due to a premature stop codon arising either directly from a point mutation or indirectly from a frameshift mutation, or from a mutation causing an abnormality in mRNA splicing. Mutations identified in the present study were characterised as null mutations on the basis of the clinical phenotype (see Table 1), although neither COL1A1 mRNA nor polypeptide product had been analysed. OI was differently manifested in related patients with the identical genotype and ranged from a mild to severe phenotype in two families (Tab-

Table 2. **Varying clinical manifestation of OI in the families OI-8 and OI-14**

Pedigree	Generation	OI Sillence type	Scleral color	Dentinogenesis imperfecta	Hearing loss	Fractures at birth	Fracture number	Deformity
OI-8	I	IB	dark blue	+	+	–	5	–
	II	III	dark blue	+	–	+ (during delivery)	80	+
	III	I/III	dark blue	–	–	+ (in uterus)	10 (until 1.5 years of age)	+
OI-14	I	IB	dark blue	+	+	–	0	–
	II	IB	dark blue	+	+	–	1	–
	III	IB	dark blue	+	+	–	7	–
	IV	I	dark blue	–	–	–	10	–
	IV (proband)	III	dark blue	–	–	–	50	+

Note: feature: – absent, + present.

le 2). This finding suggests that the phenotypic expression of OI may be influenced by other factors (genetic or epigenetic) which may play a role in the process of bone formation.

CONCLUSSIONS

1. Ten different COL1A1 gene mutations were identified in 12 unrelated individuals with OI residing in Lithuania. Of them, eight were novel (*i.e*. not yet registered in the Human Collagen Mutation Database).

2. Eight COL1A1 gene mutations (E500X, R183X, c.2165-2166insCTCTCTAG, c.1787delT, c. 1786-1787insC, IVS19+1G>A, IVS20-2A>G, IVS22-1G>T) are apparently null mutations and lead to a mild OI phenotype.

3. OI was differently manifested in related and unrelated patients with identical COL1A1 genotype:

(*a*) G79R mutation caused different OI forms (OI I and OI III) in three unrelated OI families from Lithuania;

(*b*) c.2165-2166insCTCTCTAG and c.1787delT and ranged from mild to severe phenotype in related patients with OI in two unrelated families; the severity of OI was increasing in subsequent generations.

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MUTACIJŲ PAIEŠKA IR JŲ KLINIKINIO PASIREIŠKIMO ĮVERTINIMAS ASMENIMS, SERGANTIEMS *OSTEOGENESIS IMPERFECTA*

S a n t r a u k a

Osteogenesis imperfecta (OI) – paveldima jungiamojo audinio liga, daugiau nei 90% atvejų nulemta dominantinių mutacijų viename iš genų (COL1A1 ar COL1A2), reguliuojančių kolageno I sintezę. Darbo tikslas – tiriant šeimas, kuriose yra sergančiųjų OI, nustatyti ligą lemiančias COL1A1 geno mutacijas ir įvertinti jų poveikį fenotipui. Lietuvoje 1980–2001 m. užregistruoti 149 ligos atvejai. Klinikinė ir genealoginė analizė OI atvejų/šeimų iš Lietuvos išaiškino 18 šeiminių (OI I tipas) ir 22 sporadinius (OI II tipas (3), OI III tipas (8), OI I tipas (7)) ligos atvejus. Nustatyta dešimt mutacijų 12 negimininguų asmenų (aštuonios naujos). Iš jų aštuonios mutacijos (E500X, R183X, c.2165-2166insCTCT CTAG, c.1787delT, c.1786-1787insC, IVS19+1G>A, IVS20-2A>G, IVS22-1G>T) buvo nulinės, lemiančios lengvą OI klinikinį pasireiškimą. Ryšys tarp mutacijos pobūdžio ir lokalizacijos bei ligos sunkumo stebėtas ekspresuotų taškinių mutacijų atveju (G79R, G481A). OI klinikinis pasireiškimas įvairavo (nuo lengvos iki sunkios ligos formos) dviejose šeimose tarp gimininguų asmenų su identišku genotipu. Tai leidžia manyti, jog fenotipiniam ligos pasireiškimui svarbūs ir kiti veiksniai, genetiniai ar epigenetiniai, kurie dalyvauja formuojantis kaulams.