Mutations in the *COL1A1 and COL1A2* genes associated with *osteogenesis imperfecta* (*OI*) types I or III

Aleksandra Augusciak-Duma[1*], Joanna Witecka[1#*], Aleksander L. Sieron[1✉],
Magdalena Janeczko[3], Jacek J. Pietrzyk[3], Karolina Ochman[4], Anna Galicka[5],
Maria K. Borszewska-Kornacka[6], Jacek Pilch[2] and Elzbieta Jakubowska-Pietkiewicz[7]

[1]Department of Molecular Biology and Genetics; [2]Department of Child Neurology; School of Medicine in Katowice; Medical University of Silesia, Katowice, Poland; [3]Jagiellonian University, Collegium Medicum, Chair of Pediatrics, Department of Medical Genetics, Polish-American Children's Hospital, Krakow, Poland; [4]Clinics and Medical Laboratories INVICTA, Genetics Clinic, Gdansk, Poland; [5]Department of Medical Chemistry, Medical University of Bialystok, Poland; [6]Neonatal and Intensive Care Department Medical University of Warsaw, Warsaw, Poland; [7]Department of Pediatric Propedeutics and Bone Metabolism Diseases, Medical University in Lodz, Poland

Although over 85% of *osteogenesis imperfecta* (*OI*) cases are associated with mutations in the procollagen type I genes (*COL1A1* or *COL1A2*), no hot spots for the mutations were associated with particular clinical phenotypes. Eight patients that were studied here, diagnosed with *OI* by clinical standards, are from the Polish population with no ethnic background indicated. Previously unpublished mutations were found in six out of those eight patients. Genotypes for polymorphisms (Sp1 – rs1800012 and *Pvu*II – rs412777), linked to bone formation and metabolism were determined. Mutations were found in exons 2, 22, 50 and in introns 13 and 51 of the *COL1A1* gene. In *COL1A2,* one mutation was identified in exon 22. Deletion type mutations in *COL1A1* that resulted in *OI* type I had no effect on collagen type I secretion, nor on its intracellular accumulation. Also, a single base substitution in I13 (c.904-9 G>T) was associated with the *OI* type I. The *OI* type III was associated with a single base change in I51 of *COL1A1*, possibly causing an exon skipping. Also, a missense mutation in *COL1A2* changing Gly→Cys in the central part of the triple helical domain of the collagen type I molecule caused *OI* type III. It affected secretion of the heterotrimeric form of procollagen type I. However, no intracellular accumulation of procollagen chains could be detected. Mutation in *COL1A2* affected its incorporation into procollagen type I. The results obtained shall help in genetic counseling of *OI* patients and provide a rational support for making informed, life important decisions by them and their families.

Key words: *osteogenesis imperfecta*, *COL1A1*, *COL1A2*, mutation, polymorphism

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✉e-mail: alsieron@sum.edu.pl
*Those Authors contributed equally to this work
#Current address: Department of Parasitology, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec Medical University of Silesia in Katowice, Jedności 8, 41-218 Sosnowiec, Poland
Abbreviations: OI, osteogenesis imperfecta; EMQN, The European Molecular Genetics Quality Network; COL1A1 or COL1A2, procollagen type I genes

INTRODUCTION

Osteogenesis imperfecta (*OI*) is a heterogenic, connective tissue related disorder, manifested by bone fragility, with autosomal dominant, rarely recessive, inheritance and $1/10^4$ to $1/3 \times 10^4$ prevalence (Roughley *et al.*, 2003; Tarnowski & Sieron, 2008; Forlino *et al.*, 2011; van Dijk & Sillence, 2014). The *OI* patients have poor quality of life due to frequent bone fractures, resulting from minimal trauma during regular daily activities. International Nomenclature Group for Constitutional Disorders of the Skeleton has proposed in 2009 classification of *OI* types based on radiological and clinical criteria (Sillence & Rimoin, 1978; van Dijk *et al.*, 2011; Warman *et al.*, 2011). At present, there are 15 types of OI in OMIM. In this work we focused on patients with *OI* types I and III. The type I is the least severe (blue sclera, low bone fracture frequency and slight or no skeletal deformities) (Sillence & Rimoin, 1978). Type III is an opposite progressive dominant form (Sillence & Rimoin, 1978; Sillence *et al.*, 1979; Warman *et al.*, 2011). In type III, in most severe cases the patients may develop respiratory problems leading eventually to death. Those patients which survive present severe health problems affecting their life quality (Tarnowski & Sieron, 2008; van Dijk & Sillence, 2014).

About 90% of *OI* cases are due to causative variants in *COL1A1* and *COL1A2* genes resulting in abnormal collagen I fibrils formation (Forlino *et al.*, 2011). The remaining 10% associate with recessive variants of 19 known or yet to be discovered genes (Dalgleish, 1997; Dalgleish, 1998; Galicka, 2012). Heterogeneity of *OI* symptoms and causative gene variants imposes complex modifying factors: genetic, epigenetic and environmental. Polymorphisms in *COL1A1* and *COL1A2* are among the factors influencing individuals to cope differently with mutational effects at the molecular level. Recently, more than 50 polymorphisms were identified in both the genes in the Indian population (Stephen *et al.*, 2014). Two most common polymorphisms associated with diminished bone mineralization and lower resistances to trauma causing fractures are Sp1 in *COL1A1* (Grant *et al.*, 1996; Dehghan & Pourahmad-Jaktaji, 2015), and *Pvu*II at position g.23065 in exon 25 of *COL1A2*. Sp1 is linked to low bone mineral density (BMD), osteoporosis, higher risk of bisphosphonate induced osteonecrosis of the jaw and an elevated risk of developing inguinal hernias (Katz *et al.*, 2011; Kurt-Sirin *et al.*, 2014; Sezer *et al.*, 2014). The *Pvu*II polymorphism is linked to variations in BMD, e.g. in pre-pubertal girls a transversion A→C in g.23065 („p" allele) is strongly correlated with fracture frequency and variable bone strength parameters (Blades *et al.*, 2010). In a dedicated database, Osteo-

genesis Imperfecta Variant Database there are 86 total public entries for *COL1A1* and 58 total public entries for *COL1A2* for Caucasian – Polish ethnic origin. No extensive comparison of both SNPs' frequency in neither Polish nor in general Caucasian population is available. Therefore, the two polymorphisms already linked to bone density but not to *OI*, were also analyzed along with the mutations in procollagen type I encoding genes. Therefore, the aim of this work was to verify if there is a link between the clinical diagnosis of the OI type, the mutation detected in the affected patient and the individual status for the two SNPs, Sp1 and *Pvu*II.

MATERIALS AND METHODS

Patient data. Clinical characteristics were collected by physician specialists in medical genetics who also co-author this report. The patient's medical and family genetic histories were prepared based on "Clinical check list" according to EMQN guidelines for the diagnosis of OI patient, which for our patients was translated to Polish.

Methods

Materials. DNA was isolated from blood samples or skin fibroblasts obtained from five females (F) and three males (M) diagnosed with OI types I or III (Table 1). The study protocol was approved by the Bioethical Committee of Medical University of Silesia in Katowice, Poland (Approval No. KBET/356/B/2003).

Isolation and purification of genomic DNA. DNA isolation from blood or skin fibroblasts was conducted using DNA purification kit "Blood Mini" (A&A Biotechnology, Poland).

Conditions for amplifying and DNA sequencing PCRs. The PCRs were conducted using FastStartTaq DNA Polymerase kit (Roche, Germany) and the amplicons were verified. The reaction mixtures were prepared as described elsewhere (Witecka *et al.,* 2008; Majka *et al.,* 2013). The amplicons were verified following electrophoresis in 1% agarose gel using 5 µl of the obtained PCR products.

Sanger's enzymatic method was used for DNA sequencing, therefore, 20 ng of each purified PCR product was mixed with 5 pM of the appropriate sequencing primer, for SpI: 5'-TCT-GGG-GAG-CCG-CTA-GCG-CGG-3'; for *Pvu*II: 5'-TTT-CAT-CCG-TGG-CAG-CAT-CAT-AAG-C-3' with Big Dye Terminator v.3.1. The sequencing PCR was conducted according to a protocol described elsewhere (Witecka *et al.,* 2008; Majka *et al.,* 2013).

Purified PCR sequencing products were analyzed using genetic analyzer ABI 3130xl (Applied Biosystems, USA) and the sequences were verified using Chromas Lite 2.01 and Nucleotide Blast (NCBI) software online to reference sequences NG_007400.1 for *COL1A1* and NG_007405.1 for *COL1A2*.

Fibroblast culture, procollagen type I separation and SDS PAGE/western blot analysis. Fibroblast cells were obtained from skin biopsies as previously described (Witecka *et al.,* 2008). The cells were cultured in T75 (75 cm²) culture flasks in Dulbecco's Modified Eagle's Medium supplemented with glucose (4.5 g/L), fetal calf serum (10%), penicillin (10 000 IU/mL), streptomycin (10 mg/L), amphotericin B (25 mg/mL) (all reagents from PAA Laboratories, GmbH). The cells were cultured until reaching 80% of confluence. Then the cell monolayer was washed twice with sterile PBS and supplemented with medium free of antibiotics and serum. Following

Table 1. Summary of characteristics of OI patients participating in this study.

Patient number/gender (M, male; F, female)/age	Patients medical history					Physical examination					X-ray changes	Suspicion of no accidental fractures	Reason for referral	Patient's family genetic history
	Total fractures number	Fractures before birth	Fractures after birth	Self-handling	Orthopedics	Hearing problems	Stature	Bone deformities	Sclera	Dentinogenesis imperfecta				
19/F/39	170	17	153 (last at 26 years of age)	Neither walks nor stands -alone (wheel chaired)	Repeated numerous orthopedic surgeries	Correct	Significantly short	YES	Blue	NO	No description	YES	Unspecified type of OI	NONE

55/F/41	3	N.D.	N.D.	Not reported	Not reported	Partial hearing loss on one side	Normal	NO	Blue	YES	N.D.	YES	Unspecified type of OI	Son, *OI* type I; Daughter, *OI* type I
56/F/8	4	N.D	4	Not reported	Not reported	Correct	Short stature 5%	NO	Blue	NO	N.D.	NO	*OI* diagnosis	Mother, *OI* type I; Brother, *OI* type I
57/M/11	3	N.D.	3	Not reported	valgus knee	Correct	Normal	unspecified	Blue	NO	N.D.	YES	*OI* diagnosis	Mother, *OI* type I; Sister, *OI* type I
73/F/11	9	0	9	Fully independent	No other problems except bone fractures	NO	Normal	NO	Blue	NO	YES (no description)	YES	*OI* diagnosis	None
91-D/F/34	20	0	20 last at 16 years of age	N.D.	N.D.	Partial loss on both sides	Normal	NO	Blue	NO	YES (no description)	NO	Unspecified type of *OI*	Son diagnosed with *OI* type I
137/M/neonate	11	11	N.D.	Infant difficult self-breathing (respiratory dyspnea)	Narrow rib cage, abnormal positioning of limbs, soft skull bones		16 percentile	Short	Blue/gray	N.A.	YES (no description)	YES	Prenatally postulated *OI* type II/III	None
G9/M/9	3	0	3	N.D.	Low bone density	NO	An ankle valgus, shortened left leg, triangle face,	Scoliosis, mild vertebral compression fractures, ptosis	Blue	YES	YES (no description)	Not reported	Postulated *OI* type III	N.D.

N.D., No Data; N.A., Not Applicable

subsequent 48 hours, the medium was harvested, supplemented with the mixture of protease inhibitors in Sigma-Fast Protease Inhibitor Coctail Tablet (Sigma, Germany) and divided in to two parts. One part was mixed with 0.25 volume of 5 × storage buffer to provide a final concentration of 25 mM EDTA and 0.04% NaN$_3$ in 0.1 M Tris/HCl buffer (pH 7.4). The samples were filtered through a 1.6 mm glass-fiber filter (Millipore). Proteins from one part were precipitated overnight at 4°C with 176 mg/ml (NH$_4$)$_2$SO$_4$. The precipitate was separated by centrifugation at 15 000 × g for 30 minutes.

The other part of the medium was precipitated with 30% polyethylene glycol (PEG) at its final concentration of 5%. The precipitated proteins were recovered by centrifugation as from the previous part and both parts were stored at –20°C until their further analysis by SDS PAGE, followed by electro-transfer to Immobilon Transfer Membrane (Millipore, USA) and western blot analysis.

Simultaneously, fibroblasts used for procollagen production were detached using 0.25% trypsin in 1 mM EDTA (PAA Laboratories, GmbH). The trypsin and EDTA were neutralized with an equal volume of serum free culture medium and the fibroblast numbers were determined in samples pre-stained with Trypan Blue Dye, (0.4%) using TC20 Automated Cell Counter (Bio-Rad). Cold lysis solution (0.1% NP40 and 10μg/ml DNase I in PBS) was used at the volume enabling to obtain the fibroblast density of 20 000 cells per 1 μL.

Proteins precipitated with ammonium sulfate were solubilized in a storage buffer (0.4 M NaCl, 0.1 M TRIS-HCl, 0.025 M EDTA, 0.04% NaN$_3$; pH 7.4). The proteins, precipitated with PEG, were solubilized in 1 x SB buffer (0.25 M Tris-HCl, pH 6.8; 50% glycerol, 5% SDS, 0.05% bromophenol blue, 0.1% β-mercaptoethanol) in a volume equal to the volume of solution used for lysis of fibroblasts recovered from the culture (all reagents were purchased from Sigma-Aldrich Co.). Total protein concentrations were determined using a spectrophotometer (Nanodrop2000, ThermoScientific, USA) at 214 nm and 280 nm wavelengths. The same amounts of total proteins were analyzed for presence of procollagen type I by Western blotting, using Monoclonal Anti-collagen type I produced in mice (#C2456, Sigma).

RESULTS

Patients' characteristics

The patient's clinical phenotypes are summarized in Table 1. Six patients were clinically diagnosed as type I OI and two as type III. The initial diagnoses of five patients were without specification of the type of OI due to inconsistent examination protocols in different medical institutions (Table 1). In such situations, further examinations and additional medical tests were performed for complete clinical diagnosis of the type of OI (Table 2). For the three patients with OI type recognized initially, had their clinical diagnosis confirmed. Finally, four women were diagnosed with type I and one female was diagnosed with type III. In addition, the males had their OI confirmed as type I and III. Our observations indicate that a full panel of recommended clinical tests and examinations is required for complete diagnosis of the OI type. Particularly, X-rays of skeleton that in our study were available only for some of the patients, are necessary for correct diagnosis of the OI type (Fig. S1 at www.actabp.pl).

New mutations detected in *COL1A1* and *COL1A2* DNA sequences

An analysis of chromatograms obtained by sequencing of the PCR products revealed six heterozygous sequence changes (Table 2). Subsequent alignment of the sequences with respective reference sequences for *COL1A1* and *COL1A2* revealed three mutations in *COL1A1* gene in four females and in one male, and two other mutations in two males (Table 2). In the *COL1A2* gene, one previously unreported mutation was found (Table 2). At least one, but possibly three mutations, two in *COL1A1* and one in *COL1A2* genes, were inherited based on the family genetic history, whereas other mutations were detected in unrelated patients, without a family genetic history

Table 2. Summary of clinical diagnosis and DNA sequencing results of OI patients participating in this study.

Order No.	Patient's No./Gender	Confirmed type of OI	Mutated gene	Location of mutation (E-exon; I-intron)	Position in cDNA	Mutation at protein level	Genotype of Sp1 polymorphism (rs1800012)	Genotype of *PvuII* polymorphism (rs412777)
1	91-D/F	I	COL1A1	E2	c.231delG	p.Thr78Pro/fs*76	SS (G/G)	Pp (A/C)
2	G9/M	I	COL1A1	I13	c.904-9 G>T	not applicable	SS (G/G)	PP (A/A)
3	55/F	I	COL1A1	E22	c.1503delT	p.Ala502Leu/fs*5	SS (G/G)	Pp (A/C)
4	56/F	I	COL1A1	E22	c.1503delT	p.Ala502Leu/fs*5	SS (G/G)	Pp (A/C)
5	57/M	I	COL1A1	E22	c.1503delT	p.Ala502Leu/fs*5	SS (G/G)	Pp (A/C)
6	73/F	I	COL1A1	E50	c.[3881A>T; 3882_3891 del	p.Glu1294Val/fs*32	SS (G/G)	pp (C/C)
7	137/M	III	COL1A1	I51	c.4248+1G>A/ possible exon skipping	not applicable	SS (G/G)	PP (A/A)
8	19/F	III	COL1A2	E22	c.1207G>T	p.Gly403Cys	SS (G/G)	Pp (A/C)
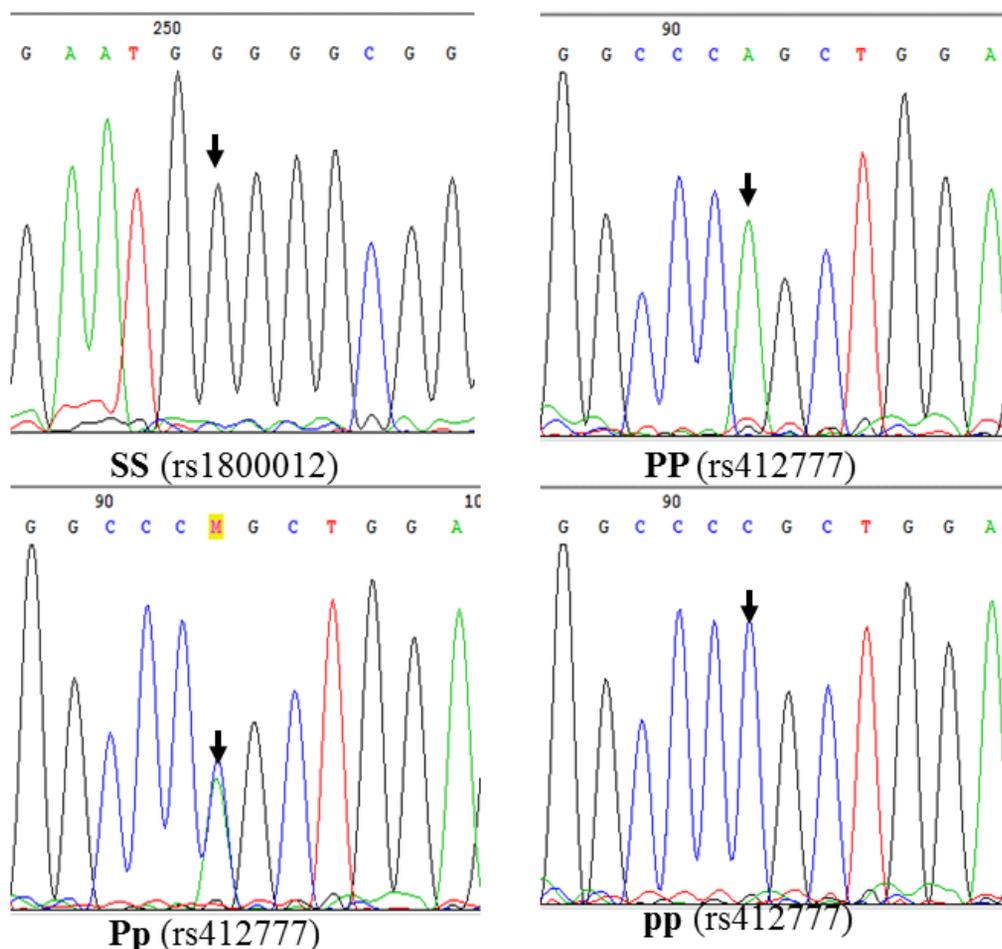
Figure 1. Selected representative chromatograms of the sequence around the *Sp1* and *Pvu*II polymorphic sites.
Polymorphism position g.6252 in *COL1A1* in patients with homozygous wild type (**upper left panel**). Polymorphism position g.23065 in *COL1A2* in patients with: homozygous wild type (**upper right**), heterozygous (**bottom left**) and homozygous recessive (**bottom right**). Arrows point to the polymorphic sites.

(Table 1). All of the mutations affected the coding sequences. In *COL1A1,* three mutations were single nucleotide deletions with subsequent frame shifts resulting in nonsense codons located 5, 32 or 76 nucleotides downstream of the deletion. Additionally, in one female patient the deletion was next to another missense mutation (73/F). In two patients, two mutations, both changing G>T/A, occurred in introns. In *COL1A2,* the mutation was a missense mutation changing glycine to cysteine in a female with type III *OI*.

The polymorphisms detected in collagen encoding genes

An analysis of sequencing chromatograms of the respective polymorphism sites in the genomic DNA, revealed that all of the patients were homozygous for wild type Sp1 polymorphism in the *COL1A1* gene (Fig. 1 and Table 2). The *Pvu*II polymorphism was detected in all three possible combinations (Fig. 1 and Table 2). Three females, two with type I and one with type III *OI,* were heterozygous for the *Pvu*II polymorphism. Two males, one with type I and the other one with type III *OI,* were homozygous for the wild type (PP) genotype. The type I *OI* 73/F patient with a missense mutation followed by an immediate single base deletion and subsequent nonsense codon was homozygous for mutated allele of the *Pvu*II polymorphism (pp). It is surprising that a single

nucleotide deletion accompanied by homozygous "pp" polymorphism resulted in just a mild clinical phenotype.

Effect of the mutations on secretion of procollagen type I

The analysis of secreted procollagen type I from fibroblasts of three patients, two with *OI* type I and one with *OI* type III, revealed that in case of mutations in *COL1A1* no retention of procollagen alpha chains in the cells could be detected (right panel in Fig. 2A). However, alpha 2 chain could be detected by western blot analysis only as a fine band in precipitates from the culture medium obtained from fibroblasts of patients 91-D/F (*OI* type I) and 19/F (*OI* type III) (left panel in Fig. 2A). Procollagen alpha chains identified in the fibroblast culture medium of 73/F the patient (*OI* type I) migrated faster than the procollagen chains from control fibroblasts (lane marked 73/F in the left panel of WB blot in Fig. 2A). This resulted from a mutation in exon 50 of *COL1A1*, c.3881A>T. The mutation changed Glu at position 1294 to Val (Table 2). There was also a deletion of 9 nucleotides from position 3882 to 3891 (Table 2), which resulted in frame shift and a subsequent stop codon following the next 32 codons. In the culture medium collected from fibroblasts of the 19/F patient (*OI* type III) carrying a mutation in exon 22 of *COL1A2* (c.1207G>T) which changes one codon (Gly403Cys),

Figure 2. Effect of detected mutations on procollagen type I production, secretion and potential properties of the proteins.
(A) detection of procollagen alpha chains in the fibroblasts and their culture medium. **(B)** Alignment of amino acid sequences around the mutations (underlined capital letter) and the nonsense codon (*). **(C)** Location of the Gly403Cys mutation (highlighted in red) in the type I procollagen α2 chain and potential sites of interaction with interleukin 2, decorin and $α_2β_1$ integrin. Boxed are Gly-Xxx-Hyp-Pro sequences potentially involved in triple helix stabilization. The numbering of nucleotides and amino acids are for sequences in cDNA encoding a triple helical domain in two $α_1$ and one $α_2$ chains of procollagen type I, respectively. Symbols in **(A)** M, purified human procollagen type; C, proteins recovered from the culture medium of control skin fibroblasts (**left panel**) or the lysed fibroblasts (**right panel**); 73/F, proteins recovered from the culture medium of fibroblasts obtained from the 73/F patient (**left panel**) or the lysed fibroblasts (**right panel**); 19/F, proteins recovered from the culture medium of fibroblasts obtained from the 19/F patient (**left panel**) or the lysed fibroblasts (**right panel**); 91-D/F, proteins recovered from the culture medium of fibroblasts obtained from the 91-D/F patient (**left panel**) or the lysed fibroblasts (**right panel**); Pro $α_1$(I), alpha 1 chain of procollagen type I; Pro $α_2$(I), alpha 2 chain of procollagen type I; ?, cross reaction of the anti-human procollagen type I antibodies with unidentified proteins or possible products of crosslinking of procollagen type I alpha chains (**left panel**) or intracellular degradation of procollagen type I alpha chains (**right panel**); an asterisk, indicates position of migration of intracellular alpha 1 chain of procollagen type I. Western blot pictures are representative of experiments done in quadruplicate. The cultures of fibroblasts for procollagen analyses were repeated and the western blots from each experiment were also done in duplicate.

only a very fine band corresponding to procollagen type I alpha2 chain was detected, whereas pro-alpha1 chain was represented by a much stronger band, indicating the ratio of $α_1$: $α_2$ to be higher than 2:1.

Although fibroblasts from the 91-D/F patient with a mutation in exon 2 (c.231delG) causing a frame shift (p.Thr78Pro), and a stop codon 76 codons downstream of the change, secreted the heterotrimeric procollagen type I, the pro-$α_2$ chain was present in the culture medium at a lower amount than in the control (Fig. 2A).

An analysis of proteins in lysates of cultured fibroblasts following SDS/PAGE and WB did not reveal intracellular retention of pro-alpha chains in any of the analyzed fibroblasts. However, some non-specific cross-reaction of the anti-human type I procollagen antibodies was detected in lysates from all fibroblasts, including the control ones (Right panel in Fig. 2A). It is possible that the cross reaction of the antibodies was due to the pres-

ence of products of partial degradation of the type I procollagen chains. Poor detection of procollagen type I in the lysates of fibroblasts from patients 19/F and 91-D/F needs to be further investigated (Fig. 2A, right panel). In addition, problems with determination of thermal stability of procollagen type I in all three cases were probably due to the poor protein yields from the culture medium of the fibroblasts obtained from the OI patients (data not shown).

DISCUSSION

Mutation locations analyzed in this paper did not point directly to type of *OI*. The resulting changes in the encoded amino acids are not sufficient for predicting how severe the disorder will be. The consistence of the glycine codon changes to the cysteine codon (at position 403 in patient 19/F) correlates with commonly accepted

Gly to Xxx substitutions resulting in a more severe type III or a lethal type II of OI (Byers *et al.*, 1991; Roughley *et al.*, 2003;). In addition, the possible exon skipping effect of I51 mutation at the 4248+1 position could explain the more severe type III phenotype manifested by a tremendous number of bone fractures (170 incidents within 26 years).

Presence of both "PP" and "SS" genotypes was linked to a two-fold lower risk of long bone fracture in children (Mann *et al.*, 2001; Blades *et al.*, 2010; Kurt-Sirin *et al.*, 2014). The homozygous wild type status for both polymorphisms did lower the risk of bone fractures by half. Results reported by others revealed that the presence of at least one copy of the "s" allele of Sp1 polymorphism in *COL1A1* gene increased the risk of long bone fractures prior to maturation by three-folds also in healthy children (Pace *et al.*, 2008). No such correlation was found in dual energy X-ray absorptiometry results (DXA – means of measuring bone mineral density) among those children. Here, we did not find such a correlation probably because all of the patients were homozygous for wild type allele of the Sp1 polymorphism. The genotypic status for *Pvu*II polymorphism had no effect on bone fractures in patients with *OI* type III (Table 1).

Faster migration of procollagen alpha chains in patient 73 could be a result of weaker posttranslational modification, probably glycosylation, due to the C-propeptide of pro-α_1 chain shortened 139 amino acids (Top alignment in Fig. 2B). In addition, it has been reported previously that correct proα_2(I) C-propeptide is critical for procollagen assembly (Suuriniemi *et al.*, 2006). Therefore, one could speculate that the disturbance in the registry of the three procollagen chains due to different lengths of the C-propetides from the three alpha chains, could lead to procollagen suicide. However, the non-mutated chains could be abnormally under-hydroxylated as an effect of insufficient amount of newly synthetized type I procollagen. Therefore, it could be processed faster through the ER and Golgi apparatus and more quickly secreted.

Disturbed ratio of procollagen chains in patient 19/F could be explained by shortage of the α_2 chain, as α_1 homo-trimer was secreted along with trace amounts of the hetero-trimer (Fig. 2A). The mutation is located in the region flanked by Gly-Xxx-Hyp triplets in both, α_1 and α_2 chains (Fig. 2C). This fragment also contains amino acids forming sites for interactions with interleukin 2, decorin and $\alpha_2\beta_1$ integrin (Di Lullo *et al.*, 2002) (Fig. 2C). Therefore, substitution of a small glycine residue by a larger cysteine in the pro-α_2(I) might disturb the structure of the triple-helix, decrease its stability and disturb binding sites for the above listed factors.

In case of patient 91-D/F it is possible that this extremely short polypeptide, containing just 152 amino acids due to a stop codon (bottom sequence in Fig. 2B), and mutated pro-α_1 chain could be degraded, therefore, causing insufficient amount of procollagen type I to be secreted.

Results obtained by detection of mutations in genes encoding procollagen type I in patients diagnosed with either type I or III OI revealed that the causative mutations might occur anywhere in both genes. The clinical outcome resulting from these mutations is complex and the severity is difficult to predict based only on the type and location of the mutation, particularly when the mutation is dominant. Although recommendation for *OI* diagnosis based on a clinical exam and verified by DNA gene sequencing, but without protein analysis, is sufficient for clinical treatment, it seems to be insufficient for genetic counseling of both, the patient and the patient's parents. Therefore, more research is needed on effects of pathogenic mutations on cell biology.

Conflict of interest

All authors declare no conflict of interests.

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