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LIDIIA ZHYTNIK

Inter- and intrafamilial diversity based on genotype and phenotype correlations of Osteogenesis Imperfecta

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Department of Traumatology and Orthopeadics, Institute of Clinical Medicine, University of Tartu, Estonia

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II. **L. Zhytnik**, K. Maasalu, A. Pashenko, S. Khmyzov, E. Prans, S. Kõks, A. Märtson. "*COL1A1/2* pathogenic variants and phenotype characteristics in Ukrainian Osteogenesis Imperfecta patients". *Front Genet*. 10:722, 2019.

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Paper I: Conceiving the study; performing genetic analysis and interpreting data; preparing figures and drafting the manuscript.

Paper II: Conceiving the study; participation in the design of the study; interaction with the patients; carrying the genetic analysis; performing the data analysis; preparing figures and drafting the manuscript.

Paper III: Conceiving the study; participation in the design of the study; carrying the genetic analysis; performing the data analysis; preparing figures and drafting the manuscript.

Paper IV: Conceiving of the study; participation in the design of the study; interaction with the patients; carrying the genetic analysis; performing the data analysis; preparing figures and drafting of the manuscript.

Paper V: Conceiving of the study; participation in the study design; performing the data analysis and interpreting data; preparing figures and drafting the manuscript

“Natural forces within us are the true healers of the disease.”
Hippocrates

ABBREVIATIONS

aa	Amino Acid
ACMG	American College of Medical Genetics and Genomics
AD	Autosomal Dominant
AR	Autosomal Recessive
BMD	Bone Mineral Density
BRIL	Bone-Restricted Interferon-induced transmembrane protein-Like protein
CADD	the Combined Annotation Dependent Depletion
cDNA	complementary DNA
DI	Dentinogenesis Imperfecta
DN	Dominant Negative
ECM	Extracellular Matrix
EDS	Ehlers-Danlos Syndrome
EDTA	Ethylenediaminetetraacetic Acid
EE	Estonian
ER	Endoplasmic Reticulum
gDNA	genomic DNA
HPC	Hyperplastic Callus
INCDS	International Nomenclature group for Constitutional Disorders of the Skeleton
Indels	Insertions-deletions
LoF	Loss-of-Function
MALEP	Methionine, Alanine, Leucine, glutamic acid and Proline
MLBR	Major Ligand Binding Region
NCP	Noncollagenous Protein
NGS	Next-Generation Sequencing
NMD	Nonsense Mediated mRNA Decay
OI	Osteogenesis Imperfecta
(O)MIM	(Online) Mendelian Inheritance In Man database
OP	Osteoporosis
P3H1-CRTAP-PPIaseB	Prolyl 3-Hydroxylase 1-Cartilage-Associated Protein-Peptidyl-Prolyl *cis–trans* Isomerase B protein complex
PCR	Polymerase Chain Reaction
PTC	Premature Termination stop Codon activation
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
UA	Ukrainian

UT OI biobank	Osteogenesis Imperfecta database of the Clinic of Traumatology and Orthopedics, University of Tartu, Estonia
UTR	Untraslated Region
VN	Vietnamese
VUS	Variant of Unknown Significance
XR	X-linked

1. INTRODUCTION

The earliest documented case of Osteogenesis Imperfecta (OI) dates back to 1000 BC. Paleopathological analysis of the skeletal remains of an Egyptian mummy of a 3-day-old infant, has shown, that he was affected with severe lethal OI (i.e. skull and long bones deformities, Dentinogenesis Imperfecta (DI), thin bones) (Lowenstein, 2009; Tainmont, 2007). There are 1700 known, published works regarding bone brittleness, numerous fractures and bone deformity reporting in medical literature (Axmann, 1831; Ekman, 1788; Reiske, 1776; Vrolik, 1849; Weil, 1981). For almost 300 years of OI medical history the disorder was described using various eponyms, which along with phenotype variety, presented difficulties for classifying OI cases as a single disease (Table 1) (Baljet, 2002; Weil, 1981). It was not until the late 1800's that the common nature of brittle bone diseases under different eponyms was established via histological analysis (Looser, 1906; Schmidt, 1897; Stilling, 1889). In 1924 it was agreed that the OI bone phenotype was caused by abnormal collagen and disturbed osteoblast maintenance (Bauer, 1920; Knaggs, 1924). A majority of OI cases involve pathogenic mechanisms, altering collagen type I structure, synthesis, post-translational modifications, trafficking, processing and secretion (Marini et al., 2017). Collagen type I is the most abundant structural protein in the human organism, found in bone, teeth, lungs and blood vessels, thus symptoms of OI disturb not only bone tissues but many others including ligament, dentin and sclera (Van Dijk and Sillence, 2014). Since 1912 the famous classical triad of main OI characteristics has included: blue sclera, frequent fractures and deafness (Weil, 1981). Later, symptoms such as dental, cartilage and blood vessel defects were added (Bauer, 1940). The modern conception of OI is based on the works of David Sillence, who unified mild and severe bone fragility cases into a single picture of OI disorder, providing OI classification (Sillence, 1981; Sillence et al., 1979b; Sillence and Rimoin, 1978).

OI is a genetically and phenotypically heterogeneous disorder, thus the prevalence of OI depends on the OI type being considered, where milder OI forms are more prevalent compared to severe OI forms (Martin and Shapiro, 2009). Studies in countries with extensive medical databases reported the prevalence of OI as occurring in 1.5/10,000 and 0.5/10,000 living births (Folkestad et al., 2016; Kuurila et al., 2002). Marini *et al.* proposed that the overall OI incidence as 1/10,000 (Marini et al., 2017). However, other researchers estimate OI prevalence as 1/20,000 (Van Dijk and Sillence, 2014). Collagen-related OI cases are thought to be distributed evenly among populations. However, there might be some differences among OI forms. For instance, the proportion of collagen type I pathogenic variants is higher in mild OI, compared to severe and moderate forms of the disorder (Bardai et al., 2016; Marini et al., 2017).

Table 1. Eponyms of Osteogenesis Imperfecta (adapted from Weil 1981).

Adair-Dighton disease (syndrome)	Malacia myeloplastica
Aplasia periostalis	Maladie de Durante
Blegvad-Haxthausen syndrome	Micromelia annularis (chondromalacia)
Blue sclera syndrome	Molities ossium
Blue sclerotics and brittle bones (fragility of bones)	Osseous fragility
Blue scleras and fragilitas ossium	Osteogenesis imperfecta congenita (Vrolik)
Brittle bones and blue sclerae	Osteogenesis imperfecta (familiaris) (tarda)
Dark sclerotics and fragilitas ossium	Osteomalacia congenita
Dysplasie periostale	Osteomyopathia
Dystrophie periostale	Osteoporosis foetalis
Eddowe disease (syndrome)	Osteopsathyrosis idiopathica (Lobstein)
Ekman syndrome	Osteopsathyrosis (foetalis) (hereditaria)
Ekman-Lobstein syndrome	Ostitis parenchymatosa chronica
Fetal rickets	Rachitis congenita (foetalis annularis)
Fragile bones, Fragility of the bones	Spurway-Eddowes syndrome
Hereditary fibrous osteodysplasia	van der Hoeve syndrome
Hereditary hypoplasia of the mesenchymez	Vrolik disease (syndrome)
Lobstein disease	

During the early stage of genetic research, OI was considered as a hereditary collagen type I disorder (Barsh and Byers, 1981; Brookes et al., 1989; Byers et al., 1988; Pope et al., 1985; Spotila et al., 1991; Wallis et al., 1986). It was concluded, that pathogenic variants in the collagen type I genes give rise to all OI types (Wallis et al., 1986). Interestingly, estimating the genotype-phenotype correlation in collagen-related OI is still challenging to a certain extent, as patients who harbor identical pathogenic variants develop phenotypes of different severity (Dalgleish, 1998).

Understanding OI remained challenging, as the genetic cause of OI had not yet been identified in some patients. Surprisingly, these patients phenotypically resembled patients with classical *COL1A1/2* pathogenic variants and Sillence OI types (Jay R. Shapiro et al., 2013). With the availability of next generation sequencing techniques (NGS) the involvement of other genes responsible for OI has been confirmed. Within a few years 10 non-collagen OI genes were elucidated (Asharani et al., 2012; Becker et al., 2011; Martínez-Glez et al., 2012; Semler et al., 2012; van Dijk et al., 2012). An OI "gene rush", which continues nowadays, changed modern conception of OI. In the beginning of the new century, OI stopped being considered as a strictly collagen-related disorder. OI is now classified into 19 different types according to genetic cause (Van Dijk and Sillence, 2014).

The present concept of OI includes: phenotypic and genetic heterogeneity, the absence of epidemiological differences based on sex and race (Marini et al., 2017). However, knowledge about OI has grown exponentially during the past few years; many crucial aspects of the disorder remain unexplored and a cure still does not exist. Thus, there is a need for the further exploration of OI etiology, pathology and epidemiology persists.

The current thesis addresses issue of spectrum of collagen type I pathogenic variants in 30 Estonian and 94 Ukrainian unrelated OI patients and describes novel *COL1A1/2* OI pathogenic variants with correspondence to OI type for the first time. Further, this thesis explores *de novo* collagen type I pathogenic variants in 144 OI families in terms of genotype characteristics and phenotype severity. In addition, we investigate OI type V patients' genotype and phenotype characteristics and evaluate genotype-phenotype correlations in 238 unrelated OI patients. Finally, OI inter- and intrafamilial variability in collagen-related OI families is analyzed.

2. REVIEW OF LITERATURE

2.1. OI bone pathology

OI bone is characterized by increased fragility, low bone mass and abnormalities in bone structure. These factors cause a susceptibility to fractures in OI patients (Marini et al., 2017). The latest research has shown that OI defect is complex, and both bone structure and function is altered in addition to the main pathological mechanism of an OI pathogenic variant (Jay R. Shapiro et al., 2013). Notably, independent from causative pathogenic variant, general bone tissue properties are similar between different OI types (Marini et al., 2017). However, histological modifications correlate with the phenotypical severity of a disorder. Histological analysis describes OI bone as an immature, woven bone with an osteoporotic pattern. OI bone differs with a reduced amount of extracellular matrix (ECM), poor lamellar structure, thick osteoid, thin and chaotic trabecula, as well as decreased cortical bone mass (Baron et al., 1983). On a cellular level, OI bone shows hypercellularity and provides enhanced bone turnover and mineralization. However, ECM synthesis is decreased within measure of a single cell (Fratzl-Zelman et al., 2016; Rauch et al., 2000; Roschger et al., 2008). OI pathological mechanisms alter ECM not only via reduction of collagen levels but also the noncollagenous protein (NCP) level, revealing ECM qualitative differences of OI in addition to quantitative changes (Fedarko et al., 2009). Freedman *et al*. hypothesized that altered stoichiometry of collagen and NCPs are partly responsible for OI phenotypic variations (Freedman et al., 2000).

2.1.1. OI molecular mechanisms

The disruption of bone material properties in OI might be caused by several factors: collagen-related alterations (collagen structure and quantity, collagen processing, post-translational modifications, transport and chaperons); mineralization defects, abnormal osteoblast function and differentiation; defective cell signaling and proliferation (Marini et al., 2017). A detailed description of molecular mechanisms of recessive OI are presented in section 2.4.

2.1.1.1. Collagen type I alterations in OI

Collagen type I is synthetized in the rough endoplasmic reticulum (ER) of osteoblasts, skin fibroblasts and tenocytes. *COL1A1* and *COL1A2* genes are transcribed into α1 and α2 chains. Collagen fiber consists of two α1 and one α2 chains. These chains obtain a simple structure, where every third amino acid (aa) is glycine (Gly), forming Gly-Xaa-Yaa triplets, in which X and Y are any aa, but more frequently are proline and hydroxyproline (Figure 1).

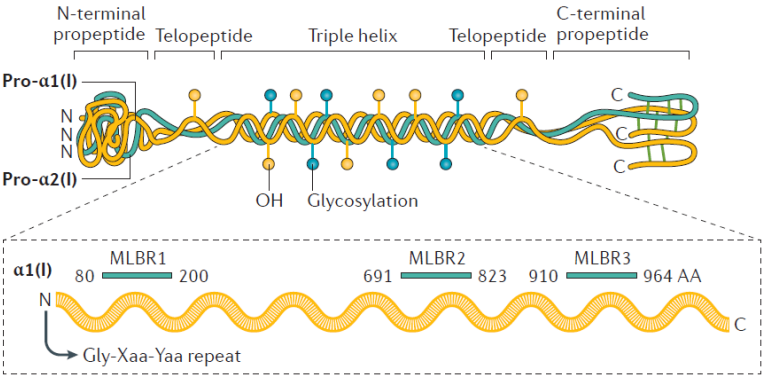


Figure 1. Collagen structure (Marini et al., 2017).

The collagen quantitative defect is caused by loss-of-function (LoF) pathogenic variants in the *COL1A1/2* genes. These pathological variants are mostly represented by splice site, nonsense and frameshift pathogenic variants. LoF pathogenic variants result in a severely truncated mRNA molecules include a premature termination stop codon activation (PTC), which is followed by the rescue mechanism of a mediated mRNA decay (NMD) (Chang et al., 2007; Fang et al., 2013; Symoens et al., 2014). As a result, develops haploinsufficiency and all collagen I chains are synthetized from one wild type allele. The collagen I amount is reduced by half. In general, phenotypes are represented with milder OI forms (Marini et al., 2007).

Structural defects include: single nucleotide variants (SNV)s and substitution of one aa, which causes chain structure due to differences in charge and structural properties of aa; C-propeptide domain changes, which cause folding and transport delay; N-propeptide defects, which result in abnormal helix formation; aa deletions and duplications, which cause shift in chains of a heterotrimer (Cabral et al., 2003; Symoens et al., 2014). Structural defects cause a delay of procollagen folding (Symoens et al., 2014). Due to this, procollagen chains are overmodified and form atypical collagen fibrils (Marini et al., 2017). These abnormal fibrils result in disorganized ECM, ER stress, alterations of osteoblast differentiation and maturation. Structurally abnormal collagen is degraded. Abnormal heterotrimers are usually not incorporated into the matrix due to the selective deposition of collagen fibrils (Bateman and Golub, 1994; Cetta et al., 1993). As a result, collagen synthesis is decreased and bone mass is reduced (Ishida et al., 2009). Collagen type I also indirectly affects mineralization processes and cell maintenance via binding of vital NCPs, cells and bone proteins, essential for ECM formation (Jay R. Shapiro et al., 2013).

Collagen structure defects are qualitative alterations of a collagen molecule. These changes are represented with missense pathogenic variants, causing substitution of aa (Prockop and Kivirikko, 1984). Clinical phenotype are affected by the character of the changes, location of substitution, altered aa types and defective chains (Marini et al., 2007). An altered aa ruins the proper organized

structure of triple helix. (Martin and Shapiro, 2009; Millington-Ward et al., 2005) (Figure 2). The most severe phenotypes arise due to changes of a Gly in Gly-Xaa-Yaa triplets with an aa with distant polarity, charge and structure (Glu, Arg, Asp, Val) (Figure 2c).

Some of the pathogenic variant positions might alter major ligand binding domains (MLBR), which bind essential NCPs and bone cells. Thus, bone fragility results not only in a structural change of a collagen I, but also in its inability to fulfill its function (Marini et al., 2007). For example, lethal pathogenic variants are associated with the MLBR2 and MLBR3 domains (Cabral et al., 2003; Marini et al., 2007). Lethal domains also interrupt the proteoglycan binding sites (Kuivaniemi et al., 1997; Marini et al., 2007). Structural alterations are responsible for the whole spectrum of OI forms (types I–IV) (Marini et al., 2017; Patel and Camacho, 2019; Sillence et al., 1979a; Van Dijk and Sillence, 2014).

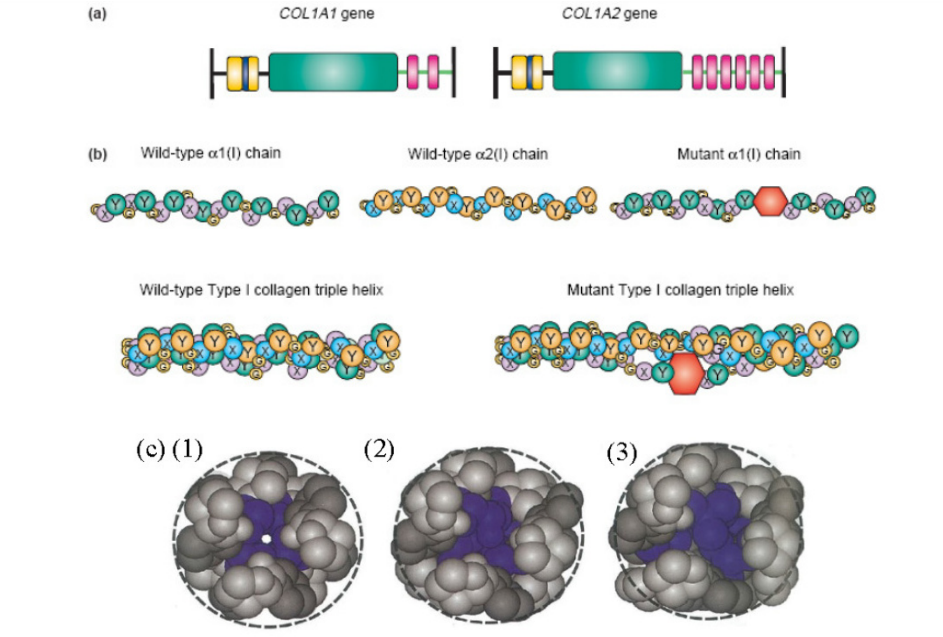


Figure 2. (a) *COL1A1* and *COL1A2* genes' structure. (b) Synthesis and assembly of a wild type and mutant collagen I with Glycine substitution (red hexagon) (Millington-Ward et al., 2005). (c) Cross-section of a collagen triple helix (Gly-Xaa-Yaa)n. Gly residues – dark blue, X residues – dark grey, Y residues – light grey. The circle illustrates the diameter of a wild type triple helix. (c1) Normal, (c2) Gly substitution with Ser, (c3) Gly substitution with Val (Jay R. Shapiro et al., 2013).

2.1.1.2. Mineralization molecular defects

A few OI cases arise due to pathogenic variants in genes that are involved in bone mineralization, among which is the *IFITM5* gene, which codes bone-restricted interferon-induced transmembrane protein-like protein (BRIL). Alteration of this protein might result in excessive ossification and abnormal mineralization (Akiyama et al., 2010; Becker et al., 2011; Semler et al., 2012). The exact function of the BRIL protein is unknown; however, the participation of BRIL in bone development and mineralization has been hypothesized (Reich et al., 2015; Semler et al., 2012).

2.2. OI as a spectrum of complex disorders

All mentioned pathological alterations lead to the development of a complex phenotype in OI patients. In addition to expected skeletal features, the spectrum of symptoms also include secondary features – extraskeletal characteristics (Sissman, 2001). The development of extraskeletal features is associated with the presence of type I collagen in many other tissues (eg. ligament, dentin, dermis, myofibrils, endomysium, sclera and cornea) (Alharbi, 2015; Gelse et al., 2003). Symptoms are individual and develop different degrees of severity in different patient, leading to diverse phenotypes among affected individuals (Van Dijk and Sillence, 2014). Due to the involvement of numerous organ systems in disease pathogenesis, OI must be considered as a complex disorder, and requires a multidisciplinary approach by health professionals from different medical areas (Alharbi, 2015; Dahan-Oliel et al., 2016; Marini et al., 2017).

2.2.1. OI skeletal features

The main symptom of OI is minimal trauma or atraumatic fractures. Fractures alter long bones more frequently, as these bones experience greater stress and loadings (Lin et al., 2009). OI patients experience a higher incidence of fractures compared to the general population; however, the pattern of fracture peak distribution among ages is in concordance with that spotted in general population (Folkestad et al., 2017). The number of fractures differs depending on the severity of the disorder, and ranges from a few to several hundred during the lifetime (Sillence, 1981). Despite being referred to as a “brittle bone disease”, some OI individuals lack bone fractures; however, they may develop secondary OI features (Deodhar and Woolf, 2000). Thus, a diagnosis could be made later in life after the transmission of a pathogenic variant to one’s offspring, who appears to be more severely affected.

The highest fracture number happens in toddlers and during adolescence (Folkestad et al., 2017). Severe OI cases stand out due to their intrauterine fractures. Babies with moderate and severe OI might also experience fractures during delivery (Sillence et al., 1979a).

Skeletal OI manifestation includes bone deformities (Figure 3). OI patients might develop spinal (kyphosis and scoliosis), chest deformities (bell-shaped rib cage, pectus excavatum/carinatum), bowing of lower and upper extremities and triangular shaping of the head (Marini et al., 2017). Deformities are more common in individuals with OI III and IV (Sillence et al., 1979b). Deformities of the lower extremities are represented with bowing of the long bones, usually anterolateral bowing of the femur and anterior bowing of the tibia. Deformities of the lower extremities are found more often because of weight bearing which can lead to more frequent fractures (Tadashi Moriwake, 1997). The extreme curvature of the bone brings additional stress to the bone tissue and adds an additional risk of bone fracture (Jay R. Shapiro et al., 2013).

Another typical feature of OI is short stature. Short stature correlates with disorder severity. Mild OI individuals possess slightly reduced to normal stature, whereas individuals with severe OI develop extremely short stature (Sillence et al., 1979b). Possible explanations of a height shortage might include spinal deformities and aberrations in the cartilage growth plates (Forlino et al., 2011).

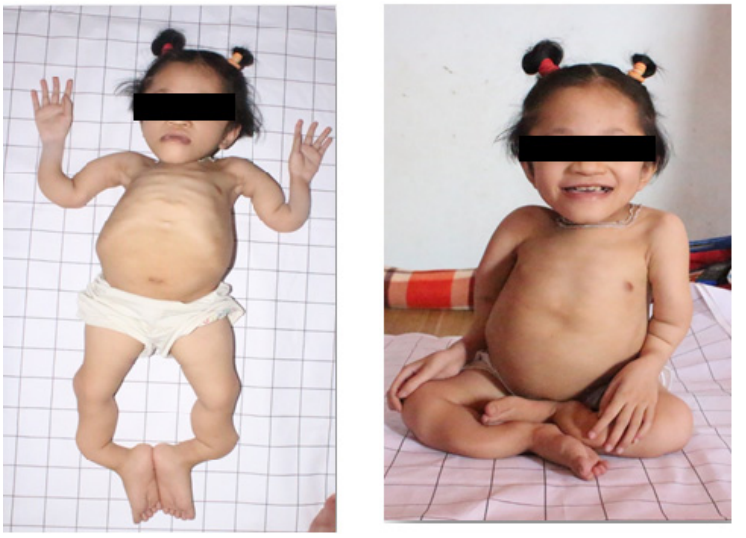
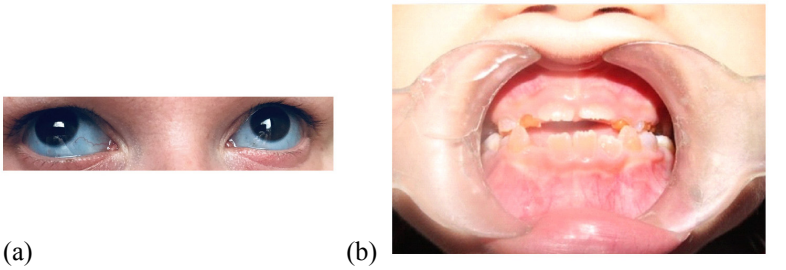


Figure 3. Skeletal deformities in a patient with severe OI (OI database of Clinic of Traumatology and Orthopedics, University of Tartu, Tartu, Estonia).

2.2.2. OI extraskeletal features

One of the typical OI symptoms is blue sclera, which is caused by one of the following proposed sources: decreased thickness and increased transparency of sclera; altered extracellular matrix composition and thinner collagen fibers; decreased light scattering by the sclera; increased viability of the underlying choroid (Figure 4a) (Apsey and Bohnsack, 2016). Blue sclera is associated with quantitative collagen defect and mild OI; whereas, individuals with moderate

and severe OI and structural collagen changes as well as pathogenic variants in the recessive OI genes usually have grey or white sclera (Das and Bhatnagar, 2017; Sillence et al., 1979a; Van Dijk and Sillence, 2014). However, correlations are still ambiguous, as some of patients with recessive OI pathogenic variants develop bluish sclera hue, such as those with pathogenic variants in the *TMEM38B* and *SERPINH1* (OMIM 600943) genes (See section 2.4.). Interestingly, sclera hue might also vary in affected members of a single family. It is also known, that blue sclera tend to decolorize with age (Marini et al., 2017; Van Dijk and Sillence, 2014).



(a) (b)

Figure 4. OI extraskeletal manifestations in patients from the OI UT database. (a) Blue hue of a sclera (b) Dentinogenesis Imperfecta (DI) (OI database of Clinic of Traumatology and Orthopedics, University of Tartu, Tartu, Estonia).

Around 34–78% of OI individuals develop hearing loss of different severity, which usually starts during their mid-twenties (Kaija Kuurila and Johansson, 2000; Kuurila et al., 2002). Hearing loss is progressive and variable among patients and OI types. In some cases, it might reach complete deafness. According to etiology, hearing loss might be conductive, sensorineural or mixed. Sensorineural hearing loss is found mostly in adult patients, whereas conductive hearing loss is found in younger patients (Kuurila et al., 2002; Pillion et al., 2011). The presence of hearing loss is most common in OI type I and type III, and it appears to be rare in type IV (Sillence, 1981). Generally, hearing loss is associated with changes in the bones that fixate the footplate of stapes which can yield the thinning, atrophy or fractures of stapes and incus (Swinnen et al., 2012). Also, hearing loss can be induced by skull fractures, seizures or basilar invagination (Pillion et al., 2011). Interestingly, there is a lack of correlation between hearing loss and OI pathogenic variants. It was hypothesized, that additional genes shape the hearing phenotype of OI individuals (Swinnen et al., 2011, 2009).

Up to 80% of OI patients might develop DI. DI is characterized with discoloration of enamel, with various shades of dentin (light grey-yellow-dark grey), spontaneous dental fractures and enamel loss, modifications of dentin matrix, poor mineralization, reduced size of teeth and tooth loss (Figure 4b) (Biria et al., 2012; Majorana et al., 2010; Surendra et al., 2013).

Craniofacial manifestations in OI patients include triangular head, Wormian bones and malocclusion (Sæves et al., 2009).

Breathing and lung problems are the main cause of OI deaths (McAllion and Paterson, 1996). Pulmonary complications are associated with kyphoscoliosis, vertebral collapse, pectus carinatum, short stature, rib fractures and pulmonary collagen abnormalities. Pulmonary hypoplasia has been observed in OI patients with lethal OI (Shapiro et al., 1989).

2.3. OI phenotypic spectrum

Already from the earliest descriptions of OI cases, clear differences between mild and severe forms have been elucidated. Patients, who suffered fractures from early childhood and had blue sclera, were classified as an OI congenita. Other patients with blue sclera, deafness and bone fragility were put into the tarda OI group (Cremin and Beighton, 1978; Zajtchuk and Lindsay, 1975). The present classification is not capable to sort discrepant OI cases with highly variable phenotypes (Sillence, 1981; Sillence et al., 1979a; Sillence and Rimoin, 1978).

2.3.1. Clinical OI classification

In 1979, Australian researcher, David Sillence, proposed a classification for OI disorder. Sillence highlighted four diverse types: I–IV based on clinical manifestations, radiological features and inheritance pattern of OI in observed in Australian patients. The number of the type does not resemble the severity of the phenotype, but rather the present order, in which patients were described (Sillence et al., 1979b). In 2000, Glorieux and Rauch discovered a fifth OI type with additional calcification of interosseous membranes and hyperplastic callus (HPC) (Glorieux et al., 2000).

Roman numerals for type designation were implemented into genetic OI classification by David Sillence in 1979. In 2014 David Sillence and Fleur van Dijk suggested updated OI nomenclature and insisted on five clinical syndromic OI forms as the most descriptive and useful for clinical practice. Sillence and van Dijk proposed the use of Arabic numerals to differentiate genetic and clinical OI nomenclature (OI 1–5). Considering terminology it must be noted, that although van Dijk and Sillence proposed a new OI classification (Van Dijk and Sillence, 2014) with Arabic numerals, it was not yet accepted by International Nomenclature group for Constitutional Disorders ICHG of the Skeleton (INCDS) and currently the old classification with Roman numerals is broadly in use.

OI type I (Mendelian inheritance in man database, MIM 166200) describes dominantly inherited, classical, non-deforming OI cases with blue sclera. OI type I is the mildest OI form. Incidence is estimated as 1/10,000 births. Patients have nearly normal height, hearing loss and mild skeletal deformities (Figure

5a). The first fracture occurs usually during the preschool period. The number of fractures is minimal. Patients do not develop problems with mobility and are able to walk independently (Sillence et al., 1979b). Based on the presence of DI, patients are divided into A (with DI) and B (without DI) subtypes. It is supposed, that individuals with IA have greater rates of fractures and more severe phenotype compared to IB (Paterson et al., 1983). Only 90% of patients suffer from fractures (Van Dijk and Sillence, 2014).

OI type II (MIM 166210) is a perinatal lethal OI. This type is the most severe, lethal OI form. Incidence is described as 1/1,000,000. An affected fetus is often aborted or dies during the first days of life due to respiratory deficiency. The subject suffers numerous intrauterine fractures, severe skeletal deformities, severe shortening of the limbs, rhizomelia, beaded ribs, platyspondyly, skin head (soft skull). These OI individuals have extremely dark blue eye sclera (Sillence et al., 1979b). Based on radiological data OI type II was divided into subtype A (short, broad long bones, angulation of tibias and continuously beaded ribs); subtype B (short, broad, crumpled femurs, angulation of tibias, normal ribs or with incomplete beading); subtype C (long and thin long bones, multiple fractures and thin beaded ribs) (Sillence et al., 1984).

OI type III (MIM 259420) describes a progressive deforming OI with normal sclera. Incidence of OI type III is estimated as 1 to 2 per 60,000 to 100,000. Patients develop severe bone fragility and severe skeletal deformities. Patients with OI type III have short stature (~1m), triangular head, low set ears and temporal, occipital bossing, disproportion of body and limbs (Figure 5c). Patients with OI type III might have intrauterine fractures and multiple fractures during birth. The number of fractures is extremely high during the patient's entire lifespan. Patients might also develop DI, hearing loss or joint laxity. Sclera hue range from pale blue and greyish to white. OI type III phenotype is the most severe of the non-lethal OI types. Patients are unable to walk and require wheelchairs (Sillence et al., 1979b).

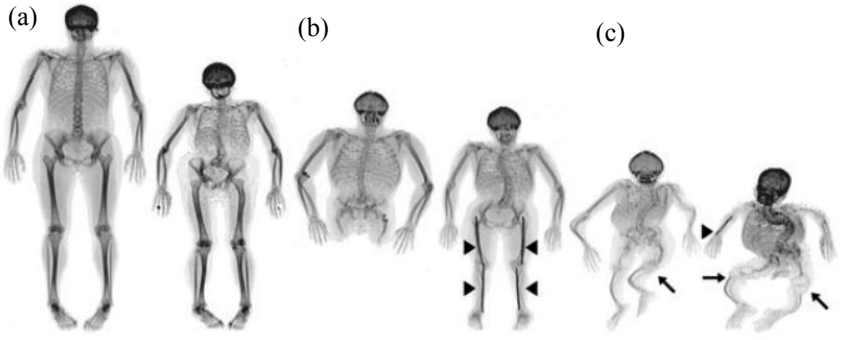


Figure 5. X-rays of adult OI patients: OI type I (a), OI type IV (b), OI type III (c) (Reeder and Orwell, 2006).

OI type IV (MIM 166220) is a common variable OI with normal sclera. Patients have moderate OI phenotype. This group includes various OI cases. Stature is higher compared to OI type III, but shorter than patients with OI type I. Skeletal deformities are moderate, affecting mostly lower limbs. Patients require assistance for walking. The number of fractures is significantly higher compared to OI type I (Figure 5b) (Sillence et al., 1979b). Intrafamilial severity is highly variable. In some families there a majority of family members present mild OI with a few having moderately severe OI (Holcomb, 1931; Seedorf, 1949).

OI type V (MIM 610967) is underlined by calcification abnormalities, like interosseous membrane calcification in the forearms and legs (Figure 6C–E). This pathologic ossification restricts pronation and supination of the affected limb. All patients were reported to develop dislocation of the radial head in the forearm. Sclera were reported as white, no DI and hearing loss were previously described in these patients. OI type V individuals have typical histomorphometry with a coarse mesh-like pattern of lamellation (Figure 6A, B) (Rauch et al., 2013). Patients with OI type V might develop HPC of different severity (Figure 6F). HPC is a massive benign callus with swelling and pain at the site of fracture or surgery, which has the tendency to increase and disappear later in life (Cheung et al., 2007; Glorieux et al., 2000; Rauch et al., 2013). Recent descriptions of OI type V cases prove the high variability of OI type V phenotypes (Balasubramanian et al., 2013; Fitzgerald et al., 2013; Guillén-Navarro et al., 2014; Rauch et al., 2013; Jay R Shapiro et al., 2013).

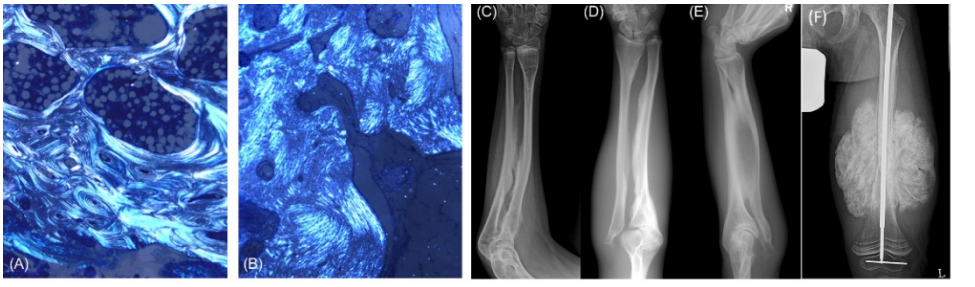


Figure 6. Histological and radiological findings in patients with OI type V. (A) Lamellar pattern of the Haversian system from a normal subject. (B) Mesh-like lamellar pattern in OI type V. (C–E) Ossification of the interosseous membranes. (F) Hyperplastic callus. Adapted from (Cho and Moffatt, 2014).

2.4. Genetic OI nomenclature and genetic heterogeneity

Research into OI genetics has caused the development of a new approach to OI classification, which is based on genetic-functional defects. Genetic OI classification differentiates 19 OI types, where every type is associated with a separate gene (Table 2) (Marini et al., 2017). Sillence types I–IV correspond with additional numerical types V–XIX (Marini et al., 2017).

Up to 85–90% of OI cases are caused by autosomal dominant (AD) pathogenic variants in the *COL1A1* and *COL1A2* genes, which encode α1 and α2 chains of type I collagen (Marini et al., 2007). The remaining 10–15% of OI cases are caused by 19 non-collagen OI genes. Most of them are AR. These genes encode proteins involved in post-translational modification (*CRTAP, P3H1, PPIB*), processing, transport and cross-linking (*FKBP10, BMP1* (Online Mendelian Inheritance in Man (OMIM) database 112264)*), SERPINH1, PLOD2* (OMIM 601865), *SEC24D*) of the type I collagen; bone tissue mineralization (*SERPINF1* (OMIM 172860)*, IFIMTM5* (OMIM 614757)*; AD*) osteoblast differentiation and functioning (*WNT1, TMEM38B, SPARC* (OMIM 182120)*, SP7* (OMIM 606633)*, CREB3L1* (OMIM 616215)*, MBTPS2* (OMIM 300294); X-linked (XR)) and osteoclast function (*PLS3;*XR) (Van Dijk and Sillence, 2014b; Marini et al., 2017). Lately, a connection between the *TENT5A* or *FAM46A,* (OMIM 611357) and *NBAS* (OMIM 608025) genes to OI has been confirmed (Figure 7) (Balasubramanian et al., 2017; Doyard et al., 2018). Some OI cases lack a genetic diagnosis implying the existence of still unidentified OI genes (Bardai et al., 2016).

Although OI might be caused by pathogenic variants in numerous genes, OI phenotypes overlap and resemble the classical clinical OI types I–V (Table 2) (Van Dijk and Sillence, 2014).

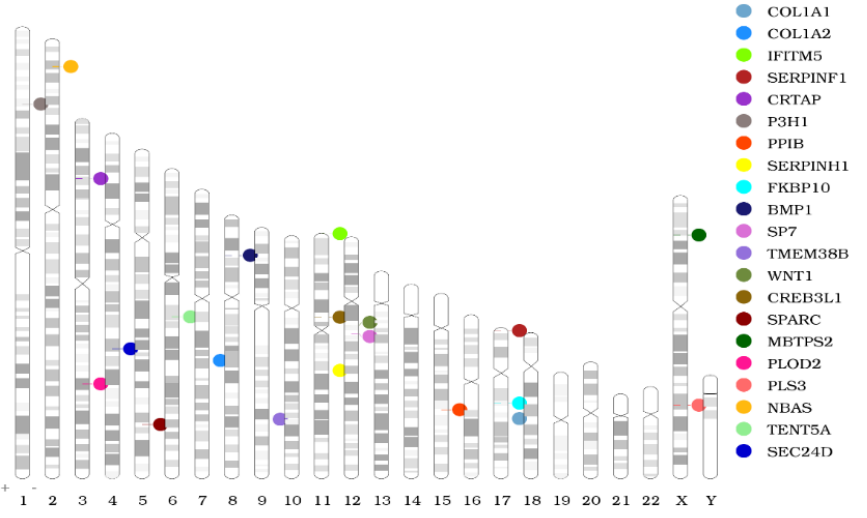


Figure 7. Idiogram of OI genes showing genetic heterogeneity. The OI idiogram includes genes with AD, AR and XR patterns of inheritance.

Table 2. OI clinical and genetic nomenclatures combined with causative genes and phenotypes. Adapted from Online Mendelian Inheritance in Man (OMIM) database (Marini et al., 2017; Van Dijk and Sillence, 2014).

OI type	Mutated gene	Genetic OI type	OMIM	Inheritance	Protein product	Phenotype
OI I OI II OI III OI IV	*COL1A1*	I–IV	166200 166210 259420 166220	AD, AR	Collagen α1(I)	Non deforming OI with blue sclera; Common variable OI with normal sclera; Progressively deforming
OI I OI II OI III OI IV	*COL1A2*	I–IV	166200 166210 259420 166220	AD, AR	Collagen α2(I)	Non deforming OI with blue sclera; Common variable OI with normal sclera; Progressively deforming
OI V OI III	*IFITM5*	V	610967	AD	Bone-restricted interferon-induced transmembrane protein-like protein (BRIL; also known as IFM5)	OI with calcification in interosseous membranes, hyperplastic callus, radial head dislocation or severe bone deformity with grey sclera
OI III	*SERPINF1*	VI	613982	AR	Pigment epithelium-derived factor (PEDF)	Progressively moderate to severe deforming, osteoid, fish-scale appearance of bone lamella
OI III OI II	*CRTAP*	VII	610682	AR	Cartilage-associated protein (CRTAP)	Progressively deforming, severe rhizomelia, white sclera
OI III OI II	*P3H1 (LEPRE1)*	VIII	610915	AR	Prolyl 3-hydroxylase 1 (P3H1)	Progressively deforming, severe rhizomelia, white sclera
OI III OI II	*PPIB*	IX	259440	AR	Peptidyl-prolyl *cis–trans* isomerase B (PPIase B)	Severe bone deformity with grey sclera
OI III	*SERPINH1*	X	613848	AR	Serpin H1 (also known as HSP47)	Severe skeletal deformity, blue sclera, DI, skin abnormalities and inguinal hernia
OI III	*FKBP10*	XI	610968	AR	65 kDa FK506-binding protein (FKBP65)	Mild-to-severe skeletal deformity, normal-to-grey sclera and congenital contractures

OI type	Mutated gene	Genetic OI type	OMIM	Inheritance	Protein product	Phenotype
OI III	*BMP1*	XII	614856	AR	Bone morphogenetic protein 1 (BMP1)	Mild-to-severe skeletal deformity and umbilical hernia
OI III OI IV	*SP7*	XIII	613849	AR	Transcription factor SP7 (also known as osterix)	Severe skeletal deformity with delayed tooth eruption and facial hypoplasia
OI III	*TMEM38B*	XIV	615066	AR	Trimeric intracellular cation channel type B (TRIC-B; also known as TM38B)	Severe bone deformity with normal-to-blue sclera
OI III OI IV	*WNT1*	XV	615220	AD, AR	Proto-oncogene Wnt-1 (WNT1)	Severe skeletal abnormalities, white sclera and possible neurological defects
OI III	*CREB3L1*	XVI	616229	AR	Old astrocyte specifically induced substance (OASIS; also known as CR3L1)	Progressively severe deforming
OI III	*SPARC*	XVII	616507	AR	SPARC (also known as osteonectin)	Progressively severe deforming, severe fragility
OI III	*TENT5A (FAM46A)*	XVIII	617952	AR	Terminal nucleotidyltransferase 5A	Progressively moderate to severe, congenital bowing of the lower limbs
OI III	*MBTPS2*	XIX	301014	XR	Membrane-bound transcription factor site-2 protease (S2P)	Progressively moderate to severe deforming, light blue sclera
OI III	*PLOD2*	No type	609220	AR	Lysyl hydroxylase 2 (LH2)	Progressively moderate to severe deforming, joint contractures
OI IV	*PLS3*	No type	300910	XR	Plastin 3	Common variable OI with normal sclera, normal height
OI III	*NBAS*	No type	614800	AR	Neuroblastoma amplified sequence	Progressively moderate to severe deforming, intellectual disability, liver failure, optic nerve atrophy
OI III	*SEC24D*	No type	616294	AR	Protein transport protein Sec24D	Bone fragility, skull ossification defects, craniofacial dysmorphism

2.4.1. Autosomal dominant OI

A majority of OI individuals are affected by an autosomal dominant form of OI (Sillence et al., 1979b). These patients are carriers of only one mutated allele on the somatic chromosomes, which is enough to develop OI symptoms. Dominant pathogenic variants might be inherited from patients or arise *de novo* in 35–60% of cases (Steiner et al., 1993). In the case of familial OI, the probability of transmission of a pathogenic variant to next generation is 50%. OI patients with types I and IV show predominantly an AD pattern of inheritance. Some of the families with OI type III also show AD inheritance (Tsipouras et al., 1984; van Dijk et al., 2012). A majority of the AD pathogenic variants alter *COL1A1* and *COL1A2* genes and cause classical OI types (Steiner et al., 1993). All reported OI type V cases were caused by the same AD pathogenic variant in the *IFITM5* gene (Semler et al., 2012). One more non-collagen OI gene with AD pattern is a *WNT1*. Pathogenic variants in this gene are associated with different severity of bone fragility. AD pathogenic variants cause mild phenotype or early-onset osteoporosis (OP), whereas AR pathogenic variants are responsible for severe OI (Keupp et al., 2013).

2.4.1.1. Collagen I – related OI

The importance of collagen type I is underlined by the high conservation of this protein among vertebral organisms (Bonod-Bidaud and Ruggiero, 2013; Gelse et al., 2003; Stover and Verrelli, 2011). Naturally collagen type I lacks variation especially in the N- and C-domains, as these termini are involved with collagen assembly, transport and signaling (Stover and Verrelli, 2011). More than 1500 different pathogenic variants were described in the *COL1A1/2* genes in the database of OI variants (Dalgleish, 1997).

Collagen-related OI is characterized with classical Sillence phenotypes and typical OI features like: atraumatic fractures, blue sclera, DI, hearing loss, skeletal deformities, easy bruising and joint hypermobility (Sillence et al., 1979b; Van Dijk and Sillence, 2014). Pathogenic variants in the collagen I cause a highly variable spectrum of bone fragility (Bonod-Bidaud and Ruggiero, 2013; Lisse et al., 2008; Steiner et al., 1993). Pathogenic variants of the *COL1A1/2* genes can cause any range of OI types I–IV, including a lethal OI type II, see Table 2 (Marini et al., 2007). Parental mosaicism may be found in 3–5% of OI cases. Parents with somatic mosaicism are usually mildly affected, whereas parents with somatic mosaicism may reveal mild clinical symptoms of OI. In cases of parental mosaicism the offspring may develop recurrent AD OI (Chen et al., 2013; Frederiksen et al., 2016). In addition to OI, pathogenic variants in the *COL1A1* and *COL1A2* can lead to Caffey disease, Ehlers-Danlos syndrome (EDS), bone mineral density (BMD) variations and OP (Jay R. Shapiro et al., 2013).

2.4.1.1.1. *COL1A1*: characteristics and relation to OI

The *COL1A1* gene is located on the 17q21.33, reverse strand (Figure 7). The gene codes for a collagen type I α1 chain (Figure 1). *COL1A1* mRNA is monocistronic (Lazarides and Lukens, 1971). Collagen type I from bone, skin and tendon tissue differ in pattern of hydroxylation of Pro and Lys residues, cross-linking formation and glycosylation (Boedtker et al., 1983). The characteristic feature of the collagen genes is numerous small exons. The *COL1A1* consists of a 51 coding exon. Up to 90% of OI, patients harbor pathogenic variants in the *COL1A1* gene (Steiner et al., 1993). According to Körkkö, 1/5 of the *COL1A1* pathogenic variants are identical in unrelated OI individuals. 80% of these recurrent OI pathogenic variants are located in the CpG islands (Körkkö et al., 1998). OI pathogenic variants have been identified in all exons. Severity of the phenotype depends on the location and type of the pathogenic substituting aa residue (Marini et al., 2007).

2.4.1.1.2. *COL1A2*: characteristics and relation to OI

The *COL1A2* gene is located on the 7q21.3, forward strand (Figure 7). The gene codes for collagen type I α2 chain (Figure 1). The gene is composed of 52 exons. Compared to *COL1A1*, fewer individuals harbor *COL1A2* pathogenic variants, only up to 30% (Körkkö et al., 1998; Steiner et al., 1993). Carriers of the *COL1A2* pathogenic variants generally have less severe phenotype compared to carriers of the pathogenic variants in the *COL1A1* gene. This is explained by a fewer number of α2 chains in the collagen triple helix, so that only 25% will have normal α2 chain, compared to 75% in the case of an abnormal α1 chain (Marini et al., 2007; Jay R Shapiro et al., 2013). Lethal pathogenic variants may appear in both genes, with a predominance in the *COL1A2* (Marini et al., 2007). The number of the LoF pathogenic variants in the *COL1A2* gene is reduced, as they are supposed to cause an asymptomatic form of the disease or the mildest symptoms (i.e. early onset OP) and are not captured by clinical detection (Pope et al., 1985). There were no previous descriptions of the complete *COL1A2* deletions (Jay R Shapiro et al., 2013).

2.4.1.2. Association of the IFITM5 gene with OI

OI type V individuals harbor a heterozygous pathogenic variant in the 5' untranslated region (UTR) of the *IFITM5* gene, c.–14C>T (Cho et al., 2012; Semler et al., 2012). The gene is located on the 11p15.5, reverse strand and consists of two exons (Figure 7). The physiological function as well as the pathogenic mechanism of the 5'UTR pathogenic variant of the *IFITM5* gene are not yet well understood. The *IFITM5* gene product – BRIL protein, is believed to be involved with the development and mineralization of the bone tissue. Interestingly, a co-expression of *ifitm5* with *col1a1* and other OI-related genes was observed

in a murine model of OI (Farber et al., 2014). It was shown that the c.–14C>T pathogenic variant causes an emergence of a new upstream start codon and facilitates the inclusion of five redundant amino acids to the N-termini of the protein – Methionine, Alanine, Leucine, Glutamic acid and Proline (MALEP) (Hanagata, 2016; Lazarus et al., 2014). The consequence of the pathogenic variant on protein function remains debated. One hypothesis suggests that there is evidence of a gain-of-function effect of the pathogenic variant. However, others point towards neomorphic nature of the c.–14C>T pathogenic variant in the *IFITM5,* proving that mutated MALEP-IFITM5 protein gains a new molecular function (Lietman et al., 2015). The first evidence of association of OI type V with *IFITM5* was published in 2012. Incidence of patients who harbor the c.–14C>T *IFITM5* pathogenic variant remains rare. Moreover some of the pathogenic variant carriers do not express typical symptoms of the OI type V phenotype and lack a diagnosis (Figure 6) (Cho et al., 2012; Grover et al., 2013; Semler et al., 2012). In addition, a c.119C>T, p.(Ser40Leu) heterozygous pathogenic variant in the *IFITM5* gene has been described to cause a typical OI type III with progressive deformities, grey sclera, limb shortening and lacks the typical symptoms of OI type V (Guillén-Navarro et al., 2014). A pathogenic variant in the same position p.(Ser40Trp) caused multiple prenatal fractures in an affected individual. However, the patient had normal BMD after birth and only a few fractures appeared during adolescence, after a significant trauma (Lim et al., 2019). Thus, the phenotypical range of *IFITM5*-related OI might be greater than estimated.

2.4.2. Autosomal recessive OI

Only 10% of OI cases have AR inheritance. In AR cases, an affected person must inherit two copies of a mutated allele (biallelic) in order to develop a disease phenotype.

These pathogenic variants may be homozygous (at the same position) or compound heterozygous (at different positions). AR is observed in collagen type I genes, as well as non-collagen OI genes, which are also called AR OI genes, due to the predominance of a recessive inheritance pattern among them (Table 2) (Marini et al., 2017; Van Dijk and Sillence, 2014).

The inheritance pattern of the collagen-related OI is mostly AD; however, some AR pathogenic variants were also reported. Individuals, harboring homozygous and compound heterozygous variants in the collagen I genes develop much more severe phenotypes compared to carriers of the same heterozygous variants (Ackermann and Levine, 2017; De Paepe et al., 1997; Pope et al., 1985). A few cases from consanguineous families with heterozygous parents with mild OI resulted in homozygous, severely affected children (De Paepe et al., 1997; Pope et al., 1985).

Non-collagen OI genes are mostly AR. The current number of known AR non-collagen OI genes is 16. Mutations in the AR OI genes cause the development

of severe OI phenotypes OI types II and III, which are usually indistinguishable from collagen-related OI (Table 2) (Marini et al., 2017). Pathogenic variants of the AR OI genes lack LoF variants, which lead to null allele and a significant decrease in protein synthesis or the total absence of a gene product (Van Dijk and Sillence, 2014).

2.4.3. Pathogenic variant types

Pathogenic variants are important factors of disease progression, altering gene expression, size, structure and genetic interactions. Their effect on organism fitness can be lethal, deleterious, neutral (i.e. benign) or beneficial. Pathogenic variants can be classified as insertions-deletions (indels), SNVs and chromosomal aberrations (Nishant et al., 2009). OI is usually caused by indels and SNVs. However, cases of chromosomal aberrations that adjust *COL1A1/2* loci have also been described (Knisely et al., 1988). Pathogenic variants can be located in introns (non-coding regions) or exons (coding regions). As a rare disorder, OI is caused predominantly by pathogenic variants in the exons, splice sites, 3' or 5' UTR. Collagen I pathogenic variants may be classified according to the type of defect – quantitative (LoF, i.e. haploinsufficiency) or qualitative (missense, i.e. dominant negative (DN)) pathogenic variants (Marini et al., 2007).

According to the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG) and the Association of Medical Pathology, all genetic variants which cause Mendelian disorders are classified into variants using specific, standard terminology – "pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign". Five categories are based on criteria, which summarize types of variant evidence, such as population, computational, functional and segregation data (Richards et al., 2015).

2.4.3.1. Dominant negative pathogenic variants: missense pathogenic variants

When *COL1A1/2* genes are altered, DN pathogenic variants disrupt triple helix of the collagen I polypeptides. As a result propeptide is overprocessed with enzymes during post-translational modification and an abnormal collagen I is produced, which is rapidly degraded (dominant-negative effect) (Van Dijk and Sillence, 2014). Being a structural defect, DN pathogenic variants correspond with collagen type I qualitative abnormalities. DN pathogenic variants cause the synthesis of structurally abnormal collagen, which may cause ER stress and apoptosis of osteoblasts. The structural defects have a more severe effect on ECM than on ECM insufficiency (Forlino and Marini, 2000). DN pathogenic variants are therefore more deleterious than LoF pathogenic variants (Ben Amor et al., 2011; Marini et al., 2007; Mendoza-Londono et al., 2015; Jay R. Shapiro et al., 2013).

DN pathogenic variants in the collagen I triple helical domain result in a range of phenotypes, from lethal to moderately severe OI. Out of the missense pathogenic variants, the most common forms with clinical significance are Gly substitutions (Kuivaniemi et al., 1997; Prockop and Kivirikko, 1984). Almost 80% of Gly is substituted with Ser, Cys and Arg. Around 9% of Gly substitution leads to OI type I (Marini et al., 2007). However, in a Swedish population-based study 32% of mild OI cases were caused by qualitative pathogenic variants (Lindahl et al., 2015).

DN pathogenic variants appear more frequently as compared to LoF (i.e. frameshift and splice site) pathogenic variants in sporadic genetic diseases (de Ligt et al., 2013; Veltman and Brunner, 2012). The missense substitutions were claimed to be the most common among patients of European and North American origin (Van Dijk and Sillence, 2014).

2.4.3.2. Haploinsufficiency pathogenic variants: indels, splice site, frameshift pathogenic variants

Haploinsufficiency effect is caused by LoF pathogenic variants, which are represented with indels, splice site and frameshift variants, leading to instability of the mRNA. LoF pathogenic variants in type I collagen genes lead to a reduced production of collagen, but the structure of molecules is not altered. Thus, LoF pathogenic variants in *COL1A1/2* genes correspond to haploinsufficiency and a collagen type I quantitative defect.

Nonsense pathogenic variants lead to the PTC and NMD of an affected chain. Not all nonsense pathogenic variants result in OI type I. Sometimes PTC of a nonsense pathogenic variant results in a moderately severe or severe phenotype. This underlines the issue that in case of nonsense pathogenic variants, phenotype cannot be explained with null allele directly (Kocher and Shapiro, 1998).

Frameshifts are more common for the *COL1A1* gene, compared to *COL1A2*. Frameshifts appear in all exons and usually result in PTC, NMD and mild OI. However, frameshifts in exon 50 and 51 will not result in NMD and truncated protein will cause severe DN effects (Hartikka et al., 2004; Pollitt et al., 2006).

Splice site pathogenic variants cause intron or exon cryptic splice site activation, exon skipping and intronic retention. Donor (5') and acceptor (3') splice site pathogenic variants in the *COL1A1/2* genes appear with the same frequency. It would be expected, that exon skipping caused by splice site pathogenic variants might lead to a DN effect, as translation protein includes internal amino acid deletion. In contrast, 80% of splice site pathogenic variants result in mild OI. This is expected from the production of null-alleles, rather than truncated proteins (Kocher and Shapiro, 1998). Phenotype predictions might be dependent on additional variants, which modify the splicing process. These variants may include: cis-factors, single nucleotide polymorphisms (SNPs), which directly influence splicing; SNPs in regulatory regions, which alter gene expression; trans-factors, which alter tissue-specific splicing (Garcia-Giralt et al., 2002; Grant et al., 1996).

About 1–2% of individuals with *COL1A1/2* pathogenic variants have exon/ whole gene deletions. Deletions of the whole gene or exons also result in haploinsufficiency and mild OI type I phenotype (van Dijk et al., 2010).

2.4.3.3. De novo and familial pathogenic variants

De novo pathogenic variants are spontaneously appearing variants. *De novo* pathogenic variants are also referred to as "sporadic" pathogenic variants. *De novo* pathogenic variants differ by having a greater effect on phenotype and are more deleterious compared to inherited (i.e. familial) pathogenic variants (Acuna-Hidalgo et al., 2016; Veltman and Brunner, 2012). Due to their sporadic nature, the amount of the *de novo* pathogenic variants remain frequent in the population. There are no differences in the prevalence of *de novo* pathogenic variants among different ethnic groups and populations (Acuna-Hidalgo et al., 2016). These variants might cause congenital malformations, rare disorders and sporadic syndromes in previously unaffected families (Veltman and Brunner, 2012). Interest towards *de novo* pathogenic variants has increased alongside the availability of NGS techniques. Understanding the contribution and importance of *de novo* pathogenic variants in rare and common disorders is growing. *De novo* pathogenic variants are the main source of neurodegenerative and developmental disorders (Acuna-Hidalgo et al., 2016).

OI is considered to be a mainly hereditary disorder with a family history. However, OI pathogenic variant etiology might not only be inherited but in 35–60% of cases *de novo* (Steiner et al., 1993). Less attention is paid to the input of *de novo* pathogenic variants in OI pathology. Investigations of *de novo* pathogenic variants in the context of musculoskeletal disorders is essential for understanding the disorder's etiology and nature, diagnostic techniques and family planning.

2.5. Genotype-phenotype associations in OI

Understanding genotype-phenotype correlations is important for the evaluation of disease progression and prediction of risks as well as diagnosis, including the reduction of genetic diagnosis costs, family planning, management and treatment strategies. However, it is difficult to elucidate OI genotype-phenotype associations, as high variability of phenotype expressivity exists. Researchers are still trying to compose OI genotype-phenotype correlations (Jay R Shapiro et al., 2013).

Phenotype severity depends on the affected gene. Interestingly, due to common pathological mechanisms, the phenotypes of patients with different mutated OI genes might mimic each other or overlap. Prolyl 3-hydroxylase 1-Cartilage-associated protein-Peptidyl-prolyl cis-trans isomerase B protein complex (P3H1-CRTAP-PPIaseB) complex is a product of *P3H1, CRTAP* and *PPIB* genes.

Pathogenic variants in these genes might cause similar phenotypes: lethal or extremely severe OI (Table 2, Figure 8) (Baldridge et al., 2008; Morello et al., 2006; van Dijk et al., 2009)

However, in some cases distinctive features are developed depending on the altered gene. For example: ossification of interosseous membranes and HPC in patients with *IFITM5* gene pathogenic variants; optic atrophy and loss of color vision, growth retardation, facial dysmorphism in individuals with *NBAS* gene pathogenic variants; contractures in carriers of the *PLOD2* and *FKBP10* gene pathogenic variants (Table 2) (Balasubramanian et al., 2017; Hanagata, 2016; Y. Liu et al., 2017; Shaheen et al., 2010).

According to a study of 50 Indian OI individuals, phenotype severity ranged as follows: *IFITM5* = *LEPRE1* > *WNT1* > *SERPINF1* > *COL1A1* (qualitative) > *BMP1* > *FKBP10* > *COL1A2* (qualitative) > *COL1A1* (quantitative) > *COL1A2* (quantitative) (Mrosk et al., 2018). This presented range might be subjective, due to the limited number of patients and high variability of clinical manifestations between carriers of the pathogenic variants in the same gene. In addition to the affected gene, phenotype is strongly influenced by the type of pathogenic variant and position (Jay R Shapiro et al., 2013). For example, alterations in the *IFITM5* and *SP7* may cause both severe and moderate phenotype (Figure 8) (Marini et al., 2017).

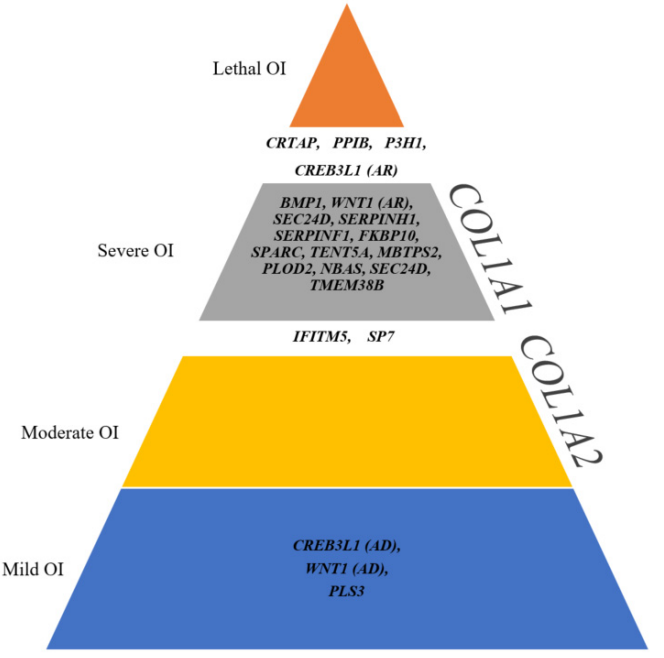


Figure 8. General representation of OI phenotype severity according to the affected OI gene.

There is an advanced understanding of genotype-phenotype correlations in collagen-related. According to previous studies, phenotype in collagen I related OI is influenced by pathogenic variant type and position, so genotype-phenotype associations exist to some extent (Marini et al., 2017, 2007). It was shown, that generally quantitative defects are associated with milder OI, whereas qualitative collagen defects are associated with moderate and severe OI (Sillence et al., 1979a). Modern approaches to OI are variant-centric; however, OI is a complex disorder and the identification of modifier factors, both genetic and epigenetic ones, is essential for a full understanding of the disease.

Generally, individuals with *COL1A2* pathogenic variants have more severe phenotypes compared to patients with *COL1A1* pathogenic variants (Maioli et al., 2019; Marini et al., 2007; Mrosk et al., 2018). In addition, there is a strong association between phenotype and the position of the variant. Individuals harboring N-propeptide pathogenic variants develop EDS/OI overlap phenotype, whereas pathogenic variants in the C-propeptide are associated with a high bone mass OI. Correlations between blue sclera, DI and fracture number are believed to be identified to some degree. N-terminal helical pathogenic variants are associated with blue sclera, and C-terminal helical pathogenic variants are associated with DI (Rauch et al., 2010). Dental abnormalities are associated with qualitative defect in the collagen I. Th severity of the DI depends on the location of the pathogenic variant (Andersson et al., 2017). However, in a recent study of 364 Italian OI patients, a correlation between pathogenic variant location and DI presence was absent (Maioli et al., 2019). Interestingly, Maioli *et al.* also confirmed a lack of correlation between lethal OI and these "lethal clusters", as proposed by Marini *et al.* (Maioli et al., 2019; Marini et al., 2007). Patients with *COL1A1* pathogenic variants have a greater prevalence of blue sclera compared to carriers of the *COL1A2* pathogenic variants. Carriers of the *COL1A2* pathogenic variants, however, are shorter compared to those with *COL1A1* pathogenic variants. The association between genotype and hearing is still unknown, as there no correlation has been found between gene, pathogenic variant type or position and presence of hearing loss. Hearing loss is suspected to be a multifactorial trait and depends on complex genetic factors (Hartikka et al., 2004). However, in a recent study of 668 Chinese OI patients, a correlation between the absence of hearing loss and Gly substitutions in the α1 chain N-terminal end was observed (Li et al., 2019).

2.6. Inter- and intrafamilial diversity

Incomplete penetrance and clinical variability has been described previously for many AD disorders (Castori, 2012; Cooper et al., 2013; McGettrick et al., 2000). Clinical variability causes mis- and underdiagnosis in cases of asymptomatic patients, especially for mildly affected families, who still transmit disease causative pathogenic variants to the next generation (Cooper et al., 2013; Deodhar and Woolf, 2000).

With enrichment of the OI variant database, OI genotype-phenotype correlations have become less clear. Variability of phenotypic variants under a single pathogenic variant have been elucidated. The existing variability of OI phenotypes between related and unrelated individuals has been observed for a long time (Daley et al., 2010; Deodhar and Woolf, 2000; Moraes et al., 2012; Rogozhina et al., 2016; Jay R Shapiro et al., 2013; Swinnen et al., 2011). The OI variant database (https://oi.gene.le.ac.uk) has reported unrelated patients, who harbor the same pathogenic variant; however, they suffer from different severities of the disease. One such case of a c.757C>T (p.Arg253*) nonsense pathogenic variant in the *COL1A1* gene was reported 11 times. Patients developed following the OI types: six OI type I, four OI type IV, one OI type III (Dalgleish, 1998; Lindahl et al., 2015; Mauri et al., 2016; Ries-Levavi et al., 2004; Jay R. Shapiro et al., 2013; Venturi et al., 2006). One of the most outstanding examples of familial OI diversity is from a study of 64 individuals from 22 Old Order Amish sibships, all harboring a single founder AD pathogenic variant c.2237G>T, p.(Gly610Cys) in the *COL1A2* gene. The phenotype spectrum between carriers of the pathogenic variant ranged from mild to moderate in the community (Daley et al., 2010). Affected patients develop phenotypes with different severity of bone fragility, fracture numbers, severity of skeletal deformities, hearing loss, DI, sclera hue and joint hypermobility (Dalgleish, 1997; Pillion et al., 2011; Swinnen et al., 2011). High intrafamilial variability is also exposed by hearing loss characteristics, underling the presence of additional genetic factors in a predisposition for hearing loss in OI individuals (Swinnen et al., 2011). Variability of phenotypes cannot be explained by differences in treatment, as asymptomatic, untreated parents might have more severely affected children, who develop the need of pharmacological treatment and orthopedic interventions.

In some cases, intrafamilial diversity is explained with mosaic parents, who are mildly affected or asymptomatic. Also in cases of *WNT1* and *CREB3L1* pathogenic variants, heterozygous family members might be mildly affected when compared to severely affected homozygous individuals (Laine et al., 2013; Symoens et al., 2013). Variable expressivity, including intrafamilial diversity of phenotype, was spotted in families with OI type V. Some of these patients are able to ambulate independently, whereas others use walking aids or wheelchairs (Jay R Shapiro et al., 2013).

Families from the OI database of the Clinic of Traumatology and Orthopedics (University of Tartu, Estonia) also expose cases of inter- and intrafamilial variability. Patients carrying the same type of collagen I defect (i.e. DN or LoF *COL1A1/2* pathogenic variants) or even carriers of the same pathogenic variant developed different OI symptoms. Intrafamilial variability was observed in the differences of OI phenotype between affected members of the single family. Phenotype severity increased, or oppositely decreased, throughout generations. Also, phenotypes might differ between affected siblings from the same generation. In some families, no differences in phenotype severity were registered and the clinical picture of all affected members was identic.

2.7. Summary of the literature review

OI is a spectrum of heterogeneous complex of bone fragility disorders. OI is a rare disease, which affects 1 in 10,000 to 20,000 people. As a disorder of the connective tissue, the majority of OI cases are connected to abnormalities in the collagen type I. OI patients may develop skeletal (fractures, deformities, joint laxity) and extraskeletal (hearing loss, blue sclera, DI, cardio-pulmonary complications) symptoms. Patients are challenged with diagnostic issues, a need for complex treatment approaches, an absence of a cure and a poor quality of life.

OI unifies different disorders of bone fragility, which range from mild to severe and even lethal cases. The disorder is classified into five clinical types (I – mild, II – lethal, III – severe, IV – moderate, V – OI with unusual mineralization pattern), and 19 different genetic OI types, according to an affected gene. The pathogenic variants in the genes, coding for collagen type I (*COL1A1/2* genes) are mainly dominant and might cause OI types I–IV. Collagen type I pathogenic variants may cause quantitative (LoF) or structural collagen defect (DN). Although phenotype-genotype correlations exist to a certain extent, there are many concerns regarding phenotype prediction based on a genotype, as symptoms develop individually.

The OI causing pathogenic variants may occur as *de novo* or can be hereditary. However, it is generally known that the more severe the phenotype the higher is the proportion of *de novo* cases, characterization of the *de novo* collagen-related OI was never addressed before.

OI type V is a rare OI type, which compromises 2–5% from general OI population. OI V arises due to 5'UTR c.–14C>T pathogenic variant in the *IFITM5* gene. Type V patients differ phenotypically with HPC, interosseous membrane calcifications and dislocation of the radial head. Type V patients lack hearing loss, DI and blue sclera. It is known, that clinical manifestations may vary between affected individuals, raising the value of phenotype descriptions for all patients with a confirmed genetic diagnosis of OI type V.

OI phenotype expressivity differs between carriers of the same pathogenic variant. OI inter- and intrafamilial diversity has been previously underlined, mainly in case reports; however, there is an urgent need for a systematic analysis of collagen-related OI phenotypical variability in terms of genotype. The current studies might benefit diagnosis and future therapeutics.

3. AIMS OF THE STUDY

The current thesis represents a large cohort studies of Osteogenesis Imperfecta patients with the overall objective to characterize a common profile of pathogenic variants and to evaluate genotype-phenotype correlations with inter- and intrafamilial diversity in OI families.

The specific aims were:

1. To characterize the *COL1A1* and *COL1A2* spectrum of pathogenic variants in a population of Estonian OI patients (Paper I);
2. To describe the clinical characteristics and molecularly specify *COL1A1*- and *COL1A2*-related OI in a cohort of Ukrainian OI families (Paper II);
3. To investigate *de novo* and inherited *COL1A1* and *COL1A2* pathogenic variants in terms of genotype characteristics and phenotype severity (Paper III);
4. To screen Estonian, Ukrainian and Vietnamese OI patients for the presence of an OI type V causative pathogenic variant (Paper IV);
5. To analyze correlations between inter- and intrafamilial variability and the genotype characteristics of *COL1A1*- and *COL1A2*-related OI families (Paper V).

4. MATERIALS AND METHODS

4.1. Study cohort

The study cohort was formed using patients from the Osteogenesis Imperfecta database of the Clinic of Traumatology and Orthopedics, University of Tartu, Estonia (UT OI database). The UT OI database consists of 238 OI families from Estonia (EE), Ukraine (UA) and Vietnam (VN). The database includes OI affected individuals and their healthy relatives. The total number of affected individuals in the database is 337.

4.1.1. Paper I

COL1A1/2 sequencing analysis was performed on 30 unrelated Estonian OI patients. Estonian OI families come from a register-based cohort of OI patients in Estonia of the Clinic of Traumatology and Orthopedics, Tartu University Hospital (Tartu, Estonia). The Estonian OI system was established in 1995, when it was agreed that all patients diagnosed with OI or suspected to have OI would be referred to Clinic of Traumatology and Orthopedics, Tartu University Hospital. The number of OI individuals in the Estonian database is 128. We assume that we have contacted almost all known OI families in Estonia.

4.1.2. Paper II

COL1A1/2 sequencing analysis was performed on 94 unrelated Ukrainian OI patients. Phenotype description was performed on 143 Ukrainian OI patients from 94 families. Ukrainian families were enrolled into the UT OI database via a collaboration with the Ukrainian Association of Crystal People and the Kharkiv Sytenko Institute of Spine and Joint Pathology (Ukraine). We have performed research trips to the West (Chernivci), East (Kharkiv) and Central (Kyiv) regions of the country to meet the patients. In May 2016 and September 2017, Ukrainian OI patients and their relatives (from all regions of Ukraine) attended interviews and clinical examination with researchers from the University of Tartu (Estonia) in cooperation with Ukrainian medical staff. Although the recruitment of the patients was population-based and there is no single register of OI patients in Ukraine, we have included all available patients from the Ukrainian Association of Crystal People and Ukrainian hospitals, of which the local community was made aware. Patients with other skeletal disorders were excluded from the study during screening (five families). A total number of 143 OI patients (66 males and 77 females; aged from 2 months to 65 years) from 94 unrelated families were included in the study.

4.1.3. Paper III

Analysis of *de novo* and inherited OI cases was performed in 146 unrelated subjects harboring *COL1A1/2* pathogenic variants from Estonian, Vietnamese and Ukrainian cohorts of the UT OI database.

4.1.4. Paper IV

Screening for the c.–14C>T pathogenic variants in the 5'UTR region of the *IFITM5* gene was performed in 90 unrelated OI patients negative for *COL1A1/2* pathogenic variants. Patients originated from Estonian (27), Ukrainian (62) and Vietnamese (57) OI cohorts of the UT OI database.

4.1.5. Paper V

An analysis of interfamilial diversity was performed in 81 families with *COL1A1/2* pathogenic variants, whose pathogenic variant and caused OI type were reported at least twice in the UT OI database or Dalgleish's OI variant database (http://www.le.ac.uk/ge/collagen/) (Dalgleish 1998). Intrafamilial diversity was explored in 64 families with collagen I-related OI, which was reported in at least two generations of the family. In addition, in four families OI was identified in a few siblings from the same generation without a previous OI history, whose parents were tested and did not carry the variant (Figure 10).

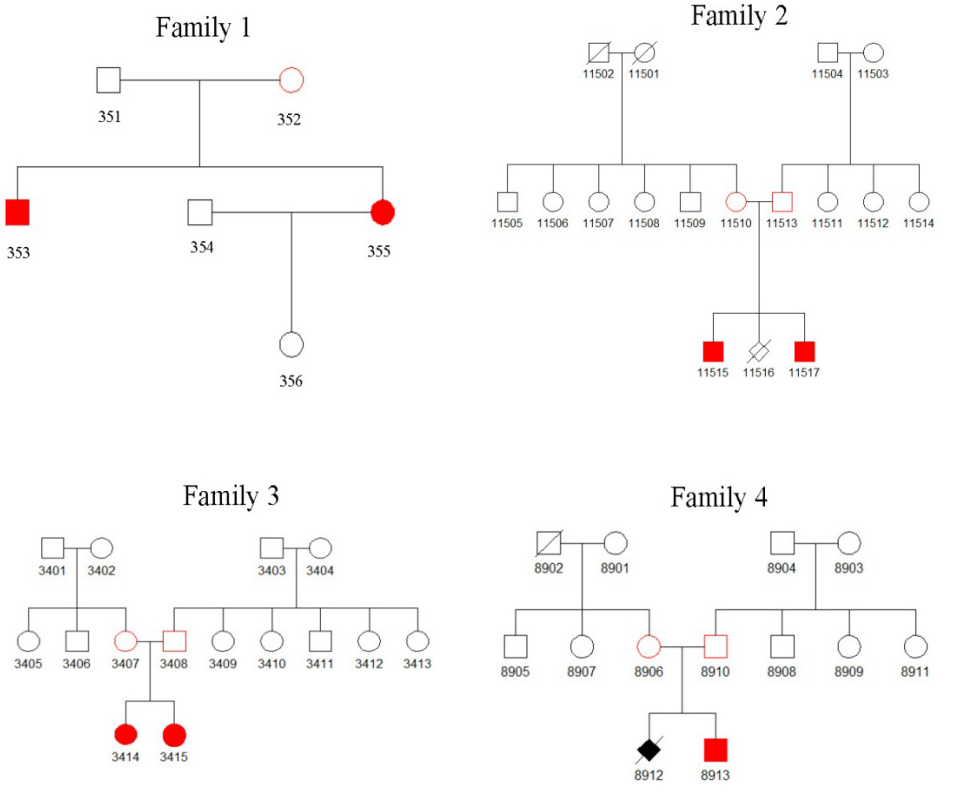


Figure 10. Pedigree trees of families with a few affected individuals in the same generation, but whose parents were not carriers of the variant, with suspected parental mosaicism.

4.2. Methods

All laboratory procedures and data analysis were performed at the University of Tartu, Estonia. In the current thesis Rome numerals (I–V) stand for clinical OI classification. The term "pathogenic variant" is used in the thesis in concordance with current guidelines of the ACMG, whereas the first publication contains the term "mutation" as a synonym (Richards et al., 2015).

4.2.1. Data and sample collection

Genealogical data was obtained in spoken word from subjects during oral interviews and included family consanguinity; OI family history; parental ages; health status; history of miscarriages. Pedigree trees were constructed using the "Kinship2" package in the R statistical program v3.3.2. (R team, Austria).

To evaluate OI clinical features, patients underwent clinical and physical examinations. OI cases were classified as OI types I–V, according to the observed clinical manifestations. Clinical data was recorded from available medical records. Phenotype description was assessed by observation. Skeletal fractures and deformations (severity, location) and extra-skeletal OI features (sclera color, DI) were noted. All sclera shades on the blue-gray scale were defined as "blue". Phenotypic data was obtained from the available medical documentation or from the patients or their relatives, including birth data (weight, height, intrauterine and birth fractures, preterm pregnancy); anthropometric data (weight and height); fracture history (time and location of the first fracture, total number of fractures, number of fractures per year); patient physical mobility; occurrence of hearing loss; joint laxity. The OI type and phenotype registration was conducted by a single medical professional to avoid bias.

Blood samples were obtained from the affected family members and their close healthy relatives. An ethylenediaminetetraacetic acid (EDTA)-preserved tubes were used for the entire blood sample collection and genomic DNA (gDNA) purification was performed using the Gentra Puregene Blood Kit (Quiagen, Germany) in accordance with the manufacturer's protocol. DNA samples were stored at −80°C until their analysis.

4.2.2. Sequencing analysis

4.2.2.1. Sequencing analysis of the COL1A1/2 genes (Paper I, II)

COL1A1/2 gene sequencing analysis was performed on the youngest affected individual from every OI kindred of 30 Estonian and 94 Ukrainian OI families. 25 polymerase chain reaction (PCR) primer pairs were used to amplify the *COL1A1* gene (51 exons) in the DNA samples of affected Estonian and Ukrainian OI patients. The *COL1A2* gene (52 exons) was amplified with 36 PCR primer pairs. Primers were designed to cover all exons, 5'and 3'UTR

regions. The list of *COL1A1* and *COL1A2* primers is provided in Appendix A. The PCR reaction was performed in 20 µl final volume, with 4 µl of 5x HOT FIREPol® Blend Master Mix Ready to Load with 7.5 mM $MgCl_2$) (Solis BioDyne, Estonia), 1 µl of each forward and reverse primer (5 pmol), 1 µl of gDNA (50 ng). PCR reaction was performed using a Thermal Cycler (Applied Biosystems, USA) PCR machine. The PCR touchdown program, used for the reaction of amplification was as follows –

1=95.0°; 15:00 min
2=95.0°; 0:25 min
3=64.0°; 0:30 min
4=72.0°; 0:40 min
5=Go to 2, 4 times
6=95.0°; 0:25 min
7=62.0°; 0:30 min
8=72.0°; 0:40 min
9=Go to 6, 30 times
10=72.0°; 5:00 min
11=6.0° forever

Amplified PCR products were electrophoresed through a 1.5% agarose gel. PCR products were purified with Shrimp alkaline phosphatase and Exonuclease I (Thermo Fisher Scientific, USA). Sanger sequencing reactions were performed from purified PCR fragments using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Reactions were processed on the ABI3730xl instrument.

Sequence reads were analyzed using the Sequence Scanner v1.0 of Applied Biosystems and aligned to the GenBank human reference genome sequences of COL1A1 (gDNA NG_007400.1, complementary DNA (cDNA) NM_000088.3) and COL1A2 (gDNA NG_007405.1, cDNA NM_000089.3). Sequence analysis and pathogenic variant identification was performed with Mutation Surveyor DNA variant analysis software of Softgenetics (USA). We focused on non-synonymous and splice-site variants absent from the publicly available normal datasets (including dbSNP135 and the 1000 Genomes Project) (Consortium, 2015; Sherry et al., 2001). We used the PolyPhen-2, SIFT, Alamut Visual software of Interactive Biosoftware, Variant Effect predictor (Ensemble), MutationTaster software tools to predict the functional effects and pathogenicity of pathogenic variants (Adzhubei et al., 2010; Kumar et al., 2009; Schwarz et al., 2010). Variants absent from the Osteogenesis Imperfecta pathogenic variant database were considered novel (http://www.le.ac.uk/ge/collagen/) (Dalgleish 1998). Pathogenicity of the novel genetic variants was interpreted using guidelines and standards of the joint consensus recommendation of the ACMG and the Association for Molecular Pathology (Richards et al., 2015).

4.2.2.2. Sequencing analysis of the 5'UTR region of the IFITM5 gene (Paper IV)

The presence of a c.–14C>T variant was controlled in a screened cohort of 90 unrelated patients negative for the *COL1A1/2* pathogenic variants. Only one patient was suspected previously to have OI type V, due to significant visible HPC.

DNA samples were amplified using a PCR with a specially designed primer pair covering the 5'UTR region and exon 1 of the *IFITM5* gene: IFITM5_F 5'-GGCTCCTGTACATCCCTGAA-3' (forward) and IFITM5 _R 5'-GTGTGA GGGCTGTGTGGG-3' (reverse).

The PCR amplification, PCR products purification, Sanger sequencing reaction performance and processing was performed as stated in the section 4.2.2.1. Sequence reads were aligned to the RefSeq sequence of the *IFITM5* (NM_001025295.1). Sequence analysis and pathogenic variant identification was performed with Mutation Surveyor DNA variant analysis software (Softgenetics, USA).

4.2.3. Analysis of *de novo* OI (Paper III)

Unrelated subjects diagnosed with OI types I–IV and harboring *COL1A1/2* pathogenic variants have undergone a comparative genotype and phenotype analysis of the *de novo* and inherited OI cases. The cohort included the youngest affected proband of every kindred from Estonian (27), Vietnamese (57) and Ukrainian (62) OI families.

The inheritance pattern for every kindred was determined based on OI family history. Cases with multiple affected individuals across different generations were considered as having inherited OI. Cases without a previous OI history were considered as *de novo*. Sequencing analysis in parents was performed in order to verify the inheritance pattern of *de novo* pathogenic variants.

The absence of pathogenic variants harbored by subjects with *de novo* OI was checked for and confirmed in all available parents. The total number of parent-child trios was 45. The total number of parent-child duos, i.e. where only one parent was available, was 29. No samples of relatives were available for 8 of the subjects, all of which harbored heterozygous variants. In cases where the parents' DNA samples were not obtained and a negative OI history in the family was determined from patients' words, a likely *de novo* state of the disorder was assumed. Afterwards, correlations between pathogenic variant nature, OI type and phenotype manifestations, and genotype were evaluated.

4.2.4. Genotype-phenotype correlations (Paper II)

OI skeletal and extraskeletal manifestations were compared with the mutated genes and the type of collagen defect to determine genotype-phenotype correlations. We have investigated associations based on the following clinical and phenotypical features: OI type (I, III, IV), scleral hue (blue), mobility (wheelchair, walking with support, walking independently, immobile), deformities (upper limb, lower limb, spine, chest) and fracture number (total and per year). Current phenotypic traits were correlated with the following characteristics of genotypes: type of the collagen defect (qualitative or quantitative), mutated gene (*COL1A1, COL1A2,* and non-collagen), Gly and Gly to Ser substitutions. Also, we have tested associations with the above listed phenotype features with qualitative and quantitative OI type I separately.

4.2.5. Interfamilial variability analysis (Paper V)

The study included 81 families, who harbored 62 different *COL1A1*/*COL1A2* pathogenic variants, which appeared to be independently reported in the OI variant database before by various authors. Families were grouped according to the following criteria: harbored OI pathogenic variant; clinical OI type reported in affected individuals from both UT OI database and Dalgleish's OI database (http://www.le.ac.uk/ge/collagen/) (Dalgleish 1998). Associations between altered gene, collagen I defect type and caused OI type were analyzed in different groups.

4.2.6. Intrafamilial variability analysis (Paper V)

Intrafamilial variability analysis was performed in 64 families harboring *COL1A1/2* pathogenic variants with OI diagnosed in two or more generations. Sequencing analysis of the *COL1A1/2* genes in the families was performed with Sanger sequencing. Affected family members from families with huge intrafamilial variability underwent the previously described whole exome sequencing (WES) (Maasalu et al., 2015).

Phenotypical variability was underlined by increasing progress of the OI phenotype severity patterns (i.e. OI symptoms with every younger generation) or decreasing of OI severity (i.e. OI symptoms dissolve in every younger generation). Severity of OI phenotype was evaluated via main OI symptoms: number of fractures; age of the first fracture; limb, rib cage and spine deformity degree; mobility status; presence of bluish sclera hue; hearing loss severity and age of hearing loss start. Phenotype variability was also explored in siblings from the same generation of one family with and without OI history. Intrafamilial diversity was identified via the presence of different OI types in the members of the same OI family. Only interviewed patients were counted, as the OI types of any unavailable patients was not registered. Correlations between genotype and degree of intrafamilial diversity were checked.

4.2.7. Statistical analysis

Percentage differences were used to assess the distribution of *COL1A1/2* pathogenic variants in Estonian and Ukrainian OI cohorts and compare them to other studied OI populations. The Shapiro-Wilk's test was used to check the normality of continuous variables. Normally distributed continuous data was presented as the mean and standard deviation (SD); continuous data lacking normal distribution is presented as median and range. The Student's t-test was used to compare normally distributed continuous data, and the Mann-Whitney U test was used to compare data without normal distribution. The categorical data was expressed as percentages. The significance of associations between the genotype and phenotype manifestations was tested using the Fisher's χ^2-test for categorical variables. P-values less than 0.05 were considered to be statistically significant. All statistical analyses were preformed using R v3.3.2. software (R Team, Austria) (Chen et al., 2012). The threshold of statistical significance was a p-value of less than 0.05.

4.3. Ethics

Informed written consent was collected from all subjects and controls, or their legal representatives, prior to their participation in the study. Additional written approval was collected to use the pictures and x-rays as illustrative materials in publications. The study was conducted in accordance with the Helsinki Declaration and authorized by the Ethical Review Committee on Human Research of the University of Tartu (Estonia) (Permit no. 221/M-34), the Ethical Review Board of Hue University Hospital (Vietnam) (approval No. 75/CN-BVYD) and the Sytenko Institute of Spine and Joint Pathology of the Ukrainian Academy of Medical Sciences (Ukraine).

5. RESULTS

5.1. Spectrum of the *COL1A1/2* pathogenic variants in the Estonian OI population (Paper I)

COL1A1/2 sequencing analysis with Sanger sequencing highlighted OI causative pathogenic variants in 86.67% (26/30) of Estonian OI patients. *COL1A1* and *COL1A2* pathogenic variants were observed in 76.92% (20/26) and 23.08% (6/26) of patients respectively (Figure 11). Discovered collagen type I pathogenic variants are represented in Table 3. Half of the discovered pathogenic variants were novel, previously undescribed in the OI variant database. (http://www.le.ac.uk/ge/collagen/). Pathogenicity classification of the novel genetic variants is represented in Table 3.1.

Twenty-five pathogenic variants were AD. Only eight patients did not have previous OI history in the family. One patient (EE07) harbored a recessive *COL1A1* pathogenic variant. Pathogenic variants were not present in healthy parents, which underlined the *de novo* nature of the pathogenic variants in existing patients.

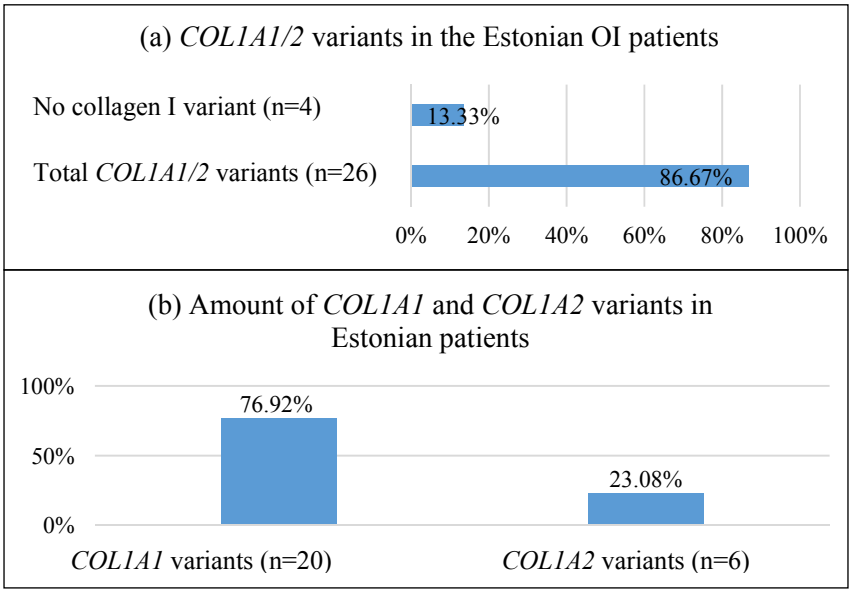


Figure 11. Diagram of collagen type I pathogenic variant distribution for Estonian OI patients. (a) Percentage of patients with *COL1A1/2* pathogenic variants vs patients negative for collagen I pathogenic variants. (b) Percentage of pathogenic variants in the *COL1A1* and *COL1A2* genes.

Table 3. Spectrum of pathogenic variants of the *COL1A1* and the *COL1A2* genes among Estonian OI patients.

#	Patient ID	Gene	Variant	Exon	Type of variant	Protein alteration	Sillence OI type
1	EE01#	*COL1A2*	c.1630G>T*	Exon 28	Missense	p.Gly544Cys	III
2	EE02	*COL1A1*	c.1821+1G>A	Intron 26	Splice site	–	III
3	EE03	*COL1A1*	c.1897G>T*	Exon 26	Nonsense	p.Glu633*	IV
4	EE04	*COL1A1*	c.750+2T>A*	Intron 10	Splice site	–	IV
5	EE05	*COL1A1*	c.1821+1G>A	Intron 26	Splice site	–	I
6	EE07#	*COL1A1*	c.2317G >T*, homozygous	Exon 33_34	Missense	p.Gly773Cys	II
7	EE08	*COL1A1*	c.3217G>A*	Exon 45	Missense	p.Gly1073Ser	III
8	EE09	*COL1A1*	c.1155+2T>G*	Intron 17	Splice site	–	I
9	EE10	*COL1A1*	c.1128_delT	Exon 17	Frameshift	p.Gly377Alafs*164	I
10	EE11#	*COL1A1*	c.3235G>A	Exon 45	Missense	p.Gly1079Ser	I
11	EE13	*COL1A1*	c.2089C>T	Exon 31	Nonsense	p.Arg697*	IV
12	EE14#	*COL1A1*	c.904-9G>A	Intron 13	Splice site	–	I
13	EE15	*COL1A2*	c.1009G>A	Exon 19	Missense	p.Gly337Ser	III
14	EE16#	*COL1A2*	c.2324G>A	Exon 38	Missense	p.Gly775Glu	III
15	EE17#	*COL1A1*	c.3045+1G>A	Intron 42	Splice site	–	IV
16	EE18	*COL1A1*	c.505G>A*	Exon 6	Missense	p.Glu169Lys	I
17	EE19	*COL1A1*	c.299-1G>C*	Intron 3	Splice site	–	IV
18	EE20	*COL1A2*	c.937-3C>T	Intron 18	Splice site	–	I

#	Patient ID	Gene	Variant	Exon	Type of variant	Protein alteration	Sillence OI type
19	EE21	*COL1A1*	c.3262G>T*	Exon 46	Nonsense	p.Gly1088*	IV
20	EE22	*COL1A1*	c.3262G>T*	Exon 46	Nonsense	p.Gly1088*	I
21	EE24	*COL1A1*	c.1767+5G>A*	Intron 25	Splice site	–	IV
22	EE25	*COL1A1*	c.1354-2A>G	Intron 20	Splice site	–	I
23	EE27#	*COL1A1*	c.3208-1G>A*	Intron 44	Splice site	–	I
24	EE29#	*COL1A2*	c.865G>A	Exon 17	Missense	p.Gly289Ser	III
25	EE30	*COL1A2*	c.2026-1_2031_hetdup*	Intron-Exon 34	Splice site, frameshift	–	III/IV
26	EE31#	*COL1A1*	c.1081C>T	Exon 17	Nonsense	p.Arg361*	I

Patients with *de novo* pathogenic variants and without OI history in the family are marked with an octothorp (#). Novel pathogenic variants previously unreported in the collagen type I variant database (http://www.le.ac.uk/ge/collagen/) are marked with an asterisk (*). In cases of heterozygous pathogenic variants, both the wild type and the mutated allele are indicated after an arrow (>).

Table 3.1. Classification of pathogenicity of novel *COL1A1* and the *COL1A2* variants identified in Estonian OI patients

#	Genetic variant	gnomAD	Poly-Phen2	SIFT	CADD	ACMG criteria	ACMG class
1	*COL1A2*, c.1630G>T, p.Gly544Cys	Absent	0.99	0	34	PM1, PM2, PM5, PM6, PP3	Likely pathogenic
2	*COL1A1,* c.1897G>T, p.Glu633*	Absent	NA	NA	41	PVS1, PM2, PP1, PP3	Pathogenic
3	*COL1A1,* c.750+2T>A, splice site	Absent	NA	NA	32	PVS1, PM2, PP1, PP3, PP5	Pathogenic
4	*COL1A1,* c.2317G>T, p.Gly773Cys	Absent	1	0	32	PM1, PM2, PM5, PM6	Likely pathogenic
5	*COL1A1,* c.3217G>A, p.Gly1073Ser	Absent	0.99	0.01	25.7	PM1, PM2, PP1, PP3	Likely pathogenic
6	*COL1A1,* c.1155+2T>G, splice site	Absent	NA	NA	34	PVS1, PM2, PP1, PP3	Pathogenic
7	*COL1A1,* c.505G>A, p.Glu169Lys (splice site change)	Present	0.29	0.29	23.5	PP1, PP3	VUS
8	*COL1A1,* c.299-1G>C, splice site	Absent	NA	NA	24.5	PVS1, PM2, PP1, PP3, PP5	Pathogenic
9	*COL1A1,* c.3262G>T, p.Gly1088*	Absent	NA	NA	52	PVS1, PM2, PP1, PP3	Pathogenic
10	*COL1A1,* c.3262G>T, p.Gly1088*	Absent	NA	NA	52	PVS1, PM2, PP1, PP3	Pathogenic
11	*COL1A1,* c.1767+5G>A, splice site	Absent	NA	NA	22.6	PVS1, PM2, PP1, PP3, PP5	Pathogenic
12	*COL1A1,* c.3208-1G>A, splice site	Absent	NA	NA	26.2	PVS1, PM2, PM6, PP3	Pathogenic
13	*COL1A2*, c.2026-1_2031_hetdup, p.Arg361*	Absent	NA	NA	–	PVS1, PM2, PP1, PP3	Pathogenic

PVS1 – null variant is a known mechanism of disease; PM1 – located in critical functional domain; PM2 – absent from controls (ExAC, 1000 Genomes); PM5 – novel missense change at an amino acid residue with previously determined pathogenic change; PM6 – assumed *de novo*; PP1 – co-segregation with disease in multiple affected family members; PP3 – multiple lines of computational evidence suggest pathogenic impact on the protein function; PP5 – reputable source recently reports variant as pathogenic; VUS-variant of unknown significance.

LoF pathogenic variants were present in 69.23% of Estonian OI patients (18/26), 16 in the *COL1A1* and two in the *COL1A2* gene (Figure 12). Splice site pathogenic variants composed 46.15% (12/26), 10 and two in *COL1A1* and *COL1A2* respectively. One patient had deletion in the intron-exon 34 region of the *COL1A2* gene (EE30). Six out of 26 patients (23.08%) harbored *COL1A1/2* nonsense pathogenic variants.

DN pathogenic variants were observed in 30.77% of patients (8/26). Four were observed in both *COL1A1* and *COL1A2* genes. Gly substitutions were indicated in seven cases (*COL1A1* – 4, *COL1A2* – 3). In four patients Gly to Ser substitutions were confirmed (Figure 12).

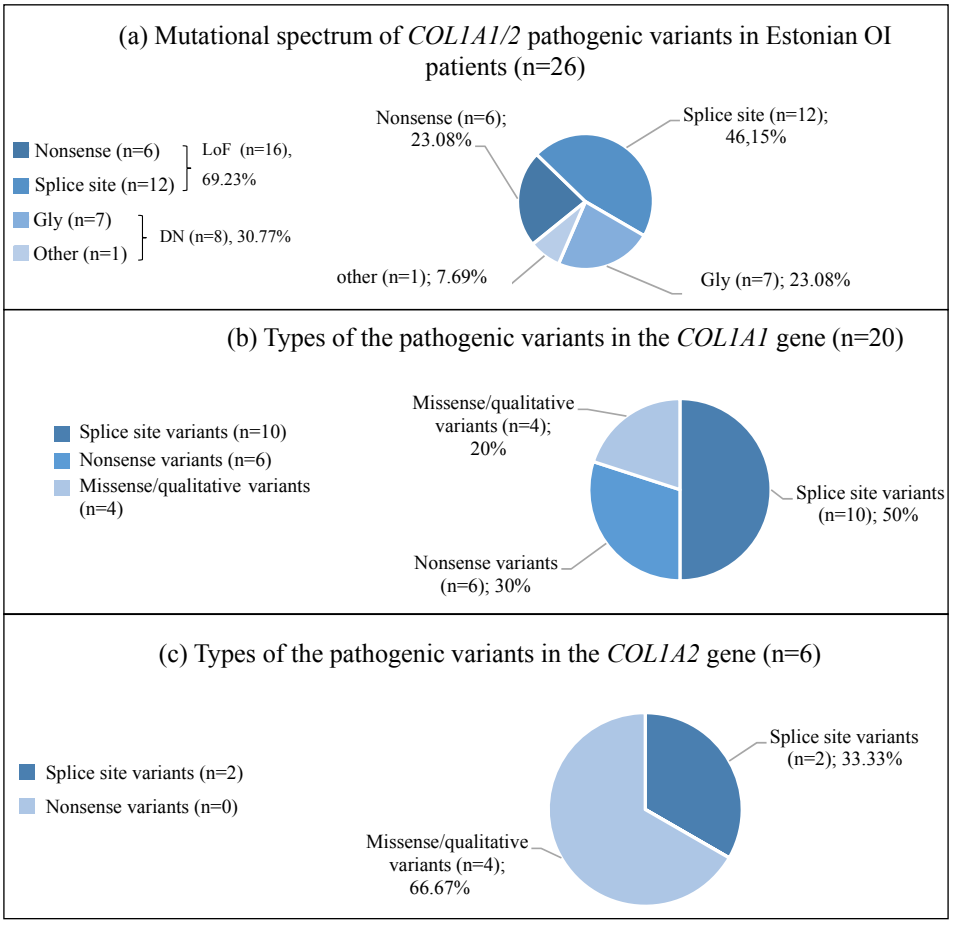


Figure 12. (a) Spectrum of pathogenic variants of *COL1A1/2* genes in Estonian OI patients. Distribution of the *COL1A1* (b) and *COL1A2* (c) mutations according to mutation type.

Interestingly, patients EE21 and EE22 appeared to be carriers of the identical pathogenic variant c.3262G>T (*COL1A1*). Investigation of pedigree trees revealed a relation between ancestors of the families four generations back, of which the patients were not aware. Patients EE02 and EE05 were carriers of the same splice site pathogenic variant c.1821+1G>A (*COL1A1*). The pathogenic variant arose independently and patients developed OI manifestations of different severity.

5.2. Spectrum of the *COL1A1/2* variants in the Ukrainian OI population (Paper II)

Sequencing analysis of the *COL1A1/2* genes in unrelated Ukrainian OI patients revealed the presence of AD collagen type I pathogenic variants in 63.83% (60/94) (Figure 13a). Out of patients with OI type I 63.89% (23/36); type III 60.87% (14/23); type IV 69.70% (23/33) harbored collagen type I pathogenic variants. The total number of pathogenic variants was 63 because double pathogenic variants in the *COL1A1/2* genes were found in patients UA08 and UA85. In addition, a compound heterozygous pathogenic variant in the *COL1A1* gene was confirmed in patient UA55. The discovered pathogenic variants are

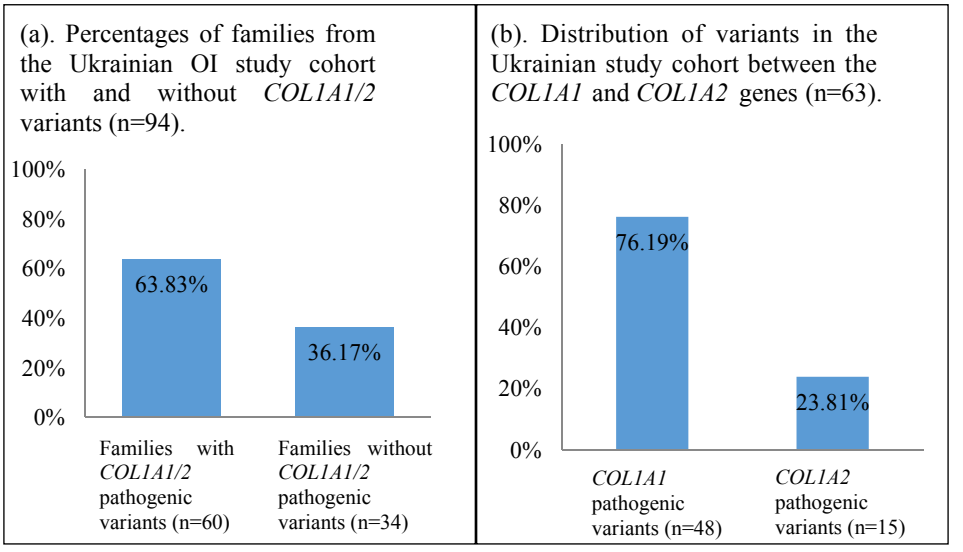


Figure 13. (a) Percentages of the families of Ukrainian OI study cohort with and without *COL1A1/2* pathogenic variants (n=94). (b) Distribution of pathogenic variants in Ukrainian OI study cohort between the *COL1A1* and *COL1A2* genes (n=67).

Forty-eight out of the 63 pathogenic variants (76.19%) were located in the *COL1A1* gene. Fifteen out of 63 (23.81%) pathogenic variants altered the *COL1A2* gene (Figure 13b). Twenty seven out of 63 (42.85%) (20 in *COL1A1*; 7 in *COL1A2*) pathogenic variants were novel and unreported in the OI collagen type I variant database previously. A pathogenicity evaluation of the novel variants is represented in Table 4.1. Thirty one out of 63 (49.21%) were DN pathogenic variants, 32 out of 63 (50.79%) were LoF pathogenic variants (Figure 14a). Out of DN pathogenic variants 58.06% (18/31) and 41.94% (13/31) were located in the *COL1A1* and *COL1A2* genes respectively. Twenty four out of 31 (77.42%) DN pathogenic variants were Gly substitutions (58.33% (14/24) *COL1A1*; 41.67% (10/24) *COL1A2*). Gly to Ser substitutions were present in 45.83% (11/24%) out of them (7 *COL1A1*; 4 *COL1A2*) (Figures 14b–14d). Two patients had missense non-Gly substitution in the C-terminal propeptide of collagen α1 (*COL1A1*): UA21 c.3655G>GT (p.(Asp1219Tyr)) and UA71 c.4356G>GC (p.(Gln1452His)). Thirty out of 32 (93.75%) of LoF pathogenic variants were in *COL1A1* gene. Twelve out of 32 (37.50%) were frameshift pathogenic variants (91.67% (11/12) in *COL1A1*; 8.33% (1/12) *COL1A2*). 34.38% (11/32) were splice site pathogenic variants (9 *COL1A1;* 1 *COL1A2*). 31.25% (10/32) of LoF pathogenic variants were nonsense pathogenic variants, allocated in the *COL1A1* gene (Figures 14 b–d).

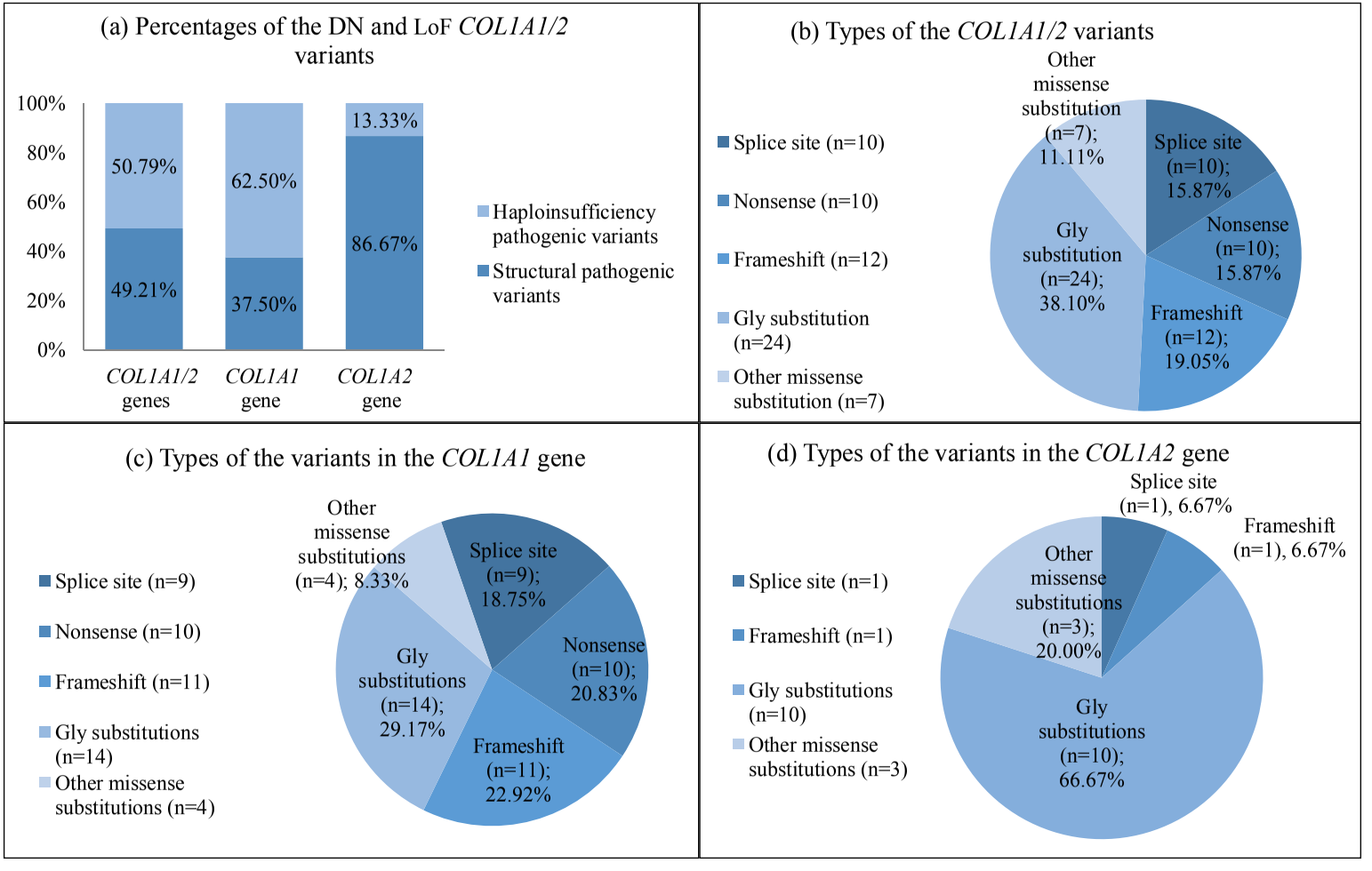


Figure 14. (a) Percentages of the DN and LoF *COL1A1/2* pathogenic variants in Ukrainian OI study cohort. (b) Spectrum of pathogenic variants of the *COL1A1/2* genes in Ukrainian OI study cohort. (c) Types of mutations in the *COL1A1* gene in Ukrainian OI study cohort. (d) Types of pathogenic variants in the *COL1A2* gene in the Ukrainian OI study cohort.

Table 4. The *COL1A1* (gDNA NG_007400.1, cDNA NM_000088.3) and *COL1A2* (gDNA NG_007405.1, cDNA NM_000089.3) spectrum of pathogenic variants among Ukrainian OI families. Patients with sporadic pathogenic variants are marked with an obelisk (†). Novel variants unreported in the collagen type I variant database (http://www.le.ac.uk/ge/collagen/) are marked with a diesis (‡).

#	Patient ID	Gene	Pathogenic variant	Exon	Pathogenic variant type	Protein alteration	OI type
1	UA48†	*COL1A1*	c.1675_delG‡	25	frameshift	p.Ala559Profs*21	I
2	UA91	*COL1A1*	c.1816_del G‡	26	frameshift	p.Ala606Leufs*160	I
3	UA95†	*COL1A1*	c.3489_del G‡	48	frameshift	p.Pro1165Leufs*74	IV
4	UA72†	*COL1A1*	c.3759_delG‡	50	frameshift	p.Asp1254Thrfs*77	I
5	UA15†	*COL1A1*	c.459_delT	5	frameshift	p.Gly154Alafs*111	I
6	UA14†	*COL1A1*	c.579_delT	7	frameshift	p.Gly194Valfs*71	I
7	UA61	*COL1A1*	c.2393_dupC‡	35	frameshift	p.Gly800Argfs*5	I
8	UA67	*COL1A1*	c.2523_delT	37	frameshift	p.Gly842Alafs*266	I
9	UA31	*COL1A1*	c.2821_delG‡	40	frameshift	p.Gly941Valfs*167	IV
10	UA101	*COL1A1*	c.3226G>A	45	missense	p.Gly1076Ser	IV
11	UA23	*COL1A1*	c.769G>A	11	missense	p.Gly257Arg	I
12	UA27†	*COL1A1*	c.2101G>T	31	missense	p.Gly701Cys	III
13	UA62	*COL1A1*	c.1057G>T	17	missense	p.Gly353Cys	IV
14	UA39	*COL1A1*	c.3652G>A	49	missense	p.Ala1218Thr	IV
15	UA57†	*COL1A1*	c.757C>T	11	nonsense	p.Arg253*	IV
16	UA21†	*COL1A1*	c.3655G>T‡	49	missense	p.Asp1219Tyr	IV
17	UA71†	*COL1A1*	c.4356G>C‡	52	missense	p.Gln1452His	III
18	UA89†	*COL1A1*	c.653G>A	9	missense	p.Gly218Asp	I
19	UA53†	*COL1A1*	c.734G>A‡	10	missense	p.Gly245Glu	I

#	Patient ID	Gene	Pathogenic variant	Exon	Pathogenic variant type	Protein alteration	OI type
20	UA33†	*COL1A1*	c.742G>A	10	missense	p.Gly284Arg	III
21	UA30†	*COL1A1*	c.1319G>C‡	20	missense	p.Gly440Ala	III
22	UA76†	*COL1A1*	c.1192G>A‡	18	missense	p.Gly398Ser	III
23	UA94†	*COL1A1*	c.1588G>A	23	missense	p.Gly530Ser	III
24	UA10	*COL1A1*	c.2362G>A	35	missense	p.Gly788Ser	IV
25	UA78	*COL1A1*	c.2434G>A‡	36	missense	p.Gly812Ser	IV
26	UA05†	*COL1A1*	c.2461G>A	37	missense	p.Gly821Ser	III
27	UA96	*COL1A1*	c.2560G>A	38	missense	p.Gly854Ser	I
28	UA32	*COL1A1*	c.1A>C‡	1	missense	p.Met1Leu	I
29	UA44†	*COL1A1*	c.1789G>T	26	nonsense	p.Glu597*	I
30	UA50	*COL1A1*	c.3076C>T	43	nonsense	p.Arg1026*	I
31	UA19	*COL1A1*	c.658C>T	9	nonsense	p.Arg220*	I
32	UA45†	*COL1A1*	c.1081C>T	17	nonsense	p.Arg361*	IV
33	UA64	*COL1A1*	c.1243C>T	19	nonsense	p.Arg415*	I
34	UA17†	*COL1A1*	c.1426G>T‡	21	nonsense	p.Arg476*	I
35	UA93	*COL1A1*	c.2179C>T‡	32	nonsense	p.Gln727*	IV
36	UA73†	*COL1A1*	c.3807G>A	49	nonsense	p.Trp1269*	I
37	UA22†	*COL1A1*	c.495T>A‡	6	nonsense	p.Tyr165*	I
38	UA25	*COL1A1*	c.904-9G>A	13i	splice site	–	I
39	UA38	*COL1A1*	c.2613+6T>C	38i	splice site	–	IV
40	UA40†	*COL1A1*	c.1614+1G>A	23i	splice site	–	IV
41	UA41†	*COL1A1*	c.3815-1G>A‡	48i	splice site	–	IV

#	Patient ID	Gene	Pathogenic variant	Exon	Pathogenic variant type	Protein alteration	OI type
42	UA43†	*COL1A1*	c.858+1G>A	12i	splice site	–	IV
43	UA56†	*COL1A1*	c.858+1G>A	12i	splice site	–	IV
44	UA80	*COL1A1*	c.804+1G>A	11i	splice site	–	IV
45	UA92†	*COL1A2*	c.2026-1_2042_dup‡	33i–34	splice site	–	III
46	UA86†	*COL1A2*	c.2045G>T‡	34	missense	p.Gly682Val	III
47	UA37	*COL1A2*	c.2233G>C	37	missense	p.Gly745Arg	III
48	UA83†	*COL1A2*	c.2027G>A	34	missense	p.Gly676Asp	IV
49	UA51†	*COL1A2*	c.3034G>A	46	missense	p.Gly1012Ser	III
50	UA54†	*COL1A2*	c.982G>A	19	missense	p.Gly328Ser	III
51	UA65	*COL1A2*	c.1009G>A	19	missense	p.Gly337Ser	IV
52	UA102†	*COL1A2*	c.2224G>A‡	37	missense	p.Gly742Arg	I
53	UA74	*COL1A2*	c.2288G>T	37	missense	p.Gly763Val	III
54	UA47	*COL1A2*	c.2314G>A	38	missense	p.Gly772Ser	IV
55	UA69†	*COL1A2*	c.2324G>A	38	missense	p.Gly775Glu	IV
56	UA90†	*COL1A2*	c.1220T>C‡	22	missense	p.Leu407Pro	I
57	UA82	*COL1A2*	c.2093_2110_dup‡	35	frameshift	p.Leu699_Leu704dup	III
58	UA55†	*COL1A1*	c.2195_delA‡	32	frameshift	p.Glu732Aspfs*34	IV
		COL1A1	c.2548_2549_dupC‡	36	frameshift	p.Gly851Luefs*258	
59	UA85	*COL1A1*	c.370-1G>A	4i	splice site	–	I
		COL1A2	c.2642A>C‡	41	missense	p.Glu881Ala	
60	UA08†	*COL1A1*	c.334-1G>A‡	3i	splice site	–	IV
		COL1A2	c.2642A>C‡	41	missense	p.Glu881Ala	

Table 4.1. Classification of pathogenicity of novel *COL1A1* and the *COL1A2* variants identified in Ukrainian OI patients.

#	Genetic variant	gnomAD	Poly-Phen2	SIFT	CADD	ACMG criteria	ACMG class
1	*COL1A1,* c.1675_delG, p.Ala559Profs*21	Absent	NA	NA	–	PVS1, PM2, PM6, PP3	Pathogenic
2	*COL1A1,* c.1816_del G, p.Ala606Leufs*160	Absent	NA	NA	–	PVS1, PM2, PP1, PP3	Pathogenic
3	*COL1A1,* c.3489_del G, p.Pro1165Leufs*74	Absent	NA	NA	–	PVS1, PM2, PM6, PP3	Pathogenic
4	*COL1A1,* c.3759_delG, p.Asp1254Thrfs*77	Absent	NA	NA	–	PVS1, PM2, PM6, PP3	Pathogenic
5	*COL1A1,* c.2393_dupC, p.Gly800Argfs*5	Absent	NA	NA	–	PVS1, PM2, PP1, PP3	Pathogenic
6	*COL1A1,* c.2821_delG, p.Gly941Valfs*167	Absent	NA	NA	–	PVS1, PM2, PP1, PP3	Pathogenic
7	*COL1A1,* c.3655G>T, p.Asp1219Tyr	Absent	0.99	0	29	PM1, PM2, PM5, PM6, PP3	Likely pathogenic
8	*COL1A1,* c.4356G>C, p.Gln1452His	Absent	0.99	0	25	PM1, PM2, PM6, PP3	Likely pathogenic
9	*COL1A1,* c.734G>A, p.Gly245Glu	Absent	0.99	0	29	PM1, PM2, PM6, PP3	Likely pathogenic
10	*COL1A1,* c.1319G>C, p.Gly440Ala	Absent	0.99	0	27	PM1, PM2, PM6, PP3	Likely pathogenic
11	*COL1A1,* c.1192G>A, p.Gly398Ser	Absent	0.99	0.01	30	PM1, PM2, PM5, PM6, PP3	Likely pathogenic
12	*COL1A1,* c.2434G>A, p.Gly812Ser	Absent	0.99	0	32	PM1, PM2, PP1, PP3	Likely pathogenic
13	*COL1A1,* c.1A>C, p.Met1Leu	Absent	0.743	0.05	26	PM1, PM2, PM5, PP1, PP3	Likely pathogenic
14	*COL1A1,* c.1426G>T, p.Arg476*	Absent	NA	NA	39	PVS1, PM2, PM6, PP3	Pathogenic
15	*COL1A1,* c.2179C>T, p.Gln727*	Absent	NA	NA	41	PVS1, PM2, PP1, PP3	Pathogenic
16	*COL1A1,* c.495T>A, p.Tyr165*	Absent	NA	NA	34	PVS1, PM2, PM6, PP3	Pathogenic
17	*COL1A1,* c.3815-1G>A, splice site	Absent	NA	NA	33	PVS1, PM2, PM6, PP3	Pathogenic

#	Genetic variant	gnomAD	Poly-Phen2	SIFT	CADD	ACMG criteria	ACMG class
18	*COL1A2,* c.2026-1_2042_dup, splice site	Absent	NA	NA	–	PVS1, PM2, PM6, PP3	Pathogenic
19	*COL1A2,* c.2045G>T, p.Gly682Val	Absent	0.99	0	34	PM1, PM2, PM5, PM6, PP3	Likely pathogenic
20	*COL1A2,* c.2224G>A, p.Gly742Arg	Absent	0.99	0	34	PM1, PM2, PM6, PP3	Likely pathogenic
21	*COL1A2,* c.1220T>C, p.Leu407Pro	Absent	0.92	0.07	27	PM2, PM6, PP3	Likely pathogenic
22	*COL1A2,* c.2093_2110_dup, p.Leu699_Leu704dup	Absent	NA	NA	–	PM2, PM4, PM6, PP3	Likely pathogenic
23	*COL1A1,* c.2195_delA, p.Glu732Aspfs*34	Absent	NA	NA	–	PVS1, PM2, PM6, PP3	Pathogenic
24	*COL1A1,* c.2548_2549_dupC, p.Gly851Luefs*258	Absent	NA	NA	–	PVS1, PM2, PM6, PP3	Pathogenic
25	*COL1A2,* c.2642A>C, p.Glu881Ala	Present	0.265	0.03	23	PM6, PP3	VUS
26	*COL1A1,* c.334-1G>A, splice site	Absent	NA	NA	27	PVS1, PM2, PM6, PP3	Pathogenic
27	*COL1A2,* c.2642A>C, p.Glu881Ala	Present	0.265	0.03	23	PM6, PP3	VUS

PVS1 – null variant is a known mechanism of disease; PM1 – located in critical functional domain; PM2 – absent from controls (ExAC, 1000 Genomes); PM4 – protein length changes; PM5 – novel missense change at an amino acid residue with the previously determined pathogenic change; PM6 – assumed *de novo*; PP1 – co-segregation with disease in multiple affected family members; PP3 – multiple lines of computational evidence; PP5 – reputable source recently reports variant as pathogenic; VUS-variant of unknown significance.

5.3. Phenotypical and genotypic signatures in *de novo* and inherited *COL1A1/2* pathogenic variants (Paper III)

Etiology of the OI causative pathogenic variant was analyzed in 146 OI cases of Estonian, Ukrainian and Vietnamese origin harboring *COL1A1/2* pathogenic variants.

Eighty-two (56.16%) patients appeared to be *de novo* cases of OI in their families. Estonian (EE) OI patients had 10/27 (37.04%) *de novo* cases; Ukrainian (UA) 36/62 (58.06%); Vietnamese (VN) 36/57 (63.16%) (Figure 15, Table 5).

Seven patients were carriers of compound heterozygous pathogenic variants, so the total number of analyzed pathogenic variants was 153.

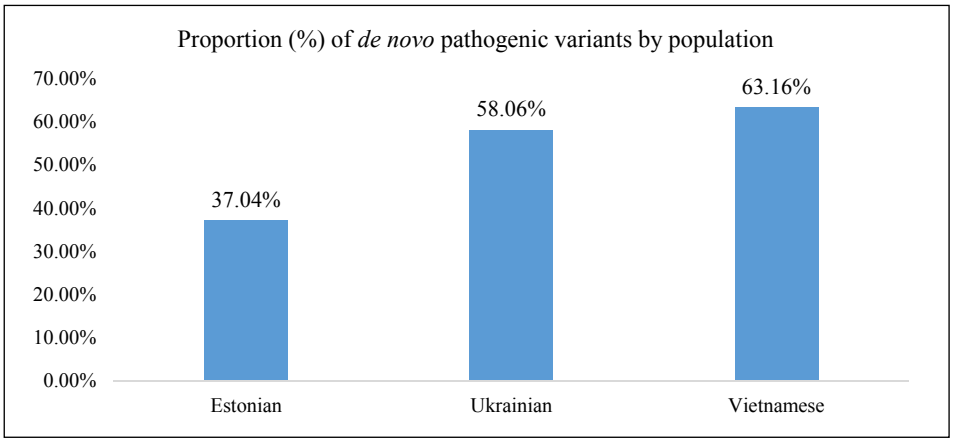


Figure 15. Proportion (%) of *de novo* pathogenic variants by population.

5.3.1. Phenotypical characteristics and OI manifestations in *COL1A1/2 de novo* pathogenic variants

We have found strong correlations between OI type and proportion of *de novo* pathogenic variants (p=0.0002) (Table 5). The number of patients with OI type I, III and IV were almost even: OI type I (26.83%), OI type III (35.36%) and OI type IV (36.59%). Among inherited OI cases OI types were distributed as follows: OI type I – 45.31%, OI type III – 7.81%, and OI type IV – 46.88%. OI type I and OI type IV were more prevalent among inherited OI cases compared to *de novo* OI. *De novo* variants composed 85.29% of the OI type III cases (Figure 16).

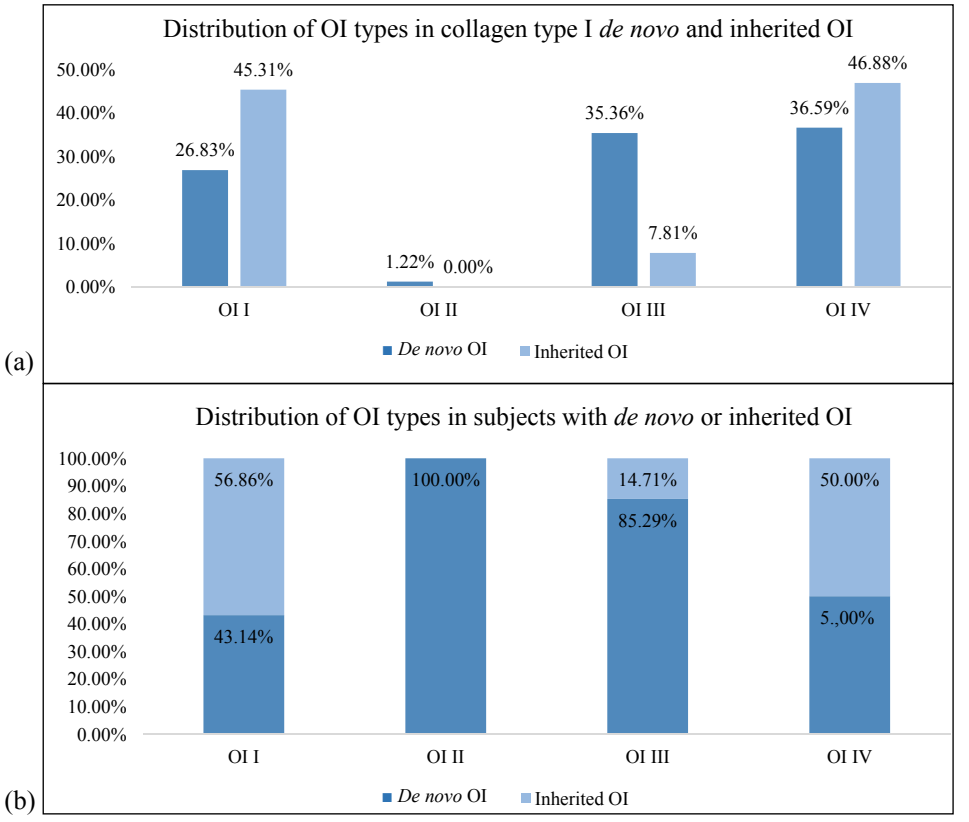


Figure 16. (a) Distribution of OI types in collagen type I *de novo* and inherited OI. (b) Distribution of OI types in subjects with *de novo* or inherited OI.

Analysis of DI, hearing loss and sclera hue did not show any associations with the etiology of the pathogenic variants. Out of the *de novo* cases 80.49% had normal hearing and 84.38% had blue sclera. Among inherited OI cases, blue sclera composed 90.24% and normal hearing was spotted in 95.31% of patients. DI was found in 58.54% of *de novo* and 54.69% of inherited OI cases.

The total fracture number (p=0.0224), as well as the number of fractures per year (p=0.0014) were significantly greater in *de novo* OI cases (means of 21.96 and 1.74, respectively) compared to inherited OI (means of 12.50 and 1.07, respectively) cases. In cases of *de novo* OI type III (mean 2.35, p=0.0023) the number of fractures per year was significantly higher compared to inherited OI type III. Differences remained in the number of fractures per year (mean 0.95). Differences in the fracture number per year of *de novo* and inherited type I and IV cases were insignificant.

Table 5. Characteristics of *de novo* and inherited OI cases. EE – Estonian, UA – Ukrainian, VN – Vietnamese populations.

All patients	***De novo***	**%**	**Inherited**	**%**	**Total**	**%**	**P-value**
Total	82	56.16%	64	43.84%	146	100%	
EE	10	37.04%	17	62.96%	27	18.49%	
UA	36	58.06%	26	41.93%	62	42.47%	
VN	36	63.16%	21	36.84%	57	39.04%	
Sex							
Males	34	49.28%	35	50.72%	69	47.26%	0.1338
Females	48	62.34%	29	37.66%	77	52.74%	
OI Type							
OI I	22 (26.83%)	43.14%	29 (45.31%)	56.86%	51	34.93%	
OI II	1 (1.22%)	100.00%	0	0.00%	1	0.69%	**0.0002**
OI III	29 (35.36%)	85.29%	5 (7.81%)	14.71%	34	23.29%	
OI IV	30 (36.59%)	50.00%	30 (46.88%)	50.00%	60	41.09%	
Fractures							
Number of total fractures (mean)	21.96		12.50		17.81		**0.0224**
Fractures per year (mean)	1.74		1.07		1.45		**0.0014**
Genotype							
COL1A1	58 (65.91%)	52.25%	53 (81.54%)	47.75%	111	72.55%	**0.0360**
COL1A2	30 (34.09%)	71.43%	12 (18.46%)	28.57%	42	27.45%	
Functional type							
Loss of Function	28 (31.82%)	43.75%	36 (55.38%)	56.25%	64	41.83%	
Nonsense and frameshift	12	37.50%	20	62.50%	32		**0.0063**
Splice site	16	50.00%	16	50.00%	32		
Missense	60 (68.18%)	67.42%	29 (44.62%)	32.58%	89	58.17%	
Gly	48	68.57%	22	28.57%	70		**0.0139**
Gly-Ser	24	61.54%	15	38.46%	39		0.5796
Architecture of pathogenic variants							
Transitions	54	56.84%	41	43.16%	95	62.09%	
Transversions	24	63.16%	14	36.84%	38	24.84%	0.6039
Indel	10	50.00%	10	50.00%	20	13.07%	

5.3.2. Proportion, functional type and architecture of *de novo* and inherited *COL1A1/2* pathogenic variants.

There was a significant association between mutated gene and etiology of the pathogenic variants. (p=0.0360). *COL1A1* pathogenic variants composed 65.91% (58/88) and 81.54% (53/65) of *de novo* and inherited cases respectively (Figure 17 a, Table 5). *De novo* variants composed 52.25% of identified *COL1A1* pathogenic variants. *COL1A2* pathogenic variants composed 34.09% (30/88) and 18.46% (12/65) among *de novo* and inherited cases respectively (Table 5, Figure 17 a).

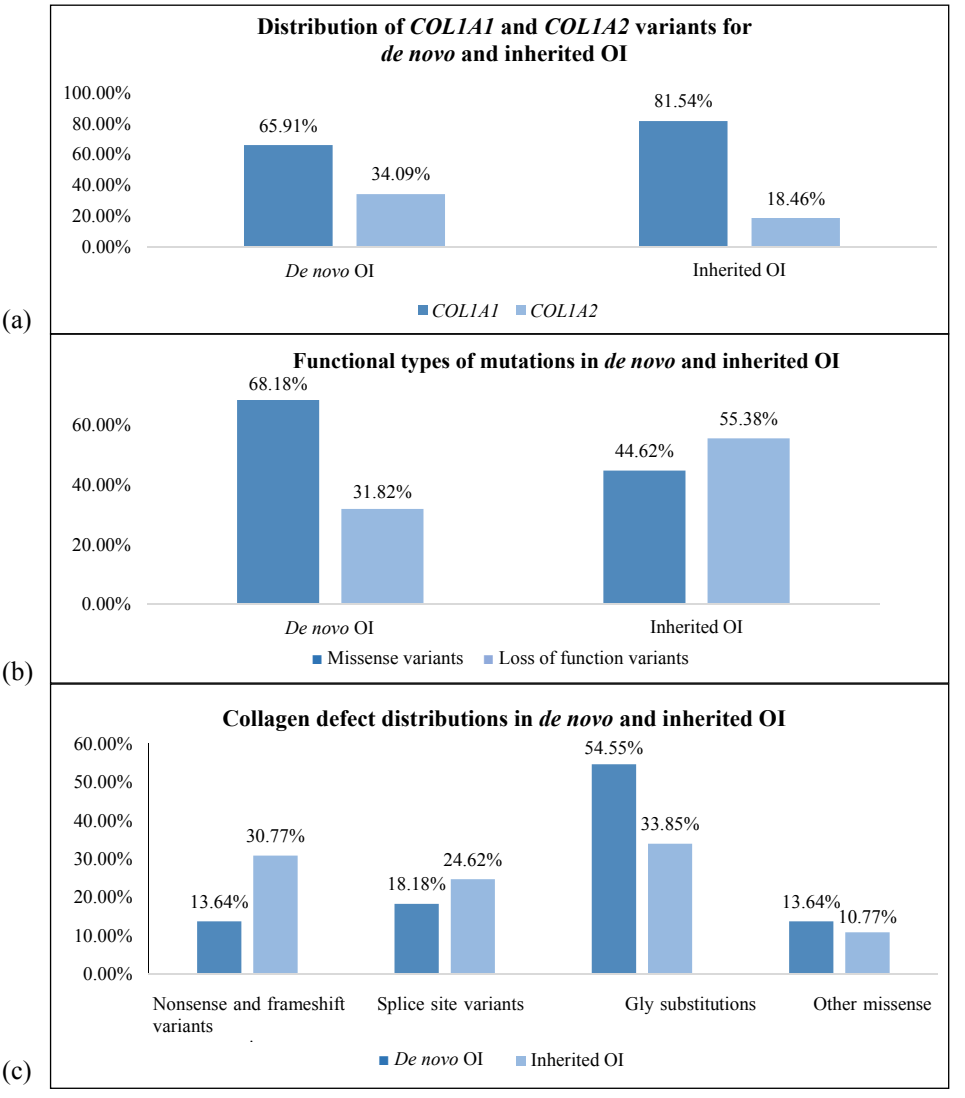


Figure 17. (a) Distribution of *COL1A1* and *COL1A2* pathogenic variants for *de novo* and inherited OI. (b) Functional types of pathogenic variants in *de novo* and inherited OI. (c) Collagen defect distributions in *de novo* and inherited OI.

Functional type of the collagen type I defect correlated with the etiology of the pathogenic variant (p=0.0063) 68.18% of the DN pathogenic variants were *de novo*. However, 55.38% of LoF pathogenic variants were inherited (Figure 17 b). Of the *de novo* pathogenic variants, 54.55% were Gly substitutions (Figure 17c). Out of DN pathogenic variants (n=89), 67.42% were *de novo* (n=60) (Table 5). Out of Gly and Gly to Ser substitutions, 68.57% (48/70) and 61.54% (24/39) arose *de novo* respectively. There were more Gly substitutions among *de novo* OI cases, compared to inherited (p=0.0139). *De novo* variants composed 43.75% (28/64) of LoF pathogenic variants. Half of splice site pathogenic variants (16/32) and 37.50% (12/32) of nonsense and frameshift variants had *de novo* etiology (Table 5). The correlation between position of the pathogenic variant as well as architecture of the pathogenic variant and its etiology was insignificant.

Nucleotide transitions composed 62.09% (n=95), of which 50.66% were G>A pathogenic variants. Transversions composed 24.84% (n=38), among which 13.07% were G>T pathogenic variants (n=20). Indels arose 20 times, of which 14 were deletions.

5.4. OI type V in Ukrainian and Vietnamese OI populations (Paper IV)

We screened 90 unrelated OI patients for the presence of a heterozygous c.–14C>T variant in the 5'UTR of the *IFITM5* gene typical for OI type V. According to clinical examination, OI type V was suspected only in one patient. Nevertheless, we have discovered four more cases of OI type V, who had an identical pathogenic variant. Three patients were Ukrainian and one patient was of Vietnamese origin. In the Estonian OI cohort we have not discovered any carriers of the 5'UTR *IFITM5* gene pathogenic variant. In one case (patient 2), the pathogenic variant was inherited and the previous family history of OI was registered. The pathogenic variant was confirmed in an affected mother (patient 3). Prevalence of OI type V among cohort of OI patients from TU OI database was 1.48% (5/337) (Table 6).

Four patients had signs of HPC and dislocation of the radial head. In three patients, interosseous membrane calcification and metaphysial radiodense bands were discovered. Three patients had bluish sclera. Patient 1 had brittleness of the teeth of an unknown etiology and hearing loss since they were 14 years old. Patient 2 and 3 showed joint laxity. The number of fractures was 0.5 to 2.3 per year, with highest amount in patient 4. Deformities of limbs were mostly mild. Three patients had chest and spine deformities. Patient 1 was immobile. All patients were shorter than normal (Table 6).

Table 6. Phenotype characteristics of OI type V patients.

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**
Age	15	4	34	7	5
Sex	m	m	f	f	f
Country	Ukraine	Ukraine	Ukraine	Ukraine	Vietnam
Phenotype	severe	mild	moderate	moderate	mild
Mimicking classic OI type	V	I	IV	IV	I
OI history in the family	no	yes	yes	no	no
Hyperplastic callus	yes, extreme	yes	yes	no	yes
Interosseous membrane calcification	yes	yes	yes (fibula-tibia)	no	no
Radial head dislocation	yes	yes	yes	no	yes
Metaphyseal radiodense band	NA	yes	yes	no	yes
Hearing loss	yes, age 14	no	no	no	no
Dentinogenesis Imperfecta (DI)	no, teeth brittle	no	no	no	
Sclera hue	greyish	bluish	bluish	greyish	bluish
Number of fractures	8	4	26	16	5
Number of fractures per year	0.5	1	0.8	2.3	1
Time of the first fracture	1.3	7 months	at birth	3 months	2
First fractured bone	femur	left femur	elbow, femur	scapula	lower leg
The most frequently fractured bone	femur	femur	femur	legs	lower leg
Pyramidal/bell-shaped chest	yes, severe	no	yes	yes	no
Scoliosis	yes	no	yes	yes	no
Upper limb long bone deformity	yes, mild	yes, mild	yes	no	yes, mild
Lower limb long bone deformity	yes	yes, mild	yes	yes, mild	yes, mild
Mobility	immobile, lying	walking	walking (didn't walk at age 9–14)	walking	walking
Height (Z score)	–3.93	0.66	–2.70	–2.83	–1.22
Birthweight and length	52/3450	51/2500	52/3300	50/2800	NA/2800
Joint laxity	no	yes	yes	no	NA

5.5. Clinical OI types and phenotype manifestations in Ukrainian patients (Paper II)

The study included 143 affected patients from 94 unrelated Ukrainian OI families. Males composed 46.15% (66/143) and 53.85% (77/143) were females. None of the families were consanguineous. OI history was observed in 38.71% (36/94) of the families. Information about one patient was missing, as she grew up in an orphanage. Patients were divided according to clinical classification into the five following OI types: type I 46.85% (n=67); type III 16.78% (n=24); type IV 34.27% (n=49); and type V 2.10% (n=3) (Figure 18a). The age range of the studied individuals was 2 months – 65 years (mean age of 19.46±14.38 years). Half of the patients (54.23%, 77/143) were in the 0–17 (pediatric) age group. Clinical characteristics and phenotype manifestations of the studied Ukrainian OI patients may be found in Table 7. The median/(range) for the total number of fractures per patient was 10.00 (0–300) (type I 5.00 (0–30), type III 20.00 (1–300), type IV 11.00 (0–60) and type V (median±SD) 12.67±11.72) (Figure 18, Figure 19). The number of fractures per year per affected individual was 1.32±2.88. The number of fractures divided by OI types was as follows: type I 0.50±0.43, type III 3.51±6.18, type IV 0.83±1.76 and type V 0.77±0.23 (Figure 18c). The highest number of fractures was observed in a 35-year-old individual with OI type III, who had 300 fractures (lock time May 2016). Only 4.96% of patients (7/143) did not have fractures, two of them were in the adult age group (a 50 year old, type I; a 33 year old, type IV). A positive OI diagnosis in patients without bone fractures was based on having a positive OI history, skeletal deformities, and/or extraskeletal manifestations. Intrauterine fractures were registered in seven affected individuals (type III 30.43%, n=7; type IV 4.17%, n=2). During delivery 19 affected individuals experienced their first fracture (type I 8.96%, n=6; type III 26.09%, n=6; type IV 12.50%, n=6; type V 33.33%, n=1). 22.70% of patients (n=32) had their first fracture at the age of 0–1 years; 24.82% (n=35) at the age of 1–2 years; 17.02% (n=24) 3–6 years; 10.64% (n=15) ≥7years old. Fractures in the tubular bones were the most common. 53.73% (n=72) of patients had the highest number of fractures in their lower extremities; 22.39%, (n=30) in upper extremities; 17.16% (n=23) in both lower and upper extremities.

Skeletal deformities were observed in 87.23% (n=123) of the patients. Patients with type I had mild or no deformities. 81.56% (n=115) had deformities of the lower extremities of varying severity: severe (type III, n=16; type IV, n=4; type V, n=1); moderate (type I, n=12; type III, n=7; type IV, n=27; type V, n=1); mild (type I, n=33; type IV, n=14). Deformities of upper extremities comprised 65.96% (n=93) of the OI patients. The majority of the patients had scoliosis and kyphosis. (77.30%, n=109). Forty-nine (34.75%) patients had chest deformities. The majority of patients (74.29%, n=104) were able to ambulate independently. Only 12.86% (n=18) needed walking aids; 12.14% (n=17) were wheelchair users. Two patients with type III were immobile.

Half of the cohort (54.68%, n=76) had DI (type I, 41.79%; type III, 70.83%; type IV, 61.22%; type V, 33.33%). Slightly more than a quarter of the patients had joint laxity (26.62%, n=37) (type I, 24.24%; type III, 27.27%; type IV, 26.53%; type V, 66.67%). Contractures were observed in two patients (type I and III). 87.32% (n=124) of patients had blue eye sclera (type I, 85.07%; type III, 86.96%; type IV, 89.80%; and type V, 100%). Hearing loss of differing severity was observed in 22.38% (n=32) of the patients (22.38% were type I (n=16), 20.83% were type III (n=5), 22.45% were type IV (n=10) and 33.33% were type V (n=1)). Three patients (types I, III and IV) had congenital hearing loss. Hearing loss started in 12 patients at the age of 1–10 years; nine patients at 11–20 years; eight patients at >20 years.

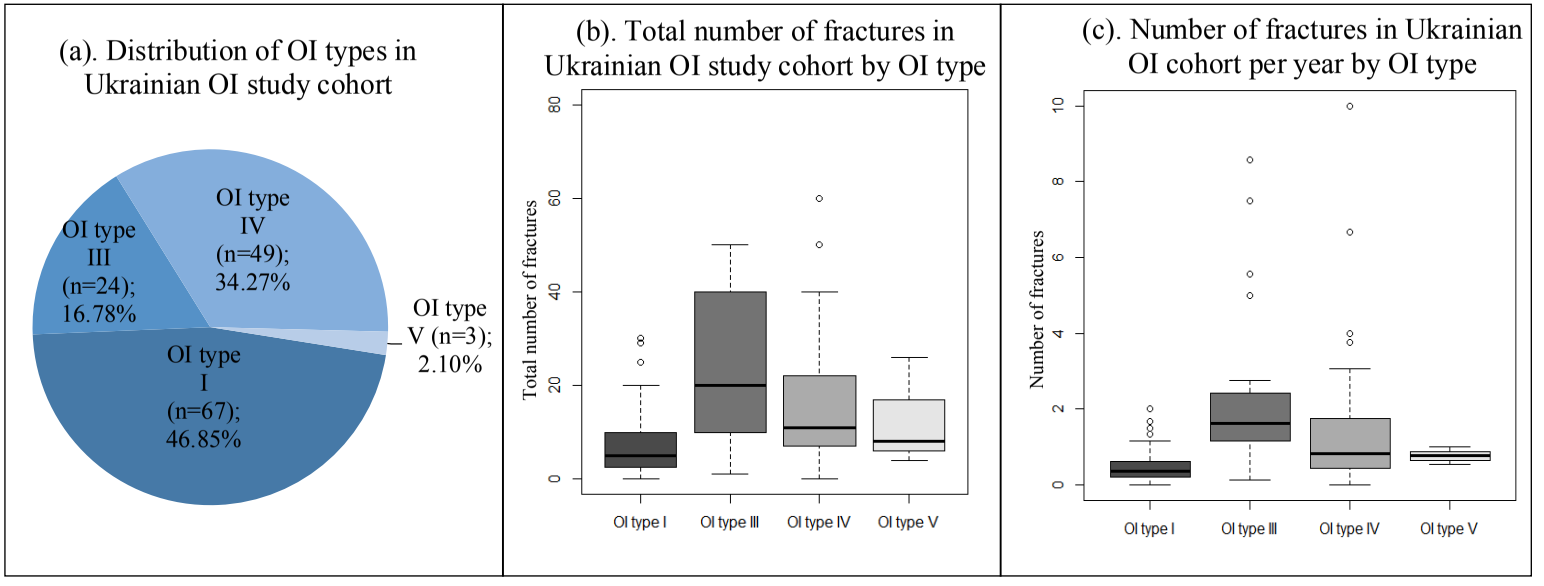


Figure 18 (a). Distribution of OI types in Ukrainian OI study cohort. (b) Total number of fractures in Ukrainian OI study cohort by OI type. (c) Number of fractures in Ukrainian OI study cohort per year by OI type.

Table 7. Clinical characteristics of the study cohort of Ukrainian OI patients divided by clinical OI type. Data is n (%) unless otherwise indicated. SD is standard deviation.

	OI type I	**OI type III**	**OI type IV**	**OI type V**	**All OI types**
Individuals	67 (46.85%)	24 (16.78%)	49 (34.27%)	3 (2.10%)	143 (100%)
Families with OI history (n=93)	19 (52.78%)	2 (8.70%)	14 (42.42%)	1 (50%)	36 (38.71%)
Gender Male/Female	35/32	4/20	25/24	2/1	66/77
Age, years (mean ± SD, range) (n=142)	20.3±15.9 (2–65y)	16.3±11.0 (2m–35y)	20.0±13.9 (6m–50y)	17.7±15.2 (4–34y)	19.5±14.4 (2m–65y)
Children (0–17y) *0–5 y* *6–10 y* *10–17 y*	37 (55.22%) 17 (25.37%) 8 (11.94%) 12 (17.91%)	13 (54.17%) 4 (16.67%) 5 (20.83%) 4 (16.67%)	25 (51.02%) 8 (16.33%) 8 (16.33%) 9 (18.37%)	2 (66.67%) 1 (33.33%) 0 (0.00%) 1 (33.33%)	77 (54.23%) 30 (20.98%) 21 (14.69%) 26 (18.18%)
Total fracture number (n=142) (median (range)/ mean ± SD)	5.00/ (0–30)	20.00/ (1–300)	11.00/ (0–60)	12.67±11.72	10.00/ (0–300)
Fractures per year (n=142) (median (range)/ mean ± SD)	0.41 (0–2)	1.63 (0.14–30)	0.83 (0–10)	0.77±0.23	0.61 (0–30)
Time of the first fracture (n=141) *No fractures* *Intrauterine* *During delivery* *≤1y* *1–2y* *3–6y* *≥7y*	 6 (8.96%) 0 (0.00%) 6 (8.96%) 11 (16.42%) 19 (28.36%) 14 (20.90%) 11 (16.42%)	 0 (0.00%) 7 (30.43%) 6 (26.09%) 6 (26.09%) 1 (4.35%) 3 (13.04%) 0 (0.00%)	 1 (2.08%) 2 (4.17%) 6 (12.50%) 14 (29.17%) 14 (29.17%) 7 (14.58%) 4 (8.33%)	 0 (0.00%) 0 (0.00%) 1 (33.33%) 1 (33.33%) 1 (33.33%) 0 (0.00%) 0 (0.00%)	 7 (4.96%) 9 (6.38%) 19 (13.48%) 32 (22.70%) 35 (24.82%) 24 (17.02%) 15 (10.64%)
Most fractured body compartment (n=134) *All tubular bones* *Lower limb* *Upper limb* *Phalanx* *Spine* *Ribs*	 9 (14.75%) 25 (40.98%) 20 (32.79%) 6 (9.84%) 1 (1.64%) 0 (0.00%)	 3 (13.04%) 18 (78.26%) 2 (8.70%) 0 (0.00%) 0 (0.00%) 0 (0.00%)	 11 (23.40%) 27 (57.45%) 7 (14.89%) 0 (0.00%) 0 (0.00%) 2 (4.26%)	 0 (0.00%) 2 (66.67%) 1 (33.33%) 0 (0.00%) 0 (0.00%) 0 (0.00%)	 23 (17.16%) 72 (53.73%) 30 (22.39%) 6 (4.48%) 1 (0.75%) 2 (1.49%)
Deformations (n=141) *Upper limb* *Lower limb* *Spine* *Chest*	 32 (47.76%) 45 (67.16%) 40 (59.70%) 6 (8.96%)	 21 (91.30%) 23(100.00%) 23(100.00%) 22 (95.65%)	 38 (79.17%) 45 (93.75%) 44 (91.67%) 19 (39.58%)	 2 (66.67%) 2 (66.67%) 2 (66.67%) 2 (66.67%)	 93 (65.96%) 115(81.56%) 109(77.30%) 49 (34.75%)

	OI type I	OI type III	OI type IV	OI type V	All OI types
Mobility (n=140)					
Immobile	0 (0.00%)	1 (4.35%)	0 (0.00%)	1 (33.33%)	2 (1.43%)
Wheelchair	0 (0.00%)	13 (56.52%)	4 (8.51%)	0 (0.00%)	17 (12.14%)
Walking with support	2 (2.99%)	9 (39.13%)	7 (14.89%)	0 (0.00%)	18 (12.86%)
Walking independently	66 (98.51%)	0 (0.00%)	36 (76.60%)	2 (66.67%)	104(74.29%)
DI (yes) (n=139)	28 (41.79%)	17 (70.83%)	30 (61.22%)	1 (33.33%)	76 (54.68%)
Blue sclera (yes)	57 (85.07%)	20 (86.96%)	44 (89.80%)	3 (100%)	124(87.32%)
Hearing loss (yes)	15 (22.38%)	5 (20.83%)	11 (22.45%)	1 (33.33%)	32 (22.38%)
Time of hearing loss start (n=32)					
at birth	1 (6.67%)	1 (20.00%)	1 (9.09%)	0 (0.00%)	3 (9.38%)
1–10 y	5 (33.33%)	2 (40.00%)	5 (45.45%)	0 (0.00%)	12 (37.50%)
11–20 y	5 (33.33%)	2 (40.00%)	1 (9.09%)	1 (100.00%)	9 (28.13%)
21–30 y	3 (20.00%)	0 (0.00%)	4 (36.36%)	0 (0.00%)	7 (21.88%)
31–40y	1 (6.67%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (3.13%)

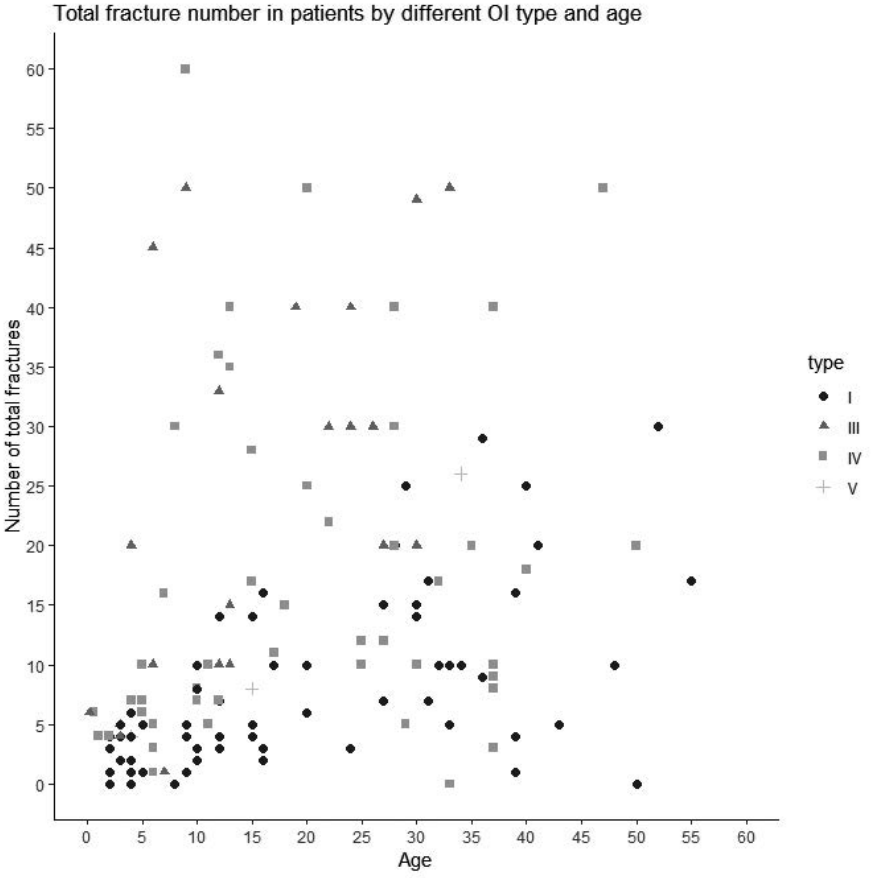


Figure 19. Total number of fractures in Ukrainian OI patient by OI type and age.

5.6. Genotype-phenotype correlations (Paper II)

Analysis of genotype-phenotype correlations in Ukrainian OI cohort has revealed clear correlation between OI type and collagen defect. LoF pathogenic variants correlated with milder OI, compared to severe OI, which was associated with DN pathogenic variants (p=0.007). LoF pathogenic variants in the *COL1A1* gene were also associated with milder OI, compared to LoF in *COL1A2* (p=0.003). Correlation between OI type and DN *COL1A1* and *COL1A2* pathogenic variants was absent (p=0.895) (Table 8).

Patients harboring LoF pathogenic variants had significantly less fractures, compared to carriers of the DN OI pathogenic variants (p=0.0247). More severe skeletal deformities were observed in patients with DN pathogenic variants, compared to those with LoF pathogenic variants (p=0.001; p=5.80e–05; p=2.37e–05; p=2.0e–04). Type I patients with DN pathogenic variants had more severe deformities of lower extremities and spine compared to type I patients with LoF pathogenic variants (p=0.022, p=0.029). Gly substitutions in *COL1A2* gene correlated with more severe chest and spine deformities, compared to Gly substitutions in the *COL1A1* gene (p=0.010, p=0.004). Patients with DN pathogenic variants were less mobile than patients with LoF pathogenic variants (p=0.023). DN pathogenic variants in the *COL1A2* were more associated with reduced mobility compared to DN pathogenic variants in the *COL1A1* (p=0.038). DI and hearing loss lack correlation with the genotype in Ukrainian OI cohort. However, blue sclera was associated with collagen type I pathogenic variants (p=0.011) (Table 8).

The total number of analyzed, unrelated patients from the Estonian, Ukrainian and Vietnamese populations was 238. Distribution of OI types was as follows: OI type I – 87 (36.56%), OI type II – 1 (0.42%), OI type III – 66 (27.73%), OI type IV – 80 (33.61%), OI type V – 4 (1.68%).

COL1A1/2 pathogenic variants were harbored in 144 individuals. Among collagen-related OI cases, OI types were distributed as follows: OI type I-51 (35.42%), OI type II-1 (0.69%), OI type III-34 (23.61%), and OI type IV-58 (40.28%). OI type correlated with the presence of the collagen type I pathogenic variants (p-value 0.0039). OI types I and IV were more common for collagen-related OI, than for non-collagen OI. OI type also correlated with an altered *COL1A1* or *COL1A2* gene (p-value 0.0013) and collagen defect DN (qualitative) or LoF (quantitative) (p-value 0.0003) (Table 9). The presence of blue sclera correlated with collagen type I pathogenic variants (p-value $4.216e^{-05}$) and DI was associated with a qualitative collagen type I disorder (p-value 0.0008) (Table 9). The number of total fractures and fractures per year correlated with presence of the collagen type I pathogenic variant, affected gene and collagen defect (Appendix B).

Table 8. Relationships between clinical characteristics and collagen I pathogenic variant types for the cohort of Ukrainian OI patients. Statistically significant p-values are marked with *. Data is n, unless otherwise indicated. SD is standard deviation.

	Structural OI type I/ haplo-insufficiency OI type I	**All Structural/ all haploin-sufficiency**	**Haploinsufficiency *COL1A1*/ haploinsufficiency *COL1A2***	**Structural *COL1A1*/ structural *COL1A2***	**Gly *COL1A1*/ Gly *COL1A2***	**Gly>Ser *COL1A1*/ Gly>Ser *COL1A2***	**Collagen OI/non-collagen OI**
All OI patients	7/16	29/31	27/2	18/11	20/10	7/4	60/34
type I	–	7/16	15/0	5/2	8/1	1/0	23/13
type III	–	12/2	0/2	7/5	6/5	3/2	14/9
type IV	–	10/13	12/0	6/4	6/4	3/2	23/10
p-value	–	**0.003***	**0.004***	0.895	0.271	1	0.317
Blue sclera	5/15	24/30	26/2	15/9	16/8	4/3	54/23
p-value	0.481	0.329	1	1	1	1	**0.011***
Mobility							
Wheelchair	–	8/2	1/1	2/6	1/6	1/1	10/5
Walking with support	1/1	7/5	5/0	6/1	7/1	3/1	12/4
Walking independently	5/15	12/23	21/0	9/3	11/2	2/2	35/22
Immobile	–	–	–	–	–	–	0/1
p-value	0.481	**0.023***	0.169	**0.038***	**0.002***	1	0.465
Deformation severity (p-values)							
Upper limb	0.065	**0.001***	0.734	0.162	0.051	0.714	0.558
Lower limb	**0.022***	**5.803e-05***	**0.048***	0.765	0.138	0.2	0.473
Spine	**0.029***	**2.37e-05***	0.285	0.224	**0.010***	0.543	0.441
Chest	1	**2.0e-04***	0.598	0.190	**0.004***	0.2	0.125
Total fracture number (median (range)/ mean ± SD)	6.00/4.00 (4–14)/(0–25)	28.00/6.00 (4–300)/(0–40)	6.00/13.00 (0–40)/(6–20)	10.00/30.00 (4–300)/(7–50)	7.00/30.00/ (2–49)/(7–50)	8.00/12.50 (4–49)/(7–30)	10/6.5 (0–300)/(0–50)
p-value	0.1184	**0.001***	0.4899	0.3131	**0.0247***	0.5918	**0.046***
Fracture number per year (median (range)/ mean ± SD)	1.00/0.73 (0.4–1.17) (0–2)	1.52/0.83 (0.4–8.57)/ (0–30)	0.8/15.37 (0–2.69)/ (0.74–30)	1.63/1.39 (0.4–8.57)/ (0.7–3.08)	1.33/1.52 (0.25–7.5)/ (0.77–3.08)	1.48/0.69 (1–3.75)/ (0.78–1.75)	1.18/0.54 (0–30)/ (0–5.56)
p-value	0.483	**0.001***	0.282	0.651	0.538	0.352	**0.004***

Table 9. Relationships between collagen I pathogenic variants and phenotypes in cohort of 238 OI patients from UT OI database. Statistically significant p-values are marked with *. Data are n.

	Collagen I		**Gene**			**Defect**		
	Collagen	**Non-collagen**	***COL1A1***	***COL1A2***	***COL1A1/2***	**DN**	**LoF**	**DN/LoF**
Type								
OI I	51	36	43	7	1	20	30	1
OI II	1	0	1	0	0	1	0	0
OI III	34	32	16	18	0	29	5	0
OI IV	58	22	42	13	3	32	25	1
OI V	0	4	–	–	–	–	–	–
Total (n=238)	144	94	102	38	4	82	60	2
p-value		**0.0039***			**0.0013***			**0.0003***
Phenotype								
Blue sclera/White sclera (n=236)	132/11	67/26	93/8	35/3	4/0	72/9	58/2	2/2
p-value		**4.2E-05***			1			0.2479
DI/Normal teeth (n=237)	82/62	47/46	53/49	27/11	2/2	57/25	24/36	1/1
p-value		0.3523			0.1033			**0.0008***
Hearing loss/ Normal hearing (n=236)	25/119	23/69	16/86	6/32	3/1	12/70	12/48	1/1
p-value		0.1851			**0.0279***			0.2578

5.7. Inter- and intrafamilial variability (Paper V)

5.7.1. Interfamilial variability

Based on phenotype-genotype characteristics, following groups were created: monophenotype (i.e. patients, with pathogenic variants, which caused the same phenotype), which included mild phenotype (type I), moderate phenotype (type IV) and severe phenotype only (type III); and polyphenotype group mild-moderate phenotype (i.e. patients with OI types I and IV), severe-moderate phenotype (i.e. patients with pathogenic variants which caused variable phenotypes, which included less variable phenotypes (mild-to-moderate (I, IV), severe-to-moderate (III, IV)) and more variable phenotypes (opposite (I, III), non-lethal (I, III, IV) and mild-to-lethal (i.e. all OI types) (Figure 20).

Ten out of 62 explored pathogenic variants (17.74%) did not cause phenotypical variability and all discovered carriers had developed identical phenotypes. These pathogenic variants are described as monophenotypic variants in Table 10. They included eight pathogenic variants causing only mild OI, two pathogenic variants causing only severe OI and one pathogenic variant causing moderate OI (Table 10, Figure 21).

All eight monophenotypic pathogenic variants which caused mild OI, type I were located in the *COL1A1* gene. Missense pathogenic variants were represented with two variants: c.590G>A, p.(Gly197Asp) and c.3766G>A, p.(Ala1256Thr); the other six were LoF pathogenic variants. Four of the patients from the UT OI database harbored the same frameshift pathogenic variant *COL1A1* c.579delT, p.(Gly194Valfs*). In the Dalgleish's database the current pathogenic variant was described in 22 patients with OI type I only (Figure 21).

Two monophenotypic *COL1A1* DN pathogenic variants caused only OI type III: c.1165G>A, p.(Gly389Ser) and c.742G>A, p.(Gly284Arg). Only moderate OI was caused by a *COL1A2* DN pathogenic variant c.1630G>A, p.(Gly544Ser).

A majority of pathogenic variants caused polyphenotypes. Among them were variants, which caused the development of less variable phenotype (I, IV and III, IV).

Twenty pathogenic variants caused OI phenotype of mild-to-moderate severity, types I, IV. LoF pathogenic variants were represented by 14 variants, all in the *COL1A1* gene. DN pathogenic variants were represented only with Gly substitutions, six in the *COL1A1* and two in the *COL1A2* genes.

Nine pathogenic variants caused severe-to-moderate OI, types III, IV. Six of the pathogenic variants were located in the *COL1A2* gene. 7/9 were DN pathogenic variants. Two LoF pathogenic variants were both splice site: *COL1A1,* c.2612+6T>C; *COL1A2,* 792+1G>A. The *COL1A2*, c.3034G>A, p.(Gly1012Ser) pathogenic variant was harbored by a pair of 13-year-old monozygotic twins. Both girls had severe OI type III. The patients had severe skeletal deformities, DI, white eye sclera and without hearing loss. Their number of fractures was moderate (10 and 15). The current variant was described in 25 other patients from the Dalgleish's database, with OI types III and IV.

More variable phenotype were represented with three groups: opposite phenotypes, non-lethal OI and all OI forms, including lethal OI. Three DN pathogenic variants in the *COL1A2* gene caused opposite phenotypes for OI type I and III: c.1072G>T, p.(Gly358Ser); c.2233G>C, p.(Gly745Arg) and c.874G>A, p.(Gly292Ser).

In the non-lethal OI phenotype group, 14 pathogenic variants were represented with types I, III, IV. 10/14 pathogenic variants were in the *COL1A1* gene. 11/14 variants were DN. Three LoF pathogenic variants: c.1821+1G>A, c.1243C>T, p.(Arg415*), c.1128_delT were located in the *COL1A1* gene.

In a group of mild to lethal OI type II, five DN pathogenic variants caused all OI types. Among this group only Gly to Ser substitutions were present. Four pathogenic variants were in the *COL1A1* gene: c.1102G>A, p.(Gly368Ser), c.1588G>A, p.(Gly530Ser), c.2299G>A, p.(Gly767Ser) and c.2596G>A, p.(Gly866Ser). Only the c.1378G>A, p.(Gly460Ser) pathogenic variant was in the *COL1A2* gene. The *COL1A1* c.2299G>A, p.(Gly767Ser) pathogenic variant was identified 35 times in patients reported in the Dalgleish's OI variant database. In three of our patients this variant caused OI types I, IV and III (Figure 22). The c.2299G>A pathogenic variant can cause the development of a whole range of OI phenotypes of – from mild to lethal.

We spotted a pattern of interfamilial variability, which was associated with the altered gene and type of pathogenic variant defect. Decreased interfamilial variability correlated with *COL1A1* gene pathogenic variants (p-value=0.001), whereas higher phenotypical variability was among carriers of the *COL1A2* pathogenic variants. Also, interfamilial variability was associated with collagen defect type (p-value=0.0007). DN pathogenic variants correlated with higher phenotypical variability compared to LoF pathogenic variants.

Table 10. Phenotypical diversity in *COL1A1*[1] and *COL1A2*[2] pathogenic variants discovered in OI patients from the UT OI biobank. Number of reported families in UT OI database and reported times in the Dalgelish's variant database are represented in brackets. Variants causing variable phenotypes in the single family are marked with a diesis (‡). Types reported in the OI variant database were obtained from the http://www.le.ac.uk/ge/collagen/. DN – Dominant Negative, LoF – Loss of function.

No	Pathogenic variant	Gene	Pathogenic variant type	Collagen defect	Protein alteration	Sillence OI type in studied cohort	Types reported in OI variant database
1. Monophenotypes							
1.1. Mild monophenotypes							
1	c.590G>A	*COL1A1*	Missense	DN	p.Gly197Asp	I (1)	I (2)
2	c.3766G>A	*COL1A1*	Missense	DN	p.Ala1256Thr	I (1)	I (1)
3	c.1354-2A>G	*COL1A1*	Splice site	LoF	–	I (1)	I (1)
4	c.904-9G>A	*COL1A1*	Splice site	LoF	–	I (2)	I (1)
5	c.579_delT	*COL1A1*	Frameshift	LoF	p.Gly194Valfs*71	I (1)	I (22)
6	c.459_delT	*COL1A1*	Frameshift	LoF	p.Gly154Alafs*111	I (1)	I (1)
7	c.3807G>A	*COL1A1*	Nonsense	LoF	p.Trp1269*	I (1)	I (2)
8	c.3076C>T	*COL1A1*	Nonsense	LoF	p.Arg1026*	I (1)	I (12)
1.2. Moderate monophenotypes							
1	c.1630G>A	*COL1A2*	Missense	DN	p.Gly544Ser	IV (1)	IV (1)
1.3. Severe-to-monophenotypes							
1	c.1165G>A	*COL1A1*	Missense	DN	p.Gly389Ser	III (1)	III (3)
2	c.742G>A	*COL1A1*	Missense	DN	p.Gly284Arg	III (1)	III (1)
2. Polyphenotypes							
2.1. Less variable phenotypes							
2.1.1. Mild-to-moderate OI phenotypes							
1	c.2560G>A‡	*COL1A1*	Missense	DN	p.Gly854Ser	I, IV (1)	I/IV (2)
2	c.3235G>A	*COL1A1*	Missense	DN	p.Gly1079Ser	I (1)	I, IV (20)
3	c.653G>A	*COL1A1*	Missense	DN	p.Gly218Asp	I (2)	IV (1)
4	c.959G>A	*COL1A1*	Missense	DN	p.Gly320Asp	IV (1)	I (1)
5	c.1451G>A	*COL1A2*	Missense	DN	p.Gly484Glu	IV (1)	I (2)
6	c.3305G>T	*COL1A2*	Missense	DN	p.Gly1102Val	I (1)	IV (1)
7	c.750+2T>A‡	*COL1A1*	Splice site	LoF	–	I, IV (1)	I (1)

No	Pathogenic variant	Gene	Pathogenic variant type	Collagen defect	Protein alteration	Sillence OI type in studied cohort	Types reported in OI variant database
8	c.3045+1G>A	*COL1A1*	Splice site	LoF	–	IV (1)	I (1)
9	c.299-1G>C†	*COL1A1*	Splice site	LoF	–	IV (1)	I (3)
10	c.1614+1G>A	*COL1A1*	Splice site	LoF	–	IV (1)	I (3)
11	c.858+1G>A‡	*COL1A1*	Splice site	LoF	–	I, IV (2)	I (2)
12	c.804+1G>A	*COL1A1*	Splice site	LoF	–	IV (1)	I (1)
13	c.1002+2T>C	*COL1A1*	Splice site	LoF	–	IV (1)	I (1)
14	c.1299+1G>C	*COL1A1*	Splice site	LoF	–	IV (1)	I, IV (4)
15	c.2614-1G>A	*COL1A1*	Splice site	LoF	–	IV (1)	I (1)
16	c.2523_delT	*COL1A1*	Frameshift	LoF	p.Gly842Alafs*266	I (1)	I, IV (4)
17	c.658C>T‡	*COL1A1*	Nonsense	LoF	p.Arg220*	I, IV (1)	I (11)
18	c.2089C>T	*COL1A1*	Nonsense	LoF	p.Arg697*	IV (1)	I, IV (8)
19	c.1789G>T	*COL1A1*	Nonsense	LoF	p.Glu597*	I (1)	IV (1)
20	c.1081C>T	*COL1A1*	Nonsense	LoF	p.Arg361*	IV (2)	I (10)
2.1.2. Severe-moderate phenotypes							
1	c.2101G>T	*COL1A1*	Missense	DN	p.Gly701Cys	III (1)	IV (1)
2	c.3226G>A	*COL1A1*	Missense	DN	p.Gly1076Ser	IV (2)	III, IV (13)
3	c.1090G>A	*COL1A2*	Missense	DN	p.Gly364Ser	III (1)	IV (1)
4	c.2288G>T	*COL1A2*	Missense	DN	p.Gly763Val	III (1)	III/IV (2)
5	c.2324G>A	*COL1A2*	Missense	DN	p.Gly775Glu	III (2)	III, IV (1)
6	c.3034G>A	*COL1A2*	Missense	DN	p.Gly1012Ser	III (2)	III/IV (24)
7	c.865G>A	*COL1A2*	Missense	DN	p.Gly289Ser	III (1)	III/IV, IV (2)
8	c.792+1G>A	*COL1A2*	Splice site	LoF	–	III (1)	IV (1)
9	c.2613+6T>C	*COL1A1*	Splice site	LoF	–	IV (1)	III (1)
2.2. More variable phenotypes							
2.2.1. Opposite OI phenotypes							
1	c.1072G>T‡	*COL1A2*	Missense	DN	p.Gly358Ser	I, III (1)	III (1)
2	c.2233G>C	*COL1A2*	Missense	DN	p.Gly745Arg	III (1)	I (1)
3	c.874G>A	*COL1A2*	Missense	DN	p.Gly292Ser	III (1)	I (5)

No	Pathogenic variant	Gene	Pathogenic variant type	Collagen defect	Protein alteration	Sillence OI type in studied cohort	Types reported in OI variant database
2.2.2. Non-lethal phenotypes							
1	c.2362G>A	*COL1A1*	Missense	DN	p.Gly788Ser	IV (2)	I, III, IV (8)
2	c.2461G>A‡	*COL1A1*	Missense	DN	p.Gly821Ser	I, IV (5)	I, III, IV (26)
3	c.757C>T	*COL1A1*	Missense	DN	p.Arg253Phe	IV (1)	I, III, IV (11)
4	c.769G>A‡	*COL1A1*	Missense	DN	p.Gly257Arg	I, IV (1)	I, III, IV (37)
5	c.977G>A	*COL1A1*	Missense	DN	p.Gly326Asp	I (1)	I, III, IV (3)
6	c.1057G>T‡	*COL1A1*	Missense	DN	p.Gly353Cys	I, IV (1)	III/IV (2)
7	c.1165G>T	*COL1A1*	Missense	DN	p.Gly389Cys	I (2)	III/ IV (2)
8	c.2314G>A‡	*COL1A2*	Missense	DN	p.Gly772Ser	I, IV (1)	I, III, IV (18)
9	c.2503G>A	*COL1A2*	Missense	DN	p.Gly835Ser	III (1)	I, III, IV (4)
10	c.982G>A‡	*COL1A2*	Missense	DN	p.Gly328Ser	IV, III (2)	I, III, IV (45)
11	c.1009G>A	*COL1A2*	Missense	DN	p.Gly337Ser	III, IV (3)	I, III, IV (29)
12	c.1821+1G>A‡	*COL1A1*	Splice site	LoF	–	III, IV, I (2)	I, IV (17)
13	c.1243C>T	*COL1A1*	Nonsense	LoF	p.Arg415*	I (1)	I, III/IV (16)
14	c.1128_delT‡	*COL1A1*	Frameshift	LoF	p.Gly377Alafs*164	I, IV, III (1)	I (9)
2.2.3. Mild-to-lethal phenotypes							
1	c.1102G>A	*COL1A1*	Missense	DN	p.Gly368Ser	IV (1)	II/III, III (2)
2	c.1588G>A	*COL1A1*	Missense	DN	p.Gly530Ser	III (1)	II, III, IV (12)
3	c.2299G>A	*COL1A1*	Missense	DN	p.Gly767Ser	I, III, IV (3)	I, II, III, IV (34)
4	c.2596G>A	*COL1A1*	Missense	DN	p.Gly866Ser	III (1)	II, III (10)
5	c.1378G>A	*COL1A2*	Missense	DN	p.Gly460Ser	IV (1)	II/III, III, IV (16)

[1] *COL1A1* GenBank reference sequence (gDNA NG_007400.1, cDNA NM_000088.3). [2] *COL1A2* GenBank reference sequence (gDNA NG_007405.1, cDNA NM_000089.3).

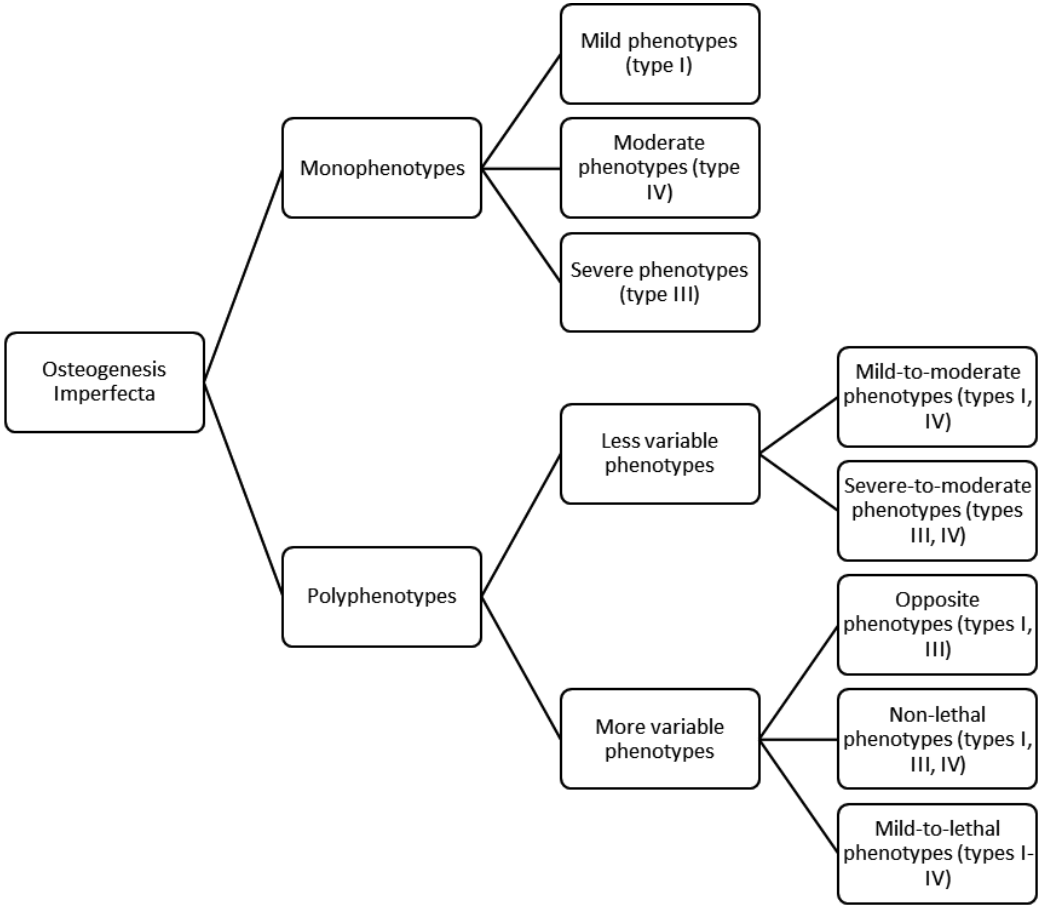


Figure 20. Phenotypical groups according to genotype-phenotype severity scale of collagen-related Osteogenesis Imperfecta. Mild – type I, moderate – type IV, severe – type III, lethal phenotype – type II.

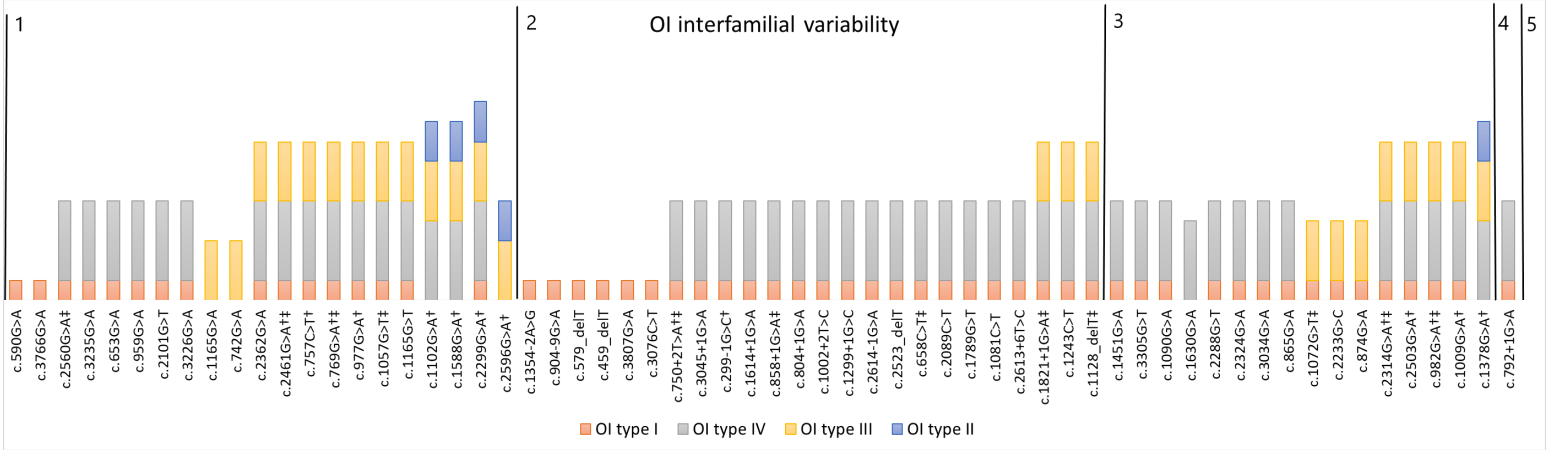


Figure 21. Interfamilial OI diversity in identified *COL1A1* and *COL1A2* pathogenic variants from the UT OI database. 1–2 *COL1A1* Dominant negative; 2–3 *COL1A1* loss-of-function; 3–4 *COL1A2* Dominant Negative; 4–5 *COL1A2* loss-of-function pathogenic variants.

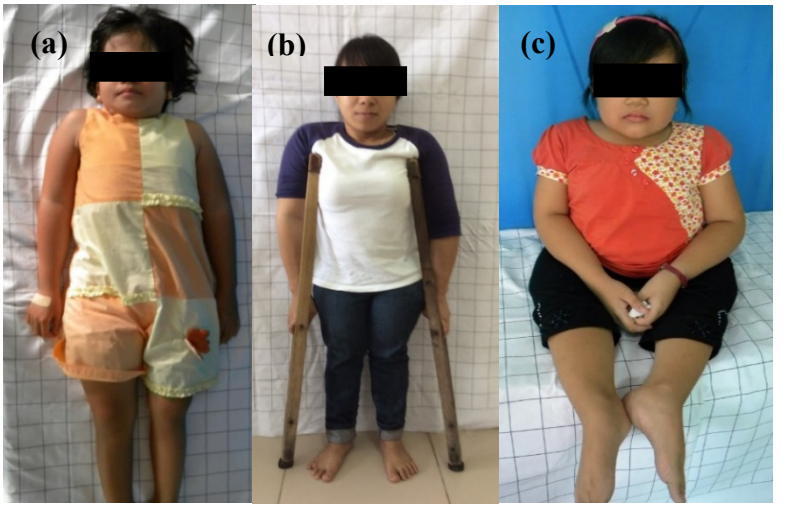


Figure 22. Patients from the UT OI database, harboring the *COL1A1* DN variant c.2299G>A, p.Gly767Ser. (a) Type I, (b) Type IV, (c) Type III.

5.7.2. Intrafamilial variability

Being a center for OI treatment and research, the Clinic of Traumatology and Orthopedics of Tartu University Hospital, has followed up with OI families during the last 25 years. Their current work with OI families provided an exclusive observation experience of OI phenotype development and variability inside families. It was noted, that OI symptom severity changes usually happen smoothly in one direction by decreasing or increasing. Intrafamilial diversity was underlined with the increase of OI phenotype severity patterns (i.e. OI symptoms progress with every younger generation) or a decrease in OI severity (i.e. OI symptoms dissolve in every younger generation). Variability of the phenotypes among siblings and cousins from the same generation was referred to as "no changes in severity" and "changing in severity".

Our data shows, that the level of phenotypical variability inside the families was lower, compared to variability between unrelated individuals. In total, 21/64 (32.81%) of the studied families had changes in OI severity throughout generations. Diversity of phenotypes between siblings and cousins from the same generation composed only 7/68 (10.29%) of the families. Intrafamilial diversity was compromised mainly of cases of mild and moderate OI inside one family. Polyphenotype, represented with types I, IV, III, were identified in two families.

In eight families (5 *COL1A1*, 2 *COL1A2*, 1 *COL1A1/2*) decreasing severity was confirmed (36.37%) (Table 11, Figure 23). Four of the families had LoF pathogenic variants. In 13 families (11 *COL1A1,* 2 *COL1A2*) progressing severity was spotted (61.90%). Data was collected from two (n=8), three (n=3) and four (n=2) generations (Table 11, Figure 23). Nine out of 13 families had DN pathogenic variants. Twenty seven out of 43 (62.79%) families lacking intrafamilial variability had LoF pathogenic variants.

We identified a correlation between the type of the collagen defect and intrafamilial variability (p-value=0.0231). LoF pathogenic variants correlated with the absence of intrafamilial variability, whereas families harboring the DN pathogenic variant had higher variability. There were no correlation between gene and intrafamilial variability (p-value=0.2376).

The *COL1A1* c.3652G>A, p.(Ala1218Thr) pathogenic variant associated with "high bone mass OI" was harbored by a family with an increasing OI severity. The mother (40 years old) had mild OI (normal height, blue sclera, hearing loss, DI, moderate number of fractures, no visible skeleton deformities, the ability to move independently). Her son and daughter with different fathers, aged 20 and 9 respectively, both had the same pathogenic variant. Both children developed DI and had normal height. In addition, the children used wheelchairs due to poor bone quality of their lower limbs and an inability of lower limbs weight bearing. Both kids had an extreme number of fractures (50–60), grey sclera and had yet to suffer hearing loss.

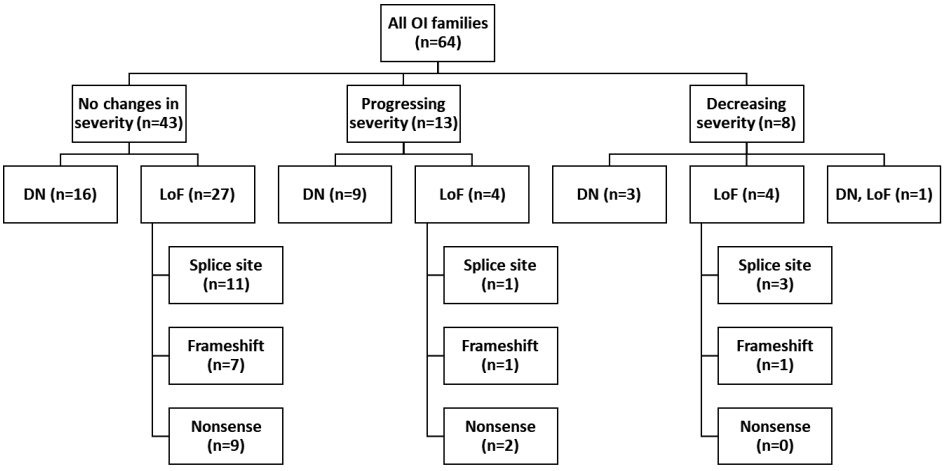


Figure 23. Genotypes and intrafamilial phenotypical variability in families with collagen-related OI. DN – dominant negative pathogenic variant; LoF – loss-of-function pathogenic variant.

Decreasing OI severity was oppositely underlined in two Estonian families. The first family harbored the LoF splice site c.1821+1G>GA pathogenic variant in the *COL1A1* gene. The first OI generation was represented by a patient with severe OI phenotype (short stature, severe skeletal deformities, high number of fractures, severe osteoporosis, triangular face, blue sclera, hearing loss). In every younger generation, symptoms of OI phenotype dissolved towards mild OI. The number of fractures reduced, patients had normal height and only mild skeletal deformities were present. The presence of blue sclera and hearing loss remained without any changes through upcoming generations. The second family had a frameshift LoF *COL1A1* pathogenic variant c.1128_delT, p.Gly337Alafs*164.

Members of this family developed polyphenotype (types I, IV, III). All affected family members were women with blue sclera, absent DI and without hearing loss. In the oldest generation, one patient had a severe OI type III (high number of fractures, reduced height, deformities of limbs, scoliosis, disproportion of the body. A patient from the second generation had mild OI type I with normal height. Two more individuals from the following generations had moderate OI type IV (reduced height, numerous fractures).

Table 11. *COL1A1*[1] and *COL1A2*[2] pathogenic variants discovered in OI patients with intrafamilial diversity. DN – Dominant Negative, LoF – loss-of-function.

No	Pathogenic variants	Gene	Pathogenic variant type	Collagen defect	Protein alteration
Pathogenic variants discovered in OI patients with decreasing OI severity in families					
1	c.1821+1G>A	*COL1A1*	Splice site	LoF	–
2	c.750+2T>A	*COL1A1*	Splice site	LoF	–
3	c.858+1G>A	*COL1A1*	Splice site	LoF	–
4	c.1128_delT	*COL1A1*	Frameshift	LoF	p.Gly377Alafs*164
5	c.769G>A	*COL1A1*	Missense	DN	p.Gly257Arg
6	c.370-1G>A /c.2642A>C	*COL1A1* /*COL1A2*	Splice site/ Missense	LoF/ DN	–/p.Glu881Ala
7	c.3305G>T	*COL1A2*	Missense	DN	p.Gly1102>Val
8	c.1072G>T	*COL1A2*	Missense	DN	p.Gly358Ser
Pathogenic variants discovered in OI patients with progressing OI severity in families					
1	c.3217G>A	*COL1A1*	Missense	DN	p.Gly1073Ser
2	c.2362G>A	*COL1A1*	Missense	DN	p.Gly788Ser
3	c.3652G>A	*COL1A1*	Missense	DN	p.Ala1218Thr
4	c.1057G>T	*COL1A1*	Missense	DN	p.353G>G/C
5	c.2560G>A	*COL1A1*	Missense	DN	p.Gly854Ser
6	c.2461G>A	*COL1A1*	Missense	DN	p.Gly821Ser
7	c.2461G>A	*COL1A1*	Missense	DN	p.Gly821Ser
8	c.2314G>A	*COL1A2*	Missense	DN	p.Gly772Ser
9	c.982G>A	*COL1A2*	Missense	DN	p.Gly328Ser
10	c.658C>T	*COL1A1*	Nonsense	LoF	p.Arg220*
11	c.495T>A	*COL1A1*	Nonsense	LoF	p.Tyr165*
12	c.1816_delG	*COL1A1*	Frameshift	LoF	p.Ala606Leufs*
13	c.103+2T>C	*COL1A1*	Splice site	LoF	–

No	Pathogenic variants	Gene	Pathogenic variant type	Collagen defect	Protein alteration
Pathogenic variants discovered in OI patients without changes in OI severity within families					
1	c.505G>A*	*COL1A1*	Missense	DN	p.Glu169Lys
2	c.1A>C	*COL1A1*	Missense	DN	p.Met1Leu
3	c.3223G>A	*COL1A1*	Missense	DN	p.Ala1075Thr
4	c.959G>A	*COL1A1*	Missense	DN	p.Gly320Asp
5	c.1102G>A	*COL1A1*	Missense	DN	p.Gly368Ser
6	c.1165G>T	*COL1A1*	Missense	DN	p.Gly389Cys
7	c.977G>A	*COL1A1*	Missense	DN	p.Gly326Asp
8	c.2005G>A	*COL1A1*	Missense	DN	p.Ala669Thr
9	c.2362G>A	*COL1A1*	Missense	DN	p.Gly788Ser
10	c.2299G>A	*COL1A1*	Missense	DN	p.Gly767Ser
11	c.3766G>A	*COL1A1*	Missense	DN	p.Ala1256Thr
12	c.1009G>A	*COL1A2*	Missense	DN	p.Gly337Ser
13	c.1009G>A	*COL1A2*	Missense	DN	p.Gly337Ser
14	c.1630G>A	*COL1A2*	Missense	DN	p.Gly544Ser
15	c.1009G>A	*COL1A2*	Missense	DN	p.Gly337Ser
16	c.1964G>T	*COL1A2*	Missense	DN	p.Gly655Val
17	c.579_delT	*COL1A1*	Frameshift	LoF	p.Gly194Valfs*71
18	c.2821_delG	*COL1A1*	Frameshift	LoF	p.Gly941Valfs*167
19	c.2393_dupC	*COL1A1*	Frameshift	LoF	p.Gly800Argfs*5
20	c.2523_delT	*COL1A1*	Frameshift	LoF	p.Gly842Alafs*266
21	c.630_delG	*COL1A1*	Frameshift	LoF	p.Glu210Aspfs*3
22	c.2523_delT,	*COL1A1*	Frameshift	LoF	p.Gly842Alafs*266
23	2093_2110_dup	*COL1A2*	Frameshift	LoF	p.Leu699_Leu704dup
24	c.1897G>T*	*COL1A1*	Nonsense	LoF	p.Glu633*
25	c.2089C>T	*COL1A1*	Nonsense	LoF	p.Arg697*
26	c.3262G>T*	*COL1A1*	Nonsense	LoF	p.Gly1088*
27	c.3262G>T*	*COL1A1*	Nonsense	LoF	p.Gly1088*
28	c.1081C>T	*COL1A1*	Nonsense	LoF	p.Arg361*
29	c.3076C>T	*COL1A1*	Nonsense	LoF	p.Arg1026*
30	c.1243C>T	*COL1A1*	Nonsense	LoF	p.Arg415*
31	c.3807G>A	*COL1A1*	Nonsense	LoF	p.Trp1269*
32	c.2179C>T	*COL1A1*	Nonsense	LoF	p.Gln727*
33	c.1821+1G>A	*COL1A1*	Splice site	LoF	–
34	c.1155+2T>G*	*COL1A1*	Splice site	LoF	–
35	c.299-1G>C*	*COL1A1*	Splice site	LoF	–

No	Pathogenic variants	Gene	Pathogenic variant type	Collagen defect	Protein alteration
36	c.1767+5G>A*	*COL1A1*	Splice site	LoF	–
37	c.1354-2A>G	*COL1A1*	Splice site	LoF	–
38	c.904-9G>A	*COL1A1*	Splice site	LoF	–
39	c.2613+6T>C	*COL1A1*	Splice site	LoF	–
40	c.804+1G>A	*COL1A1*	Splice site	LoF	–
41	c.103+2T>C	*COL1A1*	Splice site	LoF	–
42	c.2614-1G>A	*COL1A1*	Splice site	LoF	–
43	c.2026-1_2031het dup*	*COL1A2*	Splice site	LoF	–

[1] *COL1A1* GenBank reference sequence (gDNA NG_007400.1, cDNA NM_000088.3).
[2] *COL1A2* GenBank reference sequence (gDNA NG_007405.1, cDNA NM_000089.3).

6. DISCUSSION

Although the prevalence of OI was estimated as 1/10.000, among some researchers, there is still disagreements regarding this number (Marini et al., 2017). Therefore, some researchers proposed 1/20.000. Health care systems and their capacity differ among countries, and even varies between regions of the same state, so an accurate number of patients remains unknown (Van Dijk and Sillence, 2014). We also admit, that the amount of patients with mild OI might be underdiagnosed, as was previously stated (Binh et al., 2017). In addition, in some regions due to population genetic differences, OI prevalence might differ from worldwide amounts.

In our study, we have invited all available patients with OI for participation, using medical databases and patient registers. We recruited all available OI families for the study. Our study is family based. We aimed to cover as many OI families as possible from all three countries, based on hospital databases and patient organizations. It is also important to note that the number of families does not correspond to the number of affected OI individuals. We have collected blood samples only from a part of them (i.e. those available during the interview); however, some affected family members from dominant OI families were not available. We concentrated on the description of genetic pathogenic variants in OI families, which we supposed to be the same variant for a whole family. Thus, the actual number of covered patients might be even greater due to uncaptured, affected family members from families of the UT OI database.

6.1. Spectrum of the *COL1A1/2* pathogenic variants in the Estonian and Ukrainian OI populations (Paper I and II)

Previous works on OI genetics have shown that collagen type I pathogenic variants account for 60–90% of all OI cases (Dalgleish, 1998). Bardai *et al.* have reported results from their sequencing analysis of 598 OI patients from 487 families of different ethnic origin. The proportion of collagen type I pathogenic variants in their cohort was 86% (Bardai et al., 2016). The proportion of collagen type I pathogenic variants tend to vary in population-based OI studies.

COL1A1/2 pathogenic variants were present in 87% of EE OI patients. According to a study of Finnish OI patients, 90.7% of OI individuals had *COL1A1/2* pathogenic variants (Hartikka et al., 2004), which is slightly higher than what has been observed in Estonian OI population. Results of Estonian *COL1A1/2* sequencing analysis are similar to the genotype spectrum of the Swedish OI patients, which was composed of 87% *COL1A1/2* pathogenic variants (Lindahl et al., 2015). The proportion of *COL1A1/2* pathogenic variants in the UA OI cohort (63.83%) was lower than in Northern Europe (e.g. in Estonia, Sweden and Finland) (Hartikka et al., 2004; Lindahl et al., 2015). However, the UA OI population had a higher percentage of the *COL1A1/2*

compared to Russian and Asian populations. Only 41% of OI patients from Yakutia and Bashkortostan (Russia) had *COL1A1/2* pathogenic variants (Khusainova et al. 2012). Asian populations from Vietnam, Korea and Taiwan had reduced amount of *COL1A1/2* pathogenic variants: 59%, 52% and 51% respectively (Ho Duy et al., 2016; Lee et al., 2006; Lin et al., 2015). Cross-population analysis of rare disorders in large populous countries is complicated as results are often fragmented (Yang et al., 2011; Zhang et al., 2012). At the same time, countries with small population sizes and limited sample cohorts might have differences in the spectrum of pathogenic variants of rare disorders when compared to countries with a higher number of patients. The reason for differences in genotypes might hide in the different sample sizes and methods of patient recruitment and potential variations in OI genetic epidemiology between populations.

The percentages of pathogenic variants in *COL1A1* and *COL1A2* genes were similar between Estonian (77%, 23%) and Ukrainian (76%, 24%) cohorts, as well as Finnish (78%, 22%) and Swedish (79%, 21%) populations (Hartikka et al., 2004; Lindahl et al., 2015). Similar results were also observed in a study by Pollitt *et al.* (77%, 23%, n=83) (Pollitt et al., 2006). In the report of Bardai *et al.* the proportion of *COL1A1* pathogenic variants was reduced (69%, 31%) (Bardai et al., 2016). Interestingly, in a study of 83 OI patients of Turkic and Slavic origin from Russia, *COL1A2* pathogenic variants were absent (Khusainova *et al.*, 2012). These results once more underline differences in genetic epidemiology of collagen-related OI. Population differences of OI might come from recessive OI genes, as some unusual alleles might have higher frequencies because of migration processes and cultural traditions, tolerating consanguinity (Cabral et al., 2012; Essawi et al., 2018; Garbes et al., 2015; Kurt-Sukur et al., 2015; Ward et al., 2002; Willaert et al., 2009).

It was claimed by Van Dijk *et al.* (Van Dijk and Sillence, 2014), that OI patients of European and American origin harbor more DN pathogenic variants. The Estonian OI population can be characterized by a higher proportion of LoF pathogenic variants (69%), compared to DN pathogenic variants (31%). A similar pattern of collagen defects was found in the Finnish population and cohort (LoF – 67%, DN – 33%) of Pollitt *et al.* (LoF – 65%, DN – 35%) (Hartikka et al., 2004; Pollitt et al., 2006). In the Ukrainian OI patients, the number of LoF (51%) and DN (49%) pathogenic variants was almost equal. Similarly to the Swedish OI cohort, where the amount of LoF and DN pathogenic variants was 53% and 47% respectively (Lindahl et al., 2015). In Taiwanese and Vietnamese OI populations, the percentage of DN pathogenic variants was higher 78% than LoF (Ho Duy *et al.*, 2016; Lin *et al.*, 2015).

Only two Estonian and two Ukrainian patients harbored LoF pathogenic variants in the *COL1A2* gene, underlining previous results of the reduced number of LoF pathogenic variants in the collagen type I α2 chain as affected individuals develop very mild phenotype, which oftem remain undiagnosed (Hartikka et al., 2004; Lindahl et al., 2015; Pollitt et al., 2006).

Gly substitution were the most popular of all DN pathogenic variants. In the Estonian cohort they composed 7/8 cases, and in the Ukrainian OI cohort they composed 24/31 cases, which supports previous findings. All DN pathogenic variants of Estonian patients altered triple helical chain domains (aa residues 162–1218 α1; aa residues 80–1102 α2) of collagen type 1 α1/2 chains. Only one pathogenic variant (patient EE07 with OI Type II) altered the "lethal cluster" proposed by Marini *et al.* (Marini et al., 2007).

Almost half of the identified pathogenic variants (EE – 50%, UA – 43%) were novel and previously unreported in the OI variant database. Out of the identified 40 novel pathogenic variants, two were identical in two Estonian patients and two were identical in two Ukrainian OI patients. According to ACMG pathogenicity criteria, 23 variants were classified as pathogenic, 14 as likely pathogenic and three as variants of unknown significance (VUS) (Table 3.1., 4.1.) (Richards et al., 2015). In the case of the *COL1A1*, c.505G>A, p.Glu169Lys VUS the change appeared to alter a conserved aa, and the current position might tolerate the substitution, though, there was evidence of splice site changes. However, the current variant is present in gnomAD, and there is lack of clinical information of cases carrying this variant. Taking into consideration, that the current variant was identified in an individual with mild OI, there is a chance that some carriers could have undiagnosed bone fragility. A *COL1A2,* c.2642A>C, p.Glu881Ala VUS was identified in individuals carrying additional *COL1A1* variants and might have additional shaping effect on bone phenotype. Additional functional studies are needed to confirm or oppose the pathogenicity of identified uncertain variants.

Despite the numerous studies on *COL1A1/2* pathogenic variants and an increasing number of discovered variants, the amount of identified novel pathogenic variants was still high, which proves the individual nature of OI pathogenic variants and the ability of OI pathogenic variants to appear in different exons of the *COL1A1/2* genes (Bardai et al., 2016; Lindahl et al., 2015; Pollitt et al., 2006). We believe that these pathogenic variants enrich knowledge about OI genotype and improve the understanding of OI genotype-phenotype correlations.

Some of the novel pathogenic variants alter previously reported positions with new variants of substitutions. There is a particular interest to investigate the association between OI phenotype and genotypes. We hope that the current work enriches the database of OI pathogenic variants and has practical applications for OI genetic diagnostics. Patients negative for the *COL1A1/2* gene pathogenic variants will undergo further panel sequencing and WES analysis to identify the OI causing pathogenic variant. Patients with contractures will be scanned for the presence of a Bruck syndrome diagnosis (*PLOD2* and *FKBP10* genes).

We performed Sanger sequencing of the *COL1A1* and *COL1A2* genes in patients with clinical signs of OI. Sequencing primers were picked far from intron-exon splice sites, which allowed for the identification of splice site, missense, frameshift and nonsense pathogenic variants in the exons of the *COL1A1/2* genes. Being a golden standard of sequencing, Sanger method

accuracy is about 99.9%. However, it has limitations in identifying the whole gene or exon duplications and deletions, which compose 1–2% of the OI population. In this way, the number of *COL1A1/2* pathogenic variants in studied OI patients might be underestimated as the Sanger sequencing did not cover the whole *COL1A1/2* gene or separate exon deletions.

6.2. Phenotypical and genotypic signatures in *de novo* and inherited *COL1A1/2* pathogenic variants (Paper III)

The importance of investigating *de novo* cases of musculoskeletal disorders lies in advancing the understanding of the disorders' nature, etiology, the risks of common and rare bone disorders, promoting of diagnostic approaches, assisting family planning and genetic counselling.

According to the results of our study, 56.16% of unrelated OI patients were *de novo* cases. Steiner *et al.* proposed that the proportion of *de novo* OI cases is 35–60% (Steiner et al., 1993). The amount of *de novo* cases among Estonian OI patients were decreased (37.04%). A lack of *de novo* OI cases in EE population might originate from a lack of severe OI cases, which have greater effect on fitness and offspring production.

The proportion of *de novo* cases differed between OI types. The vast majority (85.29%) of OI type III cases are *de novo*. Our current results are comparable with the percentage of achondroplasia *de novo* cases, but lower than the previously reported proportion of OI type III (Ornitz and Marie, 2002; Jay R. Shapiro et al., 2013; Trotter et al., 2005). Phenotype severity characteristics, phenotype variability and treatment availability might affect the fitness of individuals with OI type III and thus modify the proportion of familial and *de novo* cases in addition to different cohort sizes.

However, cases of recurrent AD OI were referred to as inherited, the number of the *de novo* OI cases might be slightly overestimated due to a possible presence of the gonadal mosaicism (3–5% of parents) (Figure 10) (Chen et al., 2013; Frederiksen et al., 2016). In addition, in families without available DNA samples of parents (n=8) the family history of OI was registered from the patient's word, which might additionally elevate the amount of *de novo* cases in the studied cohort.

6.2.1. Phenotypical characteristics and OI manifestations in *COL1A1/2 de novo* pathogenic variants

De novo OI type I, IV and III appeared at relatively similar frequencies (26.83%, 36.59% and 35.36%, respectively). However, it was expected that in *de novo* cases the amount of type III would be higher than type I and IV, which are more common for familial OI. According to data which emerged from our study, sporadic OI cases of different non-lethal OI types arise at similar frequencies.

However, OI type I and IV are more common among inherited OI cases. Another possible explanation might result from under- or misdiagnosis of mild OI cases. The absence of OI family history and variability of clinical characteristics might promote complications in OI diagnosis, which can lead to a suspicion of child abuse or diagnosis of other skeletal dysplasia.

The majority of OI patients harbor collagen I pathogenic variants, so a comparative analysis of *de novo* and inherited *COL1A1/2* cases is vital for the daily practice of health professionals, who work with OI patients without access to genetic testing. On practice, predominantly severe OI cases are suspected to be *de novo* OI cases. However, a medical professional might run into *de novo* OI patients with OI type I, III and IV with relatively similar frequency as the main differences in OI type distributions come from inherited OI cases. The nature of pathogenic variants (i.e. *de novo* or inherited) does not affect the severity of a phenotype itself. However, the severity of a phenotype affects the fitness and reproductive decisions of OI individuals. Thus, we can see significant differences in type prevalence among *de novo* and inherited OI cases. We think that these factors are crucial for OI diagnosis, family planning and follow up.

6.2.2. Proportion, functional type and architecture of *de novo* and inherited *COL1A1/2* pathogenic variants

The amount of *de novo* pathogenic variants in *COL1A1* gene was higher compared to *COL1A2*. It must be noted, that obviously some of the *COL1A2* pathogenic variants are underdiagnosed due to an absence of OI-related symptoms. Therefore, the amount of *COL1A2* pathogenic variants in the inherited OI cases was also decreased. Patients with light bone fragility in a family might not connect it to any special bone disorder, but rather to a family trait and thus lack diagnostics.

Despite a similar distribution of OI types among *de novo* OI cases, the majority of *de novo* cases were DN pathogenic variants, which correlate with more severe OI phenotype. Among inherited OI more than 50% were LoF pathogenic variants, which are generally associated with milder OI forms. This might result from higher fitness of mild OI.

There were no differences between the results of our study compared to previous works on pathogenic variant architecture. The majority of pathogenic variants were G>A transitions in the chain domain of the collagen type I α1 or α2 chains. Transitions happen more often because of instability in methylated CpG dinucleotides (Acuna-Hidalgo et al., 2016). Highly expressed CG sites are the main source of disease-causing pathogenic variants, including OI pathogenic variants, which happen prevalently in CpG islands. Further studies might concentrate on investigating the overall mutational loading differences between *de novo* and inherited OI cases and its impact on OI phenotype.

Furthermore, WES has confirmed the presence of a *COL1A1* pathogenic variant in one more Estonian patient; however, current the current variant

was absent from Sanger sequencing data (EE06; *COL1A1*, c.112del, p. p.(Ile38Serfs*36)). Therefore, the overall proportion of *COL1A1/2* pathogenic variants in the Estonia population increased to 90% (27/30). In the Ukrainian OI cohort, the nature of a variant previously thought to be pathogenic was specified as benign according to updated data reported by other investigators (UA29, UA34; *COL1A1*, c.3223>A, p.(Ala1075Thr)). The current pathogenic variant was observed in two patients, so the total number of Ukrainian collagen-related OI cases dropped to 60/94 (63.83%) instead of 62/94 (65.96%). According to latest data, the current polymorphism affects BMD only in relation to other OI causing pathogenic variants. However, in the analysis of *de novo* OI cases both patients harboring SNP were implemented into the OI collagen cohort. The updated total number of collagen-related pathogenic variants was 144. The number of overall *de novo* cases composed 81/144 (56.25%), the amount of Ukrainian *de novo* cases was 35/60 (58.33%). The influence of the update was insignificant for the overall data statistics compared to previous results: overall 82/146 (56.16%), UA 36/62 (58.06%).

6.3. OI type V in Ukrainian and Vietnamese OI populations (Paper IV)

The percentage of OI type V cases from the general OI population varies from five to 10% (Bardai et al., 2016; Y. Liu et al., 2017). In the UT OI biobank less than 2% (5/337) of patients had OI type V. There were no patients with OI type V among Estonian OI families (n=30), as well as among Palestinian OI families (n=49) (Essawi et al., 2018). We suppose that absence of OI type V patients in these populations might come from the size of the studied population and number of recruited patients.

The number of OI type V patients in the Ukrainian OI cohort was surprisingly high. In contrast, only one patient from the Vietnamese OI cohort had OI type V; however, methods for the sequencing analysis and patient recruitment in the Ukrainian and Vietnamese cohorts were identical.

We have observed both *de novo* and familial OI type V in our patients; however, the majority of *IFITM5* pathogenic variants were *de novo*, similarly to collagen-related OI (Hanagata, 2016). OI type V does not affect reproduction as complications of the disease pass after puberty (Fitzgerald et al., 2013; Rauch et al., 2013; Zhang et al., 2013).

The majority of patients developed HPC, which was in concordance with previous results (Hanagata, 2016). Due to the development of HPC, which is untypical for classical OI, many of the OI type V patients suffered from a mis-diagnosis. Patient 1 was previously diagnosed with pseudo osteosarcoma. However, a histological analysis of patient 1 has shown a classical OI type V mesh-like lamellation pattern, primitive woven bone, inflammation, hyper-cellular trabeculae and small cartilaginous islands in the HPC (Cheung et al., 2007; Rauch et al., 2013).

In the study of Liu *et al.* interconnections between osteosarcoma cells and the OI type V pathogenic variant c.–14C>T in the *IFITM5* gene were found. The current pathogenic variant proceeded apoptosis, inhibited tumor invasion and promoted osteogenic differentiation (B.-Y. Liu et al., 2017). The effect of the MALEP-IFITM5 protein on osteosarcoma is interesting for both disorders, especially in cased of extremely severe HPC.

Interosseous membrane calcification was described to develop in all patients who are at least four years old. In our cohort OI type V patients 4 (7 yo) and 5 (5 yo) interosseous membrane calcification was absent. Correspondingly, a lack of interosseous membrane calcification was observed in Chinese OI type V patients older than 4 yo (Rauch et al., 2013; Jay R Shapiro et al., 2013).

In parallel with OI type V symptoms of atypical mineralization, we have noticed classical OI features in OI type V patients from our cohort. Patients suffered from bone fragility, numerous minimal trauma fractures, skeletal deformities, greyish or bluish eye sclera, joint laxity, hearing loss and teeth abnormalities. Previously only one patient with OI type V and hearing loss was described (Jay R Shapiro et al., 2013). Teeth abnormalities were common features in the cohort of OI type V patients from the study of Kim *et al.* (11/16) (Kim et al., 2013). However, at first sight, these collagen-related manifestations might seem obscure in non-collagen OI; interconnections of the MALEP-IFITM5 and collagen expression were registered in models of OI type V transgenic mice and OI type V primary osteoblast cultures (Lietman et al., 2015; Reich et al., 2015). Accordingly, classical collagen related OI symptoms in patients with OI type V could have arisen because of insufficient collagen expression and quantitative collagen defect. However, MALEP-IFITM5 also affects bone mineralization, where typical OI V symptoms originate.

Sequencing analysis ruled out misdiagnoses and confirmed OI type V in patient 1. This helped to build up an appropriate strategy for pharmacological and surgical treatment. Similarly, in the rest of the revealed cases (Patients 2–5 with suspected classical OI) the treatment strategy was updated. A more careful approach is needed to exclude risks and complications, coming from the molecular defect of the *IFITM5* 5'UTR pathogenic variant. Results of the study can be further used to provide family planning for those identified with OI type V families and to perform cost-effective and rapid genetic testing of family members in following generations in cases of suspected OI.

Both classical OI and OI type V symptoms varied between patients. Fracture numbers ranged from 0.5 to 2.3 per year. Growth retardation had a tendency to decrease with age and correlated with the severity of the phenotype characteristics. Despite harboring the same pathogenic variant, phenotype diversity was striking. It might underline the presence of additional factors, which may be equally important as the *IFITM5* gene pathogenic variant, for shaping of the OI type V phenotype. Rarity of OI type V complicates the investigation of this OI form. A collection of patients' cases, genetic material and deep phenotyping is essential for future research on OI type V.

6.4. Clinical OI types and phenotype manifestations in Ukrainian patients (Paper II)

One hundred forty-three OI individuals from 94 Ukrainian families. Almost half (46.85%) were affected with OI type I, 16.78% with OI type III and 34.27% with OI type IV. Three patients were suspected to have OI type V and were sent for further genetic testing of the 5'UTR of the *IFITM5* gene.

Previous works have stated the breakdown of OI type among the Polish OI cohort (n=123) as follows: OI type I – 44%, OI type III – 33% and OI type IV – 21% (Rusinska et al., 2017). In the Russian OI cohort (n=117), the break-down was 59% of OI type I, 27% of OI type III, 14% of OI type IV (Yakhyayeva et al., 2016). Among our UA OI cohort there was a lower proportion of OI type III patients. This might be caused by difficulties in some severely affected OI patients to travel to the interview venue. Cross-sectional population studies usually lack patients with OI I compared to nationwide register-based investigations. Differences are caused by diagnostic methods and reduced interest of the patients. However, in one instance the effective work of a UA patient organization (The Ukrainian Association of Crystal People) helped to recruit more patients with mild OI.

Previous works have revealed the distribution of OI types as the following: Vietnamese (OI I–31.5%, OI III–31.5%, OI IV–37%); Taiwanese (OI I–58%, OI III–7%, OI IV–35%); Chinese (OI I–28%, OI III–38%, OI IV–34%); Israeli (OI I–61%, OI III–21%, OI IV–14%); Swedish (OI I–68%, OI III–13%, OI IV–19%); Norwegian (OI I–77%, OI III–9%, OI IV–11%) and Finnish (OI I–72%, OI III–4%, OI IV–20%) (Binh et al., 2017; Hartikka et al., 2004; Lin et al., 2015; Lindahl et al., 2015). Differences in the proportion of OI types might be hidden in numerous factors, including: patient recruitment methods, sample sizes, diagnostics, classification and influence of genetic epidemiology.

The number of fractures per year was in concordance with the Swedish OI cohort (type I 0.57±0.68; type III 3.83±9.32; and type IV 1.33±1.38) (Lindahl et al., 2015). Lower limbs were the most fractured and deformed body compartments, as was seen in studies on other OI cohorts. It might be connected to greater mechanical loading of the lower limbs and their weight bearing function (Binh et al., 2017). Two individuals older than 18 yo did not develop any fractures. They were positively diagnosed with OI due to other classical OI symptoms and a positive OI history in the family. Both patients were negative for *COL1A1/2* pathogenic variants. Further panel sequencing might reveal a genetic cause in these patients. Therefore, it would be particularly interesting to the identify causative pathogenic variants in these cases and further investigate the reason behind the fracture absence.

The number of patients (55%) with DI was higher than in the Swedish (25%) and Brazilian (27%) OI cohorts, but similar to patients from the Vietnamese (61%) and Taiwanese (44%) cohorts (Binh et al., 2017; Brizola et al., 2017; Lin et al., 2015; Lindahl et al., 2015).

87% had blue sclera, which was in concordance with the Swedish, Brazilian, Taiwanese and Vietnamese cohorts (80–90%), despite the fact that the proportion of collagen I related OI cases was different (Binh et al., 2017; Brizola et al., 2017; Lin et al., 2015; Lindahl et al., 2015). The reason for the current similarities might hide in the grater spread of greyish and bluish sclera in non-collagen OI patients.

6.5. Genotype-phenotype correlations UA cohort (Paper II) and general genotype-phenotype correlations in 238 OI Estonian, Vietnamese and Ukrainian families

Genotype-phenotype associations in OI remain a main topic of interest for OI researchers. However, these correlations are elusive; previous works have identified that *COL1A1* pathogenic variants are usually more severe compared to *COL1A2* (Marini et al., 2007; Mrosk et al., 2018). In a cohort of UA patients, we have observed increased number of fractures among patients with DN pathogenic variants. Among patients with *COL1A2* DN pathogenic variants deformities of the spine and chest were more common. LoF pathogenic variants caused less severe OI forms. General correlations between OI phenotype severity and collagen defect were in concordance with previous studies (Ben Amor et al., 2011; Lindahl et al., 2015; Marini et al., 2007). In contrast with Swedish OI study, OI patients from UA cohort with LoF OI type I differed by less deformed lower limbs and less fractures compared to DN OI type I patients. It supports idea, that collagen chain structure is vital for phenotype, compared to collagen amount.

We did not observe correlation between pathogenic variant type and DI. Lack of correlation between DI and OI pathogenic variant type might be explained by the different non-OI etiology of dental issues in UA OI patients (Ben Amor et al., 2011; Lin et al., 2009; Lindahl et al., 2015).

Similarly to previous works in other cohorts, in UA OI patients blue sclera highly correlated with the presence of collagen type I pathogenic variant (Lindahl et al., 2015).

There was no correlation between genotype and hearing loss. None of the previous works succeeded to discover connections between hearing loss and genotype in OI. In the studied UA cohort, the number of pediatric patients with hearing loss was surprisingly high, so identification of non-collagen OI pathogenic variants in this cohort is of particular interest (Ben Amor et al., 2011; Hartikka et al., 2004).

The majority of OI type I and IV were caused with LoF pathogenic variants, whereas DN pathogenic variants were associated with OI type III. Interestingly, two patients with DN pathogenic variants had OI type III. Among DN cases, seven patients suffered from OI type I. Two of them had Gly substitutions, which are usually associated with severe OI. The type of the pathogenic variant,

pathogenic variant's helical location and substituted residue affect the severity of the phenotype (Marini et al., 2007; Rauch et al., 2010).

In the full cohort of the UT OI database, we have observed significant correlations between OI type, presence of a collagen type I pathogenic variant, mutated gene and collagen defect.

There remained correlations between collagen OI and blue sclera. Interestingly, in a 238 patient cohort we were able to observe a correlation between DI and defect type of *COL1A1/2* pathogenic variant, which was absent from the UA cohort. DI was associated with structural collagen defects in several previous studies (Biria et al., 2012; Li et al., 2019; Maioli et al., 2019; Majorana et al., 2010; Nguyen et al., 2017). Involvement of larger sample cohorts from different populations might raise the accuracy of studies on rare disorders.

However, while the overall impact of *COL1A1/2* pathogenic variants on phenotype is understood, genotype-phenotype correlations remain an unresolved issue. OI causing pathogenic variants do not explain all nuances of phenotype development and additional factors play a role in phenotype shaping. Great interest towards overlap of collagen and non-collagen OI phenotypes remains. Further research of deep phenotyping and the molecular characteristics of OI patients might advance our understanding of genotype-phenotype correlations in OI.

6.6. Inter- and intrafamilial diversity (Paper V)

With more information from OI genetic studies genotype-phenotype correlations become more conflicted, as patients with identic pathogenic variants can developed OI phenotypes of different severity and patients with similar phenotypes might harbor different phenotypes. Inter- and intrafamilial diversity is increasing the difficulty of both family planning and diagnostics.

6.6.1. Interfamilial variability

Eighty percent of *COL1A1/2* pathogenic variants harbored by patients from the UT OI database showed phenotypic intrafamilial variability. Th cohort of OI patients in our study consisted of patients from three populations: Estonia, Ukraine and Vietnam. A recent study by Maioli *et al.* reported lower variability, only 31.55% (Maioli et al., 2019). It could be speculated that in contrast to the Italian study, we have different populations in our cohort; additionally, we have also included publicly available data from the Dalgleish's database, which is comprised of patients from all over the world.

In the OI variant database patients were diagnosed by different medical professionals and a classification bias is present. Monophenotypic variants yielded a clear phenotype picture, especially in cases of mild OI, and all reported patients were classified with the same OI type. In polyphenotypic pathogenic

variants, clinical phenotypes differed between affected individuals. The majority of variants caused bordering types of OI, mild-moderate or severe-moderate (types I–IV and III–IV).

The number of observed patients may act as a limitation of the study, as it is impossible to catch all carriers of a single pathogenic variant. Data of phenotypes might be incomplete. More variable pathogenic variants differ with a higher number of reported cases in the OI variant database.

What is more, treatment might have changed the phenotypes of patients towards milder forms. Difficulties might be caused by classification bias of OI border types (I/IV, III/IV). In some cases, diagnosis might be subjective and debatable. Similar issues were reported in case of OI with the *IFITM5* Ser40Trp pathogenic variant, resembling clinically OI type VI, where a patient suffered multiple prenatal fractures, typical for lethal or severe OI, but remained fracture-free after birth with normal bone densitometry (Lim et al., 2019).

However, all these limitations cannot affect the presence of lethal and non-lethal OI phenotypes among phenotypes caused by a single pathogenic variant. These circumstances raise additional challenges for diagnostic procedures and disease progression prediction.

Similarly to previously reported results, according to our data variability correlated with the type of collagen defect and gene (Maioli et al., 2019). Correlations between the phenotypical variability and the gene could be party explained by the near absence of LoF pathogenic variants in the *COL1A2* gene compared to the *COL1A1* gene. Oppositely, the *COL1A2* gene dominates a number of DN pathogenic variants, especially Gly substitutions.

LoF pathogenic variants are associated with a lower phenotypical variability compared to missense pathogenic variants. These results are confirmed with the data from Dalgleish's database (Dalgleish, 1998). Pathogenic variants reported in at least 10 OI cases show that a lack of phenotypical variability is present among carriers of the LoF pathogenic variants. LoF pathogenic variants cause haploinsufficiency with the following mechanisms: degradation of the abnormal collagen type I, inefficient transport of the mRNA to cytoplasm or premature termination codons and nonsense-mediated mRNA decay (NMD) (Garnero et al., 2009). The reduced amount of collagen I causes mild OI phenotype. Interestingly, in some cases of LoF pathogenic variants in the last exons of the *COL1A1/2* genes, NMD could escape and abnormal transcripts lead to truncated collagen structure, like DN pathogenic variants (Slayton et al., 2000). DN pathogenic variants alter the triple helical structure of the collagen and affect collagen stability, causing qualitative defect and severe OI. The escape from NMD could explain the presence of some LoF pathogenic variants among cases of high phenotypical variability. Further functional mRNA studies in individuals with LoF pathogenic variants of high phenotypical variability might help to clarify the current issue.

Furthermore, it could be proposed that the modification capacity of OI phenotypes depends on collagen properties, not its amount, as patients with LoF pathogenic variants have lower variability.

6.6.2. Intrafamilial variability

Intrafamilial variability was lower (34.38%), compared to interfamilial variability. It could be explained by the higher genetic variations between unrelated individuals, when compared to members of the same family. We have met the majority of affected individuals of the same family during our interviews, and our variability estimation is expected to be accurate. Intrafamilial variability, however, correlated only with collagen defect type.

Phenotypical variability can not be disclosed based on differences in treatment on its own. In some cases, mildly affected parents, who did not need treatment, had children with moderate symptoms that were in need of surgical and pharmacological treatment.

According to our data, siblings and cousins from the same generation had lower phenotypical variability compared to the variability between different generations. However, siblings might have higher genetic variance due to recombination, compared to parent-child variance (Visscher et al., 2008). Extra differences between parent-child variance might come from *de novo* pathogenic variants (Acuna-Hidalgo et al., 2016; Veltman and Brunner, 2012).

The progression of OI characteristics in following generations was proposed before; however, our study shows that both the increasing and decreasing severity of the symptoms are possible (Moraes et al., 2012). For Estonian OI families, patterns of OI symptoms dissolve were more common. The Clinic of Traumatology and Orthopedics of Tartu University Hospital treated and followed up with Estonian OI families for 25 years. It cannot be excluded, that milder OI forms in Estonian OI patients could be supported with regular follow up, medication and early deformity corrections, which can prevent fractures and complications. All the aforementioned procedures and regular system of management of OI patients were absent in Vietnam in Ukraine until recently. At the same time, the higher number of families with progressing OI among Ukrainian and Vietnamese cohorts might be connected to the dominance of DN pathogenic variants in these OI populations, compared to Estonian cohort.

Further studies are needed to confirm the absence of compound heterozygosity and gonadal mosaicism among studied patients with intrafamilial diversity, as these are the main explanations for variability in collagen-related OI (Laine et al., 2013; Symoens et al., 2013). However, intrafamilial diversity could be also caused by modifier factors (Cutting, 2010; Nadeau, 2001).

The beneficial outcome of diagnostics and family planning from our study is in the prediction of variability risks in upcoming generations for families with OI history. In this way, families with LoF pathogenic variants and quantitative collagen defect are at a lower risk of phenotypical variability in the case of pathogenic variant transduction to following generation. Whereas, families with DN pathogenic variants need to be aware of the risks of disease progression in their children.

OI functional studies might underline causes of phenotypical variability. Genetic, epigenetic, environmental and complex interactions of all mentioned

factors might develop OI phenotype. Follow-up research concentrating on the issue of phenotypical variability of OI is needed. In addition to diagnostic and family planning benefits, it might have therapeutic potential, as addressing the origins of OI phenotypical expressivity may offer a “natural healing force”, which could change phenotype from severe to mild or even to an unaffected OI form.

7. CONCLUSIONS

I. Out of 30 unrelated Estonian OI patients ~90% harbored *COL1A1/2* pathogenic variants. Among the Estonian OI population the amount of LoF pathogenic variants was higher (69%) compared to other European OI populations. *COL1A1* gene was altered in 77% of the cases. DN pathogenic variants of Estonian OI patients altered the triple helical chain domain of α1 and α2 chains. One pathogenic variant was situated in the lethal cluster and caused OI type II. Half of the genetic variants were novel and previously undescribed.

II. Out of 94 screened, unrelated Ukrainian OI patients *COL1A1/2* pathogenic variants were identified in 63.83%. The number of DN (49.21%) and LoF (50.79%) pathogenic variants were almost equal. Novel variants composed 43% of the identified genetic variants. The distribution of OI types in Ukrainian OI patients was as follows: OI type I (46.85%), OI type III (16.78%), OI type IV (34.27%) and OI type V (2.10%). Genotype-phenotype analysis has supported the dependence of OI phenotype severity on the type of collagen I defect.

III. Slightly more than half (56.16%) of collagen-related OI cases are assumed to be *de novo*, with the highest proportion of *de novo* cases in the Vietnamese population (63.16%) and the lowest among Estonian OI patients (37.04%). Mild *de novo* OI cases are under- or misdiagnosed. There are substantial differences between the genotype and phenotype characteristics of *de novo* and inherited OI. Further studies on mutational load differences between inherited and *de novo* OI patients might advance the understanding of OI etiology, pathological mechanisms and phenotype development.

IV. c.–14C>T 5'UTR *IFITM5* pathogenic variants were identified in four families (five individuals) with OI type V of Ukrainian and Vietnamese origin with various phenotype severity, which composed less than 2% of the entire studied OI cohort. There were no cases of OI V among Estonian OI patients. Results of our study support the presence of classical OI features (hearing loss, bluish and greyish sclera, joint laxity and teeth abnormalities) in OI V patients in addition to the typical OI V features such as abnormal mineralization and HPC. Due to high variation in clinical symptoms, we suggest the testing of the 5'UTR of the *IFITM5* gene pathogenic variants in patients who lack OI V features.

V. OI inter-and intrafamilial variability is associated with genotype characteristics of the OI causing pathogenic variant. We succeeded to form phenotypical groups of collagen-related OI based on the genotype-phenotype severity scale. We have confirmed the presence of an interfamilial diversity pattern, which depends on the mutated gene. Patients lacking diversity were harboring pathogenic variants in the *COL1A1* gene, whereas patients with variable phenotypes were more likely to harbor the *COL1A2* pathogenic variants. Both inter- and intrafamilial diversity correlated with collagen I defect type. LoF pathogenic variants lacked diversity in patients with mild OI, whereas DN variants were associated with a higher degree of phenotype variability.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

SUMMARY IN ESTONIAN

Genotüübi ja fenotüübi korrelatsioonidel põhinev *Osteogenesis Imperfecta* perekondade sisene ja vaheline varieeruvus

Osteogenesis Imperfecta (OI) ehk habraste luude haigus on haruldane geneetiline sidekoehaigus. Juba vanast ajast on tuntud OI klassikaliste tunnuste triaad: hulgiluumurrud, sinised skleerad ja kuulmislangus. Lisaks on OI peamisteks sümptomiteks skeleti deformatsioonid, kolmnurkne nägu, kääbuskasv ja *Dentinogenesis Imperfecta* (DI). OI esinemissagedus on 1/20 000, kuigi erinevate OI tüüpide esinemissagedus varieerub. Kergemate OI vormide esinemissagedus on tavaliselt suurem kui raskematel OI vormidel.

OI esinemise fenotüüpide spekter on väga lai: sinna kuuluvad erinevad vormid kergest osteopeeniast kuni letaalsete vormideni. Praegune OI klassifikatsioon eristab viit kliinilist OI tüüpi: I–V. Tüüp I on kerge OI väheste luumurdudega, oluliste deformatsioonideta ja normaalse kasvuga. Tüüp II on letaalne, mida iseloomustavad hulgalised prenataalsed luumurrud, oluline kasvupeetus, rasked skeleti deformatsioonid, kolju demineraliseerumine ja respiratoorne puudulikkus. Tüüp III on raske OI hulgiluumurdudega ja progreseeruvate skeleti deformatsioonide ning kasvupeetusega. Tüüp IV on mõõdukas, mille tunnusteks on normaalne kasv ning luumurdude arv ja deformatsioonide tase on mõõdukad. Tüüp V on haruldane OI vorm, mille tüüpilisteks tunnusteks on luudevaheliste membraanide kaltsifitseerumine ja hüperplastilise kalluse tekkimine pärast luumurde. Fenotüübid on individuaalsed ning erinevad sama patogeense variandi kandjate vahel.

OI on tuntud peamiselt I tüüpi kollageenistruktuuri või -hulga häirena. Kuni 90% OI patsientidel esineb geenides autosomaalset dominantset I tüüpi kollageenivarianti *COL1A1* ja *COL1A2*. Ülejäänud ~10% OI juhtudest on seotud peamiselt retsessiivsete patogeensete variantidega mitte-kollageeni OI geenides, kuigi kliiniliselt meenutavad nende fenotüübid kollageeni patogeensete variantidega patsientide fenotüüpi. OI V tüüpi põhjustab ainult *IFITM5* geeni 5'UTR variant. Praeguseks on teada 17 erinevat mittekollageeni OI geeni, mille funktsioonid on seotud kollageeni transportimise, voltimise, posttranslatsiooni modifitseerimise, luu mineralisatsiooni ja luurakkude funktsioneerimisega.

Kollageeni I kvalitatiivne defekt on põhjustatud dominantsest negatiivsest (DN) variandist, mis on põhjustatud aminohappe vahetusest kollageeni kolmikheeliksis. Kollageeni struktuursed variandid on seotud raskemate OI fenotüüpidega. Kollageeni kvantitatiivsed defektid on seotud ühe alleeli puudulikkusega ja sellest tingitud funktsioonikao variantidega (LoF). Kollageenihulga defekt assotsieerub kergemate OI vormidega. Genotüüp-fenotüüp korrelatsioone on kirjeldatud harva ning peamiselt sama variandiga patsientidel. Isegi ühe perekonna liikmetel võib esineda erinev haiguse kliiniline väljendus. Haruldastel juhtudel võib sama variant põhjustada isegi nii letaalset kui ka mitteletaalset OI-d.

Perekondadevaheline ja -sisene varieeruvus on eelnevalt kirjeldatud üksikutel OI juhtudel. OI fenotüübi modifikatsiooni tegurid on jäänud teadmata.

Fenotüübiline varieeruvus suurendab ala- ja valediagnoosi riski asümptomaatiliste patsientide korral, takistab haiguse raskuse ning kulu ennustamist nii perekonna kui ka haiguse ravi planeerimisel. Fenotüübilise varieeruvuse uurimine võimaldab paremini aru saada OI kliinilisest pildist ning aitab seeläbi kaasa uute diagnostika- ja ravimeetodite väljatöötamisele.

Uurimuse eesmärgid

Käesoleva uurimistöö eesmärgiks oli iseloomustada OI perekondade patogeensete variantide profiili, hinnata genotüübi-fenotüübi korrelatsioone koos perekonnavahelise ja perekonnasisese varieeruvusega. Sellest tulenevalt olid töö alaeesmärgid järgnevad:

1. Iseloomustada *COL1A1* ja *COL1A2* patogeensete variantide spektrit Eesti OI patsientidel.
2. Kirjeldada kliinilisi ja molekulaarseid tunnuseid *COL1A1* ja *COL1A2* patogeensete variantidega Ukraina OI perekondade kohordis.
3. Uurida *de novo* patogeensete variantide juhtumeid *COL1A1* ja *COL1A2* patogeensete variantidega patsientide hulgas.
4. Selgitada OI V tüübi patogeensete variantide esinemist Eesti, Ukraina ja Vietnami OI patsientidel.
5. Analüüsida võimalikke seoseid perekondadevahelises ja -siseses varieeruvuses ning genotüübi mõju *COL1A1* ja *COL1A2* OI perekondades.

Materjal ja metoodika

Uuringusse kaasati Tartu Ülikooli Kliinikumi, Traumatoloogia ja Ortopeedia Kliiniku OI andmebaasist 238 perekonda, kokku 337 haiget indiviidi. *COL1A1/2* geenide sekveneerimise analüüs teostati 30 Eesti ja 94 Ukraina perekonnas. Kliiniliste tunnuste kirjeldamine teostati 143 Ukraina OI patsiendil 94 perekonnast. *De novo* OI analüüs tehti 146 mitteseotud patsiendil, kellel esines kollageeni patogeensetest variantidest tingitud OI. OI V tüüpi testiti 90 mittesuguluses oleval patsiendil, kellel ei leitud *COL1A1/2* patogeenset varianti. Perekonnavahelist fenotüübilist varieeruvust uuriti 81 *COL1A1/2* patogeensete variantidega perekonnas. Perekonnasisest varieeruvust uuriti 64 *COL1A1/2* OI perekonnas.

Perekondade haiguse kulu informatsioon koguti intervjuude käigus ja haiguslugudest. Kliinilise läbivaatuse ajal hinnati OI kliiniliste tunnuste esinemist. Patsiendid klassifitseeriti nende kliiniliste tunnuste avaldumise järgi OI tüüpideks I–V. Vereproovid koguti haigetelt ning nende tervetelt lähisugulastelt edasiseteks analüüsideks.

COL1A1/2 geenide ja *IFITM5* geeni 5'UTR patogeensete variantide analüüs tehti Sangeri sekveneerimisega verest eraldatud DNAst. Variantide patogeensust ennustati *in silico* ja ACMG klassifikatsiooni alusel. Patogeenseid variante, mis puudusid OI variantide andmebaasist, tuvastati *de novo*. *De novo* OI juhtumite analüüsis tuvastati variandi pärimiseviis. Teostati sekveneerimisanalüüs

patsiendi vanematel, et kinnitada patogeense variandi puudumist perekonnas varasemalt. Analüüsiti patogeense variandi etioloogiat, fenotüübi ning genotüübi tunnuste korrelatsioone. Genotüübi-fenotüübi korrelatsioone testiti OI kliiniliste tunnuste ja patogeensete variantide tüüpide vahel. Perekonnavahelist varieeruvust analüüsiti isikutel, kelle patogeenne variant esines vähemalt kahel mittesuguluses oleval patsiendil Tartu Ülikooli OI andmebaasis või Dalgelish OI variantide andmebaasis; hinnati patogeenset varianti põhjustavate tüüpide esinemissagedust. Perekonnasisest varieeruvust analüüsiti vastavalt kasvavale või kahanevale haiguse ilmestumisele eelmises ja/või järgnevas põlvkonnas. Testiti varieeruvuse korrelatsioone kollageeni defektitüübi ja -geeniga.

Tulemuste võrdlemiseks kasutati kirjeldavat statistikat. Kategooriliste tunnuste seoseid testiti Fisher's $\chi 2$-testiga. Shapiro-Wilk's testi kasutati normaaljaotuste määramiseks. Student's t-testi ja Mann-Whitney U testi abil kontrolliti vastavalt normaaljaotusega ja mittenormaaljaotusega pidevate tunnuste seoseid. Oluliseks määrati P-väärtus vähem kui 0.05.

Uuringu teostamiseks olid olemas Tartu Ülikooli Inimuuringute eetikakomitee (Permit no. 221/M-34), Hue Ülikooli (approval No. 75/CN-BVYD), ja Harkovi Sõtenko Selja ja Liigeste Patoloogia Instituudi eetikakomiteede load.

Peamised tulemused ja järeldused

Uurimistöö olulisemad tulemused on järgnevad:

1. Eesti 30 mittesuguluses olevast OI perekonnast esines ~90% *COL1A1/2* patogeenne variant. Eesti OI populatsiooni hulgas oli LoF variantide arv suurim (69%), võrreldes teiste Euroopa populatsioonidega. 77% variantidest esinesid *COL1A1* geenis. Üks patogeenne variant paiknes kollageeni I letaalses klastris ning põhjustas letaalset OI-d (II). Pooled patogeensetest variantidest olid uued ja varasemalt kirjeldamata.
2. Ukraina 94 perekonnast esines *COL1A1* variante 63.83% uuritavatest. DN ja LoF variantide arv oli praktiliselt sama. 43% variantidest olid *de novo*. OI tüüpide jagunemine oli Ukraina populatsioonis järgmine: OI tüüp I (46.85%), OI tüüp III (16.78%), OI tüüp IV (34.27%) ja OI tüüp V (2.10%). Genotüübi-fenotüübi korrelatsiooni analüüs näitas OI fenotüübi raskuse seost kollageeni defektitüübiga.
3. 56.16% kollageeni OI juhtumitest olid *de novo*. Kõrgeim *de novo* OI juhtumite osakaal oli Vietnami populatsioonis (63.16%) ning madalaim oli Eesti populatsioonis (37.04%). *De novo* ja pärilikud OI juhtumid erinevad oluliselt genotüübi ja fenotüübi alusel. Edasised *de novo* OI juhtumite uuringud aitavad kaasa OI etioloogia, patoloogiliste mehhanismide ja fenotüübi arenemise arusaamisele.
4. c.–14C>T 5'UTR *IFITM5* geeni variante tuvastati neljas perekonnas (kokku viis patsienti), mis moodustavad vähem kui 2% kogu Tartu Ülikooli OI andmebaasi patsientide kohordist. V OI tüübiga patsientid pärinesid Ukraina ja Vietnami OI populatsioonidest ning omasid erinevaid fenotüüpe ja raskusastet. Eesti OI populatsiooni seas OI V juhtumeid ei olnud. Meie uuringu

tulemused toetavad lisaks OI V tunnustele (anomaalne mineralisatsioon ja hüperplastlise kalluse kujunemine) ka klassikaliste kollageeni OI sümptomite (kuulmislangus, sinakashallikas skleera, liigeste lõtvus, hammaste anomaaliad ja haprus) esinemist V OI tüübiga patsientidel. Kõrge tunnuste varieeruvuse tõttu soovitame testida 5'UTR *IFITM5* geeni ka neil patsientidel, kellel puuduvad selged OI tüüp V tunnused.

5. OI perekonnasisene ja -vaheline varieeruvus korreleerub genotüübi muutustega OI patsientidel. Meil õnnestus komplekteerida kollageeni OI fenotüübilisi gruppe, toetudes genotüübi-fenotüübi raskusastme skaalale. Leidsime perekonnavahelise varieeruvuse mustri, mis sõltub muteerunud geenist. Vähema varieeruvusega patsiendid omasid variante *COL1A1* geenis võrreldes *COL1A2* geeni variantide kandjatega, kellel oli suurem fenotüübiline varieeruvus. Nii perekonnavaheline kui ka perekonnasisene varieeruvus sõltusid kollageen I defektitüübist. LoF patogeensete variantide puhul puudus fenotüübiline varieeruvus, kergetel OI juhtumitel DN patogeensete variantidega aga assotsieerus kõrgem fenotüübilise varieeruvuse tase.

Kokkuvõttes näitasid käesoleva uuringu tulemused, et suurim osa OI juhtumitest nii Eesti kui ka Ukraina populatsioonis on seotud *COL1A1/2* patogeensete variantidega ning enamus nendest tekivad sporaadiliselt. Vähem kui 2% OI patsientidest on V OI tüüp. Kvalitatiivne kollageenidefekt on seotud raskemate OI vormidega ning perekonnasisese ja -vahelise varieeruvusega OI patsientidel korreleerub fenotüübi varieeruvuse tase kollageenidefekti tüübiga.

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APPENDICES

Appendix A

Table 1. List of primers used for PCR amplification and sequencing of the *COL1A1* (gDNA NG_007400.1, cDNA NM_000088.3) and the *COL1A2* (gDNA NG_007405.1, cDNA NM_000089.3) genes in the Osteogenesis Imperfecta patients.

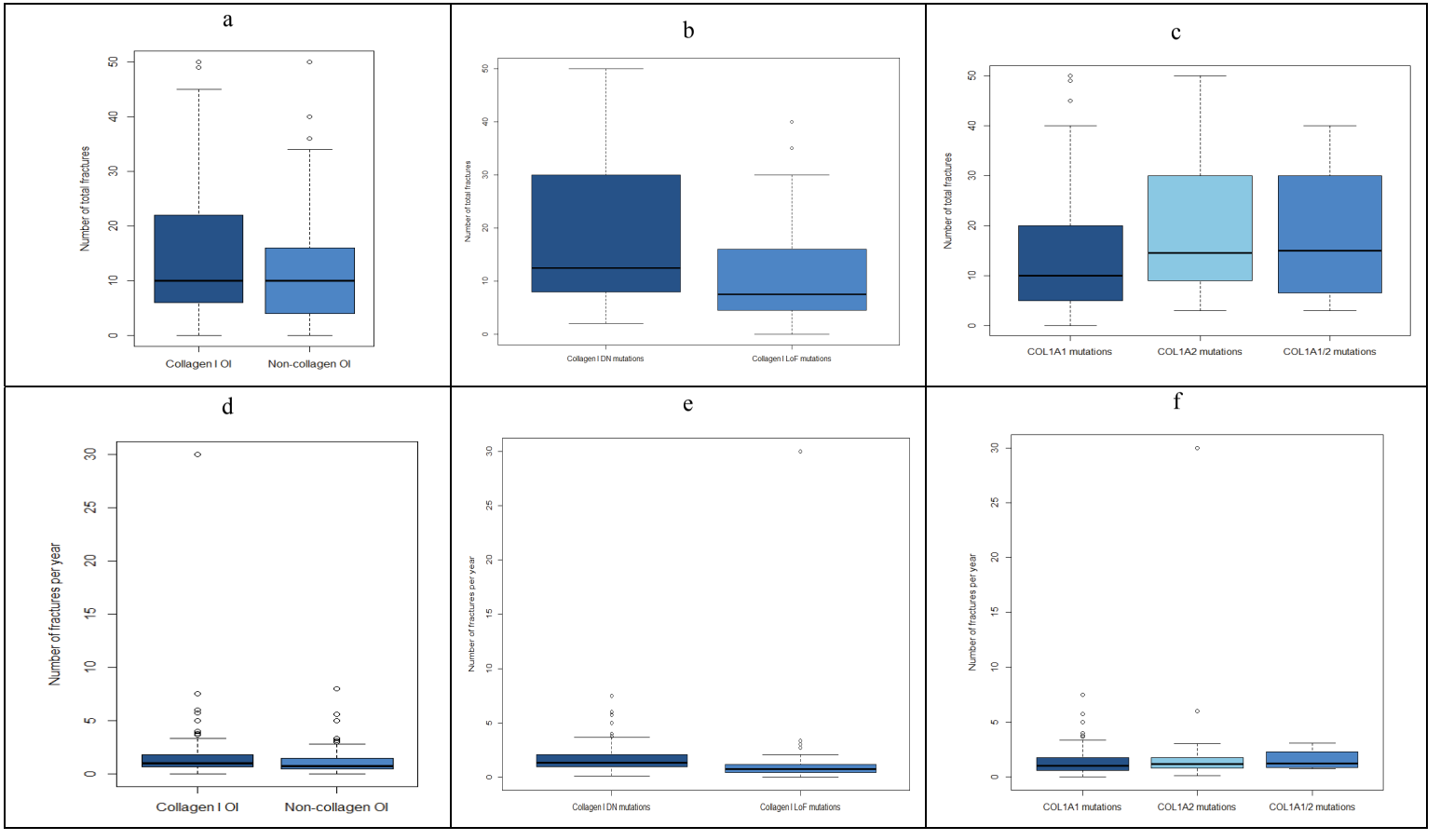
Target	Forward primer	Reverse primer	Product size (bp)
COL1A1			
5'UTR, Exon 1	5'-TCTCCATTCCAACTCCCAAA-3'	5'-GGATCATCCACGTCTCGTTT-3'	463
Exon 2, 3	5'-CGGGAAGTGAAAAATCCAAG-3'	5'-GGGAGGACTGTGAGGAGTCA-3'	554
Exon 4, 5	5'-ATCTCCTGCCCTCGAATTTT-3'	5'-CGCAAAAGAGCCTGATGTT-3'	567
Exon 6, 7	5'-GTGGCACTGGGAGTTTTGAT-3'	5'-TCTTCCCTCCAAAAGACCAA-3'	635
Exon 7–9	5'-TTTGGGACCTCAGAGTCCAG-3'	5'-CCATGGGGTCAGATGGTATC-3'	639
Exon 10, 11	5'-AACCTGACCTGCAACAATCC-3'	5'-GGTGCTTTTGGATGTCCACT-3'	481
Exon 12-15	5'-GGCTCTTTCTCAGATCTAGGTG-3'	5'-GGGGTTCAGACCAACATAACC-3'	700
Exon 16-18	5'-AACCCCAGGACTTCCTCTGT-3'	5'-CTGTGGGAGGCAGACAGC-3'	729
Exon 18-20	5'-GTCCCCTTTGCCACTTTCTA-3'	5'-TAGGGGCTCCTCTTCCTTTC-3'	589
Exon 21-23	5'-AGGAGCCCCTAGTCTTCTGG-3'	5'-TGATCCAGAACGCCTCATC-3'	700
Exon 24, 25	5'-GTCTGGGATGAGGCGTTCT-3'	5'-TGGAGCCTGGAGAAGTGTTT-3'	853
Exon 26-29	5'-AGGCCCTGGCTTCTCACT-3'	5'-CTCTGGATTCCCTGCATCTC-3'	676
Exon 30-32	5'-CCAGACCCCAGGAGGAAG-3'	5'-CAAAGCAGGCAGAGATGG-3'	791
Exon 33-35	5'-ACCCAGACACAAGCAGAACA-3'	5'-GTGGGAAACAATCCCGTCT-3'	798
Exon 36-38	5'-TTCCTGCCTCCATTACTGCT-3'	5'-AGCATTTCCCTGGATGAGG-3'	664
Exon 39, 40	5'-TCTCTCCTTCCAGGGTGCTA-3'	5'-CCTGTGATGGTTTTTCTCAGG-3'	626
Exon 41-43	5'-GCCAAGGAGAACAGATTTGG-3'	5'-GCCTCTAAGGAGGCCTGAAG-3'	640
Exon 44-46	5'-GGAGAGAGAGATCCAGCAGAG-3'	5'-GCTTGGGGCTCAGGAAGA-3'	800

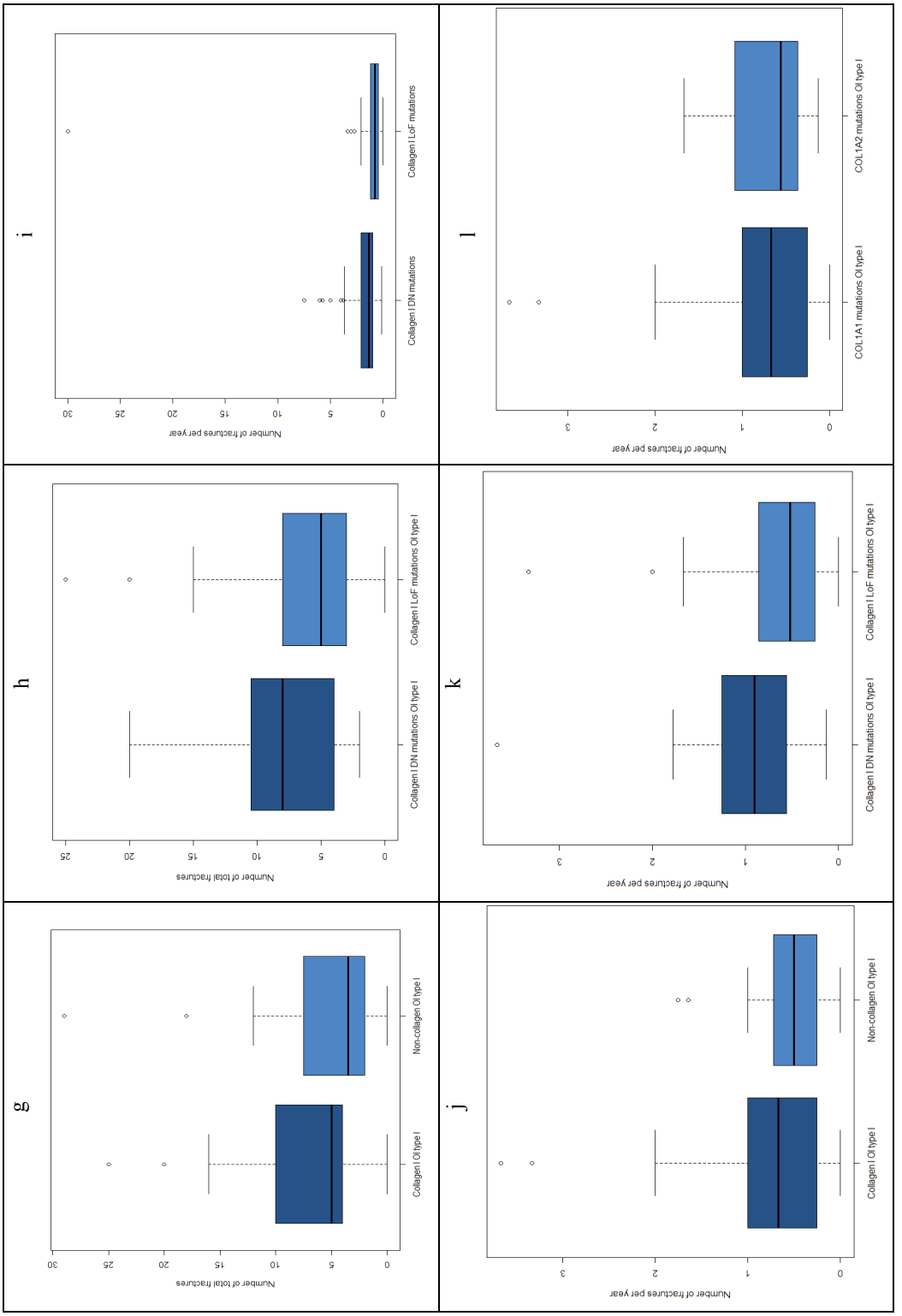
Target	Forward primer	Reverse primer	Product size (bp)
Exon 47, 48	5'-CTCCAGAGCTGGGGTTGTT-3'	5'-CAGTTCTTCTGGGCCACACT-3'	798
Exon 49	5'-ACCTCAAGAGAAGGCTCACG-3'	5'-ATGTCCCTTCTGAGCACTGG-3'	591
Exon 50, 51	5'-CTCTCTGAGGACCCTGGACA-3'	5'-TTTTTGGTCATGTTCGGTTG-3'	809
3'UTR	5'-CAAACTGAACCCCCTCAAAA-3'	5'-CCAAATCCGATGTTTCTGCT-3'	634
3'UTR	5'-ATGGCTCTTGCAACATCTCC-3'	5'-AGGGGGAAAAACTGCTTTGT-3'	706
3'UTR	5'-CCGGCTCCCTCCTAGTCT-3'	5'-CCCAGTATCCATGAGCATCC-3'	600
3'UTR	5'-CTTCACATCTGGGGTTGTCC-3'	5'-TGTCCCCCTTACCTGAGATG-3'	688
COL1A2			
5'UTR, Exon 1	5'-TGCAAATTCTGCCCATGTC-3'	5'-GAGAGTCTGCCCTCCAAGTG-3'	700
Exon 2	5'-TCCCTGCCATACTTTTGACC-3'	5'-TCAGTAGCCCCGCCTATTTT-3'	379
Exon 3	5'-TTGAATTTGAGGCATAAGTACAGG-3'	5'-TGCCTTCCATCTCCAGAATAA-3'	538
Exon 4	5'-TCCAATCCTCCAGCTGAAAA-3'	5'-CATGTGTTGGATGGGTCAAT-3'	522
Exon 5	5'-AATTTCCACCCTACTTGCACA-3'	5'-TTGACAAGGGCTCACAAAGA-3'	391
Exon 6	5'-GTGTCGGCCAAGTTTTTGAC-3'	5'-TTGATTTGCCCAAGTTATGG-3'	578
Exon 7, 8	5'-ATATCTGACCCCAGCCAACA-3'	5'-AGAAAATGGGAGACCCATCA-3'	350
Exon 9, 10	5'-TCAAGGTTTCCAAGGACCTG-3'	5'-TTGCTTATGGTATGCTTGCTG-3'	774
Exon 11, 12	5'-TTGGAGGGAAGAAGTCACTGT-3'	5'-CCATCCTTGAGGGATTTGAA-3'	853
Exon 13, 14	5'-TCTGTGTGTCTGGCATAATTGA-3'	5'-AAAGGGAAGATCAGGTGGAAA-3'	633
Exon 15, 16	5'-TGGTGAGAGAGGACGTGTTG-3'	5'-CAGTTCCAATCTTTCACATCACA-3'	693
Exon 17-19	5'-TGCTGTTTCATTATTTGCTGGT-3'	5'-ACAAGTGGAAGTCTAGATAGTGATGA-3'	633
Exon 20, 21	5'-TTCTCTTTACCTTGACCCACAAA-3'	5'-CATTGTTTCCAGGCCAATTT-3'	568
Exon 22, 23	5'-TCTAGGGGTTGGGTGAAGTG-3'	5'-TCATGCTGATAGGAGGACCA-3'	689
Exon 24	5'-AGGCAACAAACAAAAAGTCG-3'	5'-TTCCCTGCCATGTTTTTCTC-3'	379
Exon 25, 26	5'-ATCCGTGGCAGCATCATAAG-3'	5'-GGGGATGCCATCTTGAAAAG-3'	744
Exon 27-29	5'-GCTTGCAGCTAACCATCAGC-3'	5'-GGCTCATTCTCTCCATCAGC-3'	790

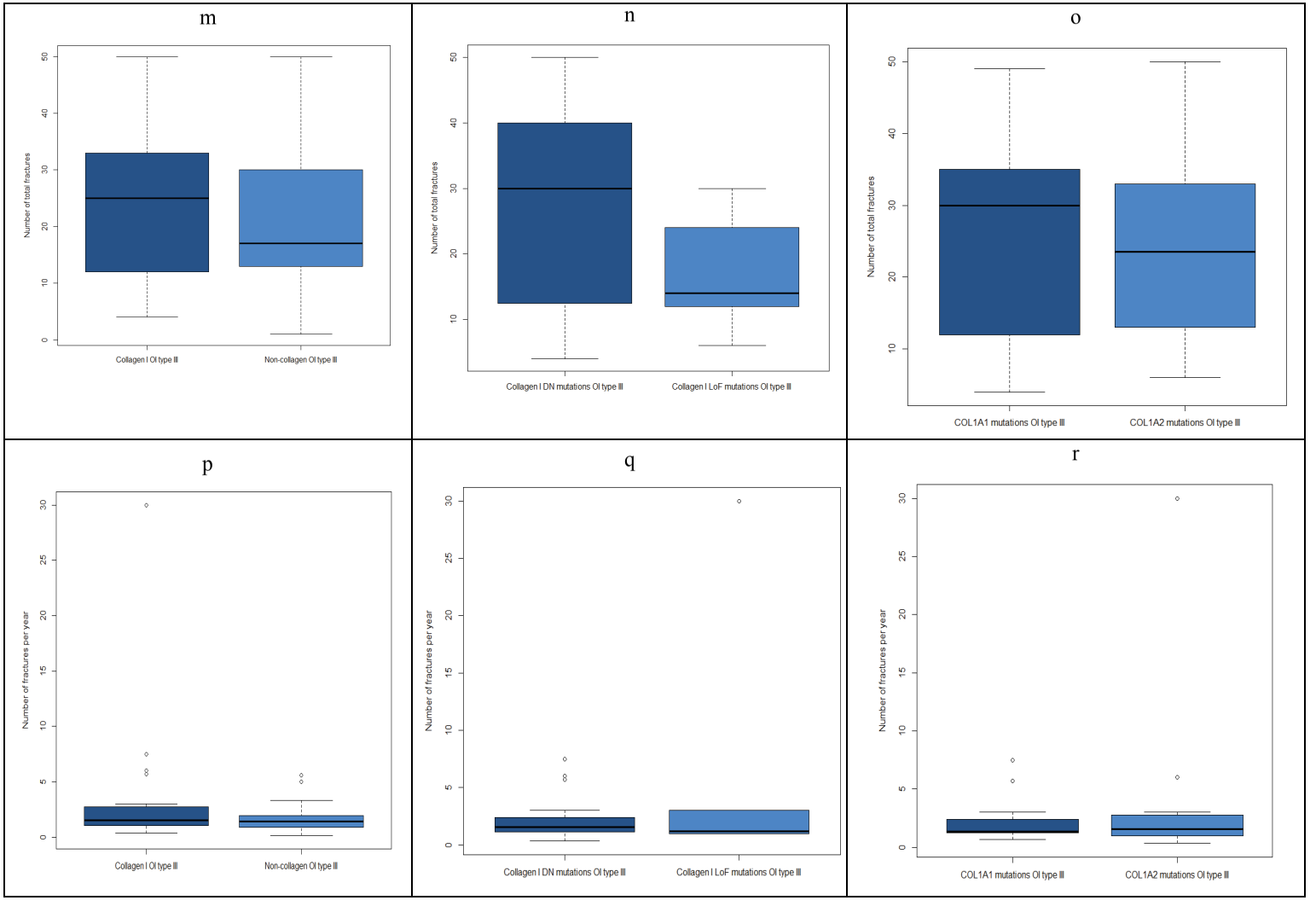
Target	Forward primer	Reverse primer	Product size (bp)
Exon 30	5’-CACTCATGTAGATACTGCCAGGTT-3’	5’-TTGAGCCACTGTCACAAATG-3’	454
Exon 31	5’-AAGGGGAATGAATTGCAAAA-3’	5’-CTCTGAATTTTCCCCCACAG-3’	586
Exon 32	5’-CAATAGCCCAGCCTTCTTTG-3’	5’-CTAATGCAGCAGCATGGTTG-3’	575
Exon 33	5’-AATCTGGAAATGGCCTTGAA-3’	5’-CACTAGGGAAGTTAGAAAATTCAGGA-3’	498
Exon 34	5’-TCCCAAATTTCTGACCTGCT-3’	5’-GGCTTTGAGAAGCCTCAGTG-3’	596
Exon 35-37	5’-TGTCTGTCCACCACTGTTCTCT-3’	5’-TTGCATAGCAGGCACTTGAC-3’	592
Exon 38	5’-AGAGATGCGGGAATGATCC-3’	5’-TTCATTTTTGGGGAGCTCTG-3’	512
Exon 39	5’-TATGGGCATTGCAACTGGTA-3’	5’-CTGAAAGGGGACTGGTGTTC-3’	503
Exon 40	5’-GGCCCTTGGTGATTAACAGA-3’	5’-GGACAGATTTCCTGGCTCAA-3’	354
Exon 41	5’-GTGCCATTGGTCTCAAGGAT-3’	5’-TTCCTAAGTGCGTGGAAAGAA-3’	573
Exon 42	5’-ACCATTGTGTGACCCATTCA-3’	5’-TTGAAAAGCCCATTCTTTGG-3’	426
Exon 43-45	5’-GAGCCTCACCAACAGCCTTA-3’	5’-CAGATGTTTTGGACTGATTCTCTC-3’	548
Exon 46	5’-TGGTGAAGTGAGTGCCATTT-3’	5’-AGTGGGGTTGAAGGACATTG-3’	417
Exon 47, 48	5’-GGAGAAAAGAGCCCCACTTTA-3’	5’-GGGTTGTCAAAGTTGTCTTGG-3’	521
Exon 49	5’-GAGAACATGCTTCCGTGTGA-3’	5’-GCTGGTTGGGAGAAGATGAC-3’	519
Exon 50	5’-AGGGGAGGGAAGGAACTGT-3’	5’-AAATTGGAACCCAGGAAAGG-3’	494
Exon 51	5’-TGACCTTGCCTCAGTCTAGTAGG-3’	5’-CCTTGGGGGCAGTCTAAGTT-3’	536
Exon 52, 3’UTR	5’-CACATGCCAAACAGTGGTTC-3’	5’-AAGGACCTCAGTTCATCTCTGTC-3’	700
3’UTR	5’-CCACTTGTGGCTTTTGAATATC-3’	5’-CACACCAAACTGCCCTTTTT-3’	643

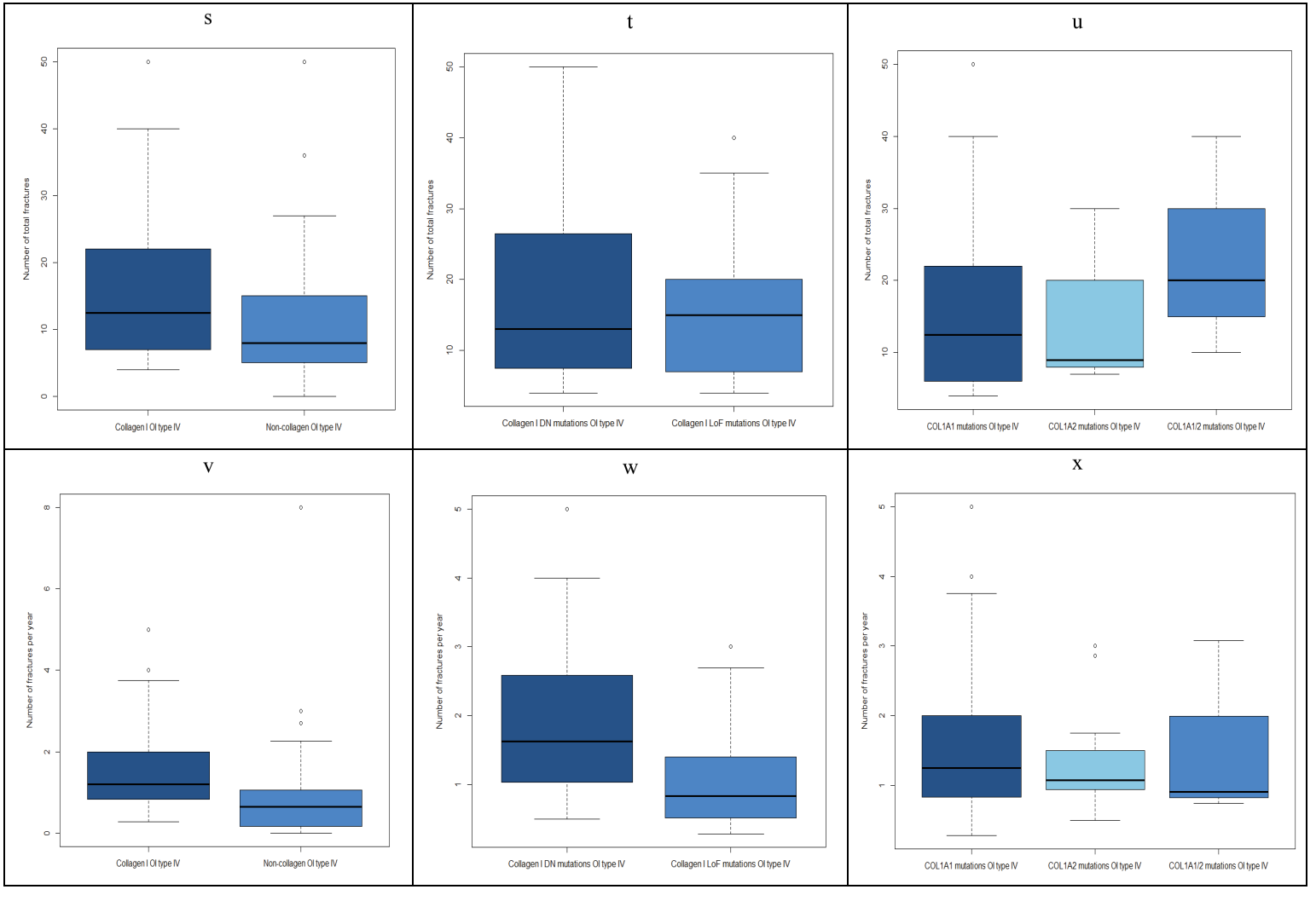
Appendix B

Figure 1. Total fractures and fractures per year in OI patients subdiveded by (a, d) genotype (collagen type I and non-collagen, (b, e) affected collagen I gene (*COL1A1, COL1A2, COL1A1/2*), (c, f) collagen defect (DN and LoF) and OI type (g–l) I, (m–r) III, (s–x) IV.




g
Number of total fractures
0
5
10
15
20
25
30
Collagen I OI type I
Non-collagen OI type I
h
Number of total fractures
0
5
10
15
20
25
Collagen I DN mutations OI type I
Collagen I LoF mutations OI type I
i
Number of fractures per year
0
5
10
15
20
25
30
Collagen I DN mutations
Collagen I LoF mutations
j
Number of fractures per year
0
1
2
3
Collagen I OI type I
Non-collagen OI type I
k
Number of fractures per year
0
1
2
3
Collagen I DN mutations OI type I
Collagen I LoF mutations OI type I
l
Number of fractures per year
0
1
2
3
COL1A1 mutations OI type I
COL1A2 mutations OI type I


m
Number of total fractures
0
10
20
30
40
50
Collagen I OI type III
Non-collagen OI type III
n
Number of total fractures
10
20
30
40
50
Collagen I DN mutations OI type III
Collagen I LoF mutations OI type III
o
Number of total fractures
10
20
30
40
50
COL1A1 mutations OI type III
COL1A2 mutations OI type III
p
Number of fractures per year
0
5
10
15
20
25
30
Collagen I OI type III
Non-collagen OI type III
q
Number of fractures per year
0
5
10
15
20
25
30
Collagen I DN mutations OI type III
Collagen I LoF mutations OI type III
r
Number of fractures per year
0
5
10
15
20
25
30
COL1A1 mutations OI type III
COL1A2 mutations OI type III


s
Number of total fractures
0
10
20
30
40
50
Collagen I OI type IV
Non-collagen OI type IV
t
Number of total fractures
10
20
30
40
50
Collagen I DN mutations OI type IV
Collagen I LoF mutations OI type IV
u
Number of total fractures
10
20
30
40
50
COL1A1 mutations OI type IV
COL1A2 mutations OI type IV
COL1A1/2 mutations OI type IV
v
Number of fractures per year
0
2
4
6
8
Collagen I OI type IV
Non-collagen OI type IV
w
Number of fractures per year
1
2
3
4
5
Collagen I DN mutations OI type IV
Collagen I LoF mutations OI type IV
x
Number of fractures per year
1
2
3
4
5
COL1A1 mutations OI type IV
COL1A2 mutations OI type IV
COL1A1/2 mutations OI type IV

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And finally, my dearest OI loved ones – my mother Larysa Zhytnik, my grandmother, Anastasia Sokolovskaya and sister, Alina Veimaer, to whom I dedicate this dissertation.

PUBLICATIONS

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2012–2014	MSc Biomedicine, Institute of Molecular and Cell Biology, Faculty of Science and Technology, University of Tartu
2008–2012	BSc Genetechnology, Institute of Molecular and Cell Biology, Faculty of Science and Technology, University of Tartu
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Employment:

2018–2019	Institute of Clinical Medicine, Faculty of Medicine, University of Tartu, Junior Researcher
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Scientific work:

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Publications:

Inter- and intrafamilial phenotypical variability in individuals with collagen-related Osteogenesis Imperfecta. L. Zhytnik, K. Maasalu, B.H. Duy, A. Pashenko, S.Khmyzov, S. Kõks, A. Märtson. Plos One (Submitted)

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Osteogenesis imperfecta – overview of a rare disorder. Experience of Estonia. (article in Estonian) Authors: Katre Maasalu, Lidiia Zhytnik, Tiia Reimand, Sulev Kõks, Aare Märtson Eesti Arst: 94 (Lisa 5), 22–27, 2015

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