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Contribution of Ho Duy Binh to these publications:

Study 1: Study design, interaction with the patients, collection of clinical data, statistical data analysis and writing the manuscript.

Study 2: Participation in the design of the study, interaction with the patients, coordinating data interpretation and drafting the manuscript

Study 3: Study design, interaction with the patients, clinical data and blood sample collection, writing the manuscript

ABBREVIATIONS

3'UTR	3' untranslated region
5'UTR	5' untranslated region
A, T, C, G	nucleotides Adenine, Thymine, Cytosine, Guanine
BiP	binding immunoglobulin protein
BMD	bone mineral density
BMP1	bone morphogenetic protein 1
COL1A1	type I collagen alpha 1 polypeptide
COL1A2	type I collagen alpha 2 polypeptide
COLFI	fibrillary collagen C-terminal domain
CREB3L1	cAMP responsive element binding protein 3 like 1
CRTAP	cartilage associated protein
CYPB	peptidyl-prolyl isomerase cyclophilin B
ddNTPs	dideoxynucleotides
DI	dentinogenesis imperfecta
DXA	dual energy x-ray absorptiometry
EDTA	ethylenediaminetetraacetic acid
ER chaperones	endoplasmic reticulum chaperones
FKBP10	FK506 binding protein 10
gDNA	genomic DNA
Gly	glycine
GRP	glucose-regulated protein
HSP	heat shock protein
IFITM5	interferon-induced transmembrane protein 5
LEPRE1	leucine proline-enriched proteoglycan 1
MLPA	multiplex ligation-dependent probe amplification
NGS	next-generation sequencing
OI	osteogenesis imperfecta
P3H1	prolyl 3-hydroxylase 1
P4HB	beta-subunit of prolyl 4-hydroxylase
PCR	polymerase chain reaction
PICP	C-terminal telopeptide
PINP	N-terminal telopeptide
PLS3	plastin 3
PLOD2	procollagen-lysine,2-oxoglutarate 5-dioxygenase 2
PPIB	peptidylprolyl isomerase B
PTH	Parathyroid hormone
SEC24D	SEC24 family member D
SERPINF1	serpin family F member 1
SERPINH1	serpin family H member 1
SP7	serine protease 7
SPARC	secreted protein acidic cysteine
TMEM38B	transmembrane protein 38B
WNT1	wingless-type family member 1

1. INTRODUCTION

Osteogenesis imperfecta, known also as a “brittle bone disease”, is a group of rare genetic disorders of bone fragility. OI estimated is around 1 person per 25,000–100,000 and is characterized by low bone mass, skeletal deformity, and growth deficiency. OI has also numerous extraskeletal features, including blue sclera, hearing loss, easy bruising, dentinogenesis imperfecta, joint laxity, and pulmonary complications (Martin & Shapiro 2009)(Basel & Steiner 2009) (Bregou Bourgeois et al. 2016). Forms of the disease vary widely with clinical severity ranging from nearly asymptomatic OI with normal stature, light osteopenia and average lifespan to recurrent fractures, skeletal deformations, profound disability, and even death (Rauch & Glorieux 2004). In 1979, four OI types (OI type I–IV) based on clinical phenotypes were identified by Sillence (Sillence et al. 1979). Current classification system is still in use today, however it was updated with three more OI types with unusual histological findings (types V–VII). Numbering does not match with severity of the types, but shows historical order of their discovery. Type I is the mildest form, which is often underdiagnosed due to very light symptoms and non-awareness of health professionals. Type II is a prenatally lethal OI form. Among survivors, type III is the most severe OI type and stands out with severe deformities and short stature. Type IV varies from mild to severe, and represents intermediate form between I and III OI types (Sillence et al. 1979) (Clarke et al. 2013).

In addition to phenotypical diversity, OI is also represented with genetic diversity. Until now mutations in more than 17 OI genes are associated with this disease. Previous studies have shown that up to 85–90% of the OI patients harbor mutations in the type I collagen alpha 1 polypeptide (*COL1A1*) and alpha 2 polypeptide (*COL1A2)* genes, located at chromosome 17q21.33 and 7q21.3, respectively. Interestingly, mutations in the *COL1A1* and *COL1A2* genes, depending on the location and type of the mutation, can cause a whole range of severity, from mild to progressive deformity and mortality forms (Marini et al. 2007) (Valadares et al. 2014). The *COL1A1* and *COL1A2* genes code for type I collagen α1 and α2 chains, respectively. Type 1 collagen is one of the most abundant protein in the human body. It is a structural component of bone, skin, tendons, cornea, and blood vessel walls as well as other connective tissues. Only 10–15% of OI mutations occur in non-collagenous genes. (Glorieux & Moffatt 2013)(Kocher & Shapiro 1998) (Van Dijk & Sillence 2014) (Glorieux & Moffatt 2013) (Shapiro 2014). However, despite the fact that there has been a significant breakthrough in OI genetics for the past few years, absence of mutations in the known OI genes in some patients is still challenging for the investigators.

Both qualitative and quantitative type I collagen defects cause OI (Marini & Smith 2015). Major amount of mild OI forms (type I) is induced by a premature stop codon in the *COL1A1/2* genes, which results in a haplotype insufficiency, underlined with a half of the normal collagen amount. However, the collagen structural sequence is not altered. Structural or qualitative defects in either of

the type I collagen chains are the cause of types II–IV, however, usually approximately 80% of them are Glycine substitutions with another amino acid (Marini & Smith 2015) (Marini et al. 2007). It was hypothesized that due to the presence of two α1 and one α2 chains in the procollagen triple helix, *COL1A1* is more susceptible to mutations as it contains more α1 chains in the collagen fibrils. *COL1A1* gene mutations cause OI more often than *COL1A2* gene mutations and tend to be more pathogenic (Marini et al. 2007).

Although genotype-phenotype correlations are great interest for all OI researchers and clinicians, connections between mutations and OI severity are still not clear and require further investigation. Information regarding OI clinical features and OI genotypes has not been studied in a Vietnamese population before. With high population density, of almost 90 million people, in Vietnam we can estimate a valuable number of OI patients. The health system in the Vietnam is organized on the basis of three levels, from the national to the community level. Health services cover emergency care, basic diagnostic procedures and therapies in the hospitals; however, advanced techniques and services are limited. Preventive health care and treatment modalities of OI traditionally have not been covered by insurance before (Tien et al. 2011). There are many hospitals that have OI patients, but focus only on the treatment of patients' fractures. Focused OI research investment in Vietnam remained still problematic. Systematic investigation of rare diseases is important for corretct diagnosis and for patient treatment and management. Are there any differences in gene defects and clinical features in OI Vietnamese patients in compare with residents of other countries? Current study revealed differences in mutation proportions of the Vietnamese OI database and the results in other countries. Our findings would seem to suggest that there might be some role of the ethnicity factor in the mutational profiles of different populations.

2. LITERATURE OVERVIEW

2.1. Normal bone structure

Bone comprises mineralized extracellular matrix and bone cells. The bone cells produce the bone matrix and replace the old matrix with new matrix. The mineralized extracellular matrix is responsible for bone characteristics (Tate 2012).

2.1.1. Cellular structure of the bone cells

Bone cells are represented with osteoblasts, osteocytes, and osteoclasts. Osteoblasts are derived from osteogenic cells, which are bone stem cells, and develop from the mesenchymal cells, located in the inner layers of the perichondrium, periosteum and endosteum. Osteoblasts produce collagen and proteoglycans, and release matrix vesicles, which play an important role in stimulation of further hydroxyapatite formation and mineralization of the matrix. Osteoblasts are also called bone building cells as they provide longitudinal and appositional growth of the bone (Tortora & Derrickson 2012) (Tate 2012) (Rizzo 2010) (Tortora & Nielsen 2012).

When the osteoblasts mature, they develop into osteocytes. Osteocytes are the main cells of the bone tissue, responsible for daily metabolism function. The osteocytes are located in lacunae; the processes of osteocytes are in canaliculi. Canaliculi allow osteocytes to maintain contact with each other, and exchange nutrients and waste with the blood (Tate 2012) (Rizzo 2010) (Tortora & Derrickson 2012).

Osteoclasts secrete an acid locally to demineralize, and enzymes (cathepsin K, collagenase etc.) to digest proteins of the bone extracellular matrix. Hence, osteoclasts are known as breakdown bone cells. They are responsible for bone resorption during normal osteogenesis process or bone repair. Osteoclasts are large cells, located in the endosteum (Tate 2012) (Rizzo 2010).

Depending on the amount of space and bone matrix, bone tissue can be divided into two different kinds: compact (or dense) bone and cancellous (or spongy) bone. Dense bone, with more bone matrix and less space, is concentrated mainly at the diaphysis of long bones. The little space it has makes it strong enough to resist outside forces and protect the bone during movement and weight bearing. Spongy bone, with more space and less bone matrix, is concentrated in flat bones, and also in epiphyses and metaphyses of long bones. The structural unit of cancellous bone is the trabecula. Trabeculae are composed of thin rods, connected together and surrounding marrow and blood vessels, which fill the holes between the rods. Such a structure increases bone flexibility, reduces the weight of the bone and protects bone marrow (Tate 2012) (Rizzo 2010) (Tortora & Derrickson 2012).

2.1.2. Bone extracellular matrix and minerals

The extracellular matrix is composed of 35% organic matter, and 65% inorganic component. The organic matter mainly consists of collagen and proteoglycans. Collagen fibers form the framework for the deposition of the mineral components. The collagen fibers are responsible for flexibility and tensile strength of the bone (Tate 2012) (Tortora & Derrickson 2012).

The inorganic component is a crystallized mineral salt, called hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. It is combination of calcium phosphate salt, $Ca_3(PO_4)_2$, and calcium hydroxide, $Ca(OH)_2$. The inorganic component also includes other salts, such as calcium carbonate, ($CaCO_3$), and four main ions: magnesium, sulphate, potassium, and fluoride. The crystallized mineral salts are responsible for the hardness and compressive strength of the bone (Tortora & Derrickson 2012) (Martini et al. 2012).

The combination of collagen and mineral salt properties creates the major functional characteristics of the bone (Tate 2012) (Tortora & Derrickson 2012).

2.1.3. Collagen type I biosynthesis

Type I collagen is the most abundant mammalian protein, found throughout the body. It is the main component of the bone, skin, tendons, cornea, blood vessel walls and other connective tissues, except for cartilaginous tissues (Makareeva & Leikin 2014) (Kadler et al. 1996). It is formed from three polypeptide chains as a heterotrimer of two identical pro-α1 chains and one pro-α2 chain. The synthesized procollagen precursor is translocated into the rough endoplasmic reticulum, where post-translation modification and folding take place. Then it is transported to the Golgi complex and secreted into the extracellular matrix where C-terminal (PICP) and N-terminal (PINP) propeptides are cleaved and collagen type I is formed. Fibrils are made by cross-linking of collagen type I molecules. Multiple fibrils are concentrated into collagen fibers, which are important contents of the bone (Makareeva & Leikin 2014) (Gelse 2003) (Van Dijk & Sillence 2014). The organization of type I collagen fibrils is shown in Figure 1.

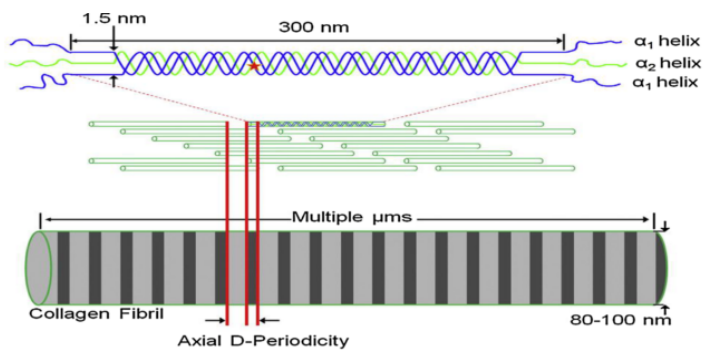

Figure 1: Organization of type I collagen fibrils (Garnero 2015)

The primary structure of collagen is characterized by the obligatory presence of glycines – the smallest amino acid – in every third position. In each α chain a repeated Gly-X-Y sequence exists. In each of the three chains, X-Y positions are occupied by any amino acid, but about 1/3 of X-positions are frequently Proline, and about 1/3 of Y-positions are frequently 4-hydroxyproline (Makareeva & Leikin 2014) (Gelse 2003) (Kadler et al. 1996) (Prockop & Kivirikko 1995) (Engel & Bächinger 2005).

The type I collagen triple-helix, with the fold proceeding from the C- to N-terminal, is unstable and slowly denatures at normal body temperature, which creates favourable conditions for the leave of C- and N- propeptides, as well as the combination of other molecules into fibers. These special properties give type I collagen its elasticity (Leikina et al. 2002) (Makareeva & Leikin 2014). Endoplasmic reticulum (ER) chaperones, binding immunoglobulin protein (BiP), glucose-regulated protein94 (GRP94), heat shock protein 47 (HSP47), FK506 binding protein (FKBP65), a complex of cartilage associated protein (CRTAP), prolyl 3-hydroxylase 1 (P3H1) and peptidyl-prolyl isomerase cyclophilin B (CYPB), appear to be required for the triple-helix folding (Makareeva & Leikin 2014) (Makareeva et al. 2011).

2.2. Collagen and malformation of osteogenesis imperfecta bone

Residues of glycine (Gly) are a component of $(Gly\text{-}X\text{-}Y)_n$ repetitions, where X and Y are random amino acids. Substitution of the Gly, positioned in the centre of the triple helix, with a different amino acid would prevent interchain hydrogen bond formation between the NH-group of Gly and the CO in the X-position of a neighbouring chain. Therefore, substitution of Gly residues will cause the helix to become bulky and unstructured (Gelse 2003) (Marini et al. 2007). In this way, it decreases the helix strength and stability, which are crucially important for protein function (Kadler et al. 1996) (Makareeva & Leikin 2014).

Osteoblasts produce collagen and noncollagenous proteins. Mutations in osteoblasts make a defective blueprint, consequently leading to production of deformed collagen and malformation of the bone. The osteoblasts contain imperfect collagen fibers, while the body demands new bone cells for development, so they are continuously involved in the formation of ineffective bone. Type I collagen is the major structural protein of bone, so the osteoblasts are responsible for the most significant part of skeletal pathophysiology in OI (Fedarko 2014) (Fedarko 2014) (Gajko-Galicka 2002).

The characteristics of OI bone are poor lamellar pattern, low trabecular volume, immature bone structure, as well as dilated endoplasmic reticulum, swollen mitochondria, and stromal calcification. Furthermore, the osteoid thickness is decreased, and the number of osteoblasts and osteoclasts are increased. OI bone demonstrates changes in mineral composition; presence of calcium, phosphorus, and stromal calcification in the bone (Baron et al. 1983) (Cassella

et al. 1996) (Fedarko 2014). The number of immature osteoblasts is increased, with evidence of a higher Rankl/Opg ratio and tumor necrosis factor (TNF) in immature osteoblasts. Osteoclast formation and differentiation stimulation is also increased (Li et al. 2010).

Normally, bone functions are maintained by a balance between the activities of osteoblasts and osteoclasts, but if osteoclasts dissolve the bone matrix faster than osteoblasts produce new matrix, bone becomes weaker. There is no difference between OI and normal bone in osteoclast quality nor quantity, but only in osteoblast deficiency. The defective collagen frame leads to an abnormal deposition of minerals and higher erosion of bone tissues by osteoclasts (Martini et al.2012) (Cassella et al. 1996).

The reduction of type I collagen in the matrix may cause diminution of the skeleton. The three main features on xray are osteoporosis, deformities and reduction in size (Cassella et al. 1996). The matrix components in affected bone include reduced collagen, osteonectin, biglycan and decorin, but increased thrombospondin, fibronectin, and hyaluronan. These changes play an important role in OI bone pathology (Gajko-Galicka 2002). Collagen fibers make a framework for deposition of mineral crystals, stick them together, and also attach to other molecules. This is essential for normal bone matrix structures and characters. Mutations lead to an abnormal quantity or quality of type I collagen, and consequently, defective collagen function, abnormal matrix bone, skeletal pathophysiology, and expression of the OI clinical phenotype. Inorganic minerals account for two thirds the weight of bone. The remaining one third is collagen fibers and other non-collagenous proteins. These inorganic components can withstand compression but are relatively inflexible. On the other hand, the collagen fibers contribute tensile strength to bone, and they sustain stretching, twisting and bending forces, but can not withstand compression. Bone is very brittle if the amount and quality of collagen are not sufficient (Martini et al.2012)(Makareeva & Leikin 2014) (Gajko-Galicka 2002) (Cassella et al. 1996).

2.3. Osteogenesis imperfecta overview

Osteogenesis imperfecta, known also as “brittle bone disease”, is a rare genetic disorder of bone metabolism. The incidence of OI worldwide varies; it ranges from 1/100,000 to 1/25,000, depending on the OI type being considered (Sillence et al. 1979) (Martin & Shapiro 2009) (Basel & Steiner 2009). OI is characterized by low bone mass, bone fragility from minimal trauma or normal weight bearing, and skeletal deformity. The variability of the condition ranges from mild osteopenia to severe deforming and lethal forms, and represents a continuum of severity. In addition to bone fragility and skeletal deformities, patients may develop secondary clinical features, such as a short stature, dentinogenesis imperfecta, joint laxity, blue or greyish eye sclerae, progressive hearing loss, and neurological and pulmonary complications. There is no evidence of an association between autosomal dominant OI with gender, race,

or ethnic group (Sillence et al. 1979) (Glorieux 2008) (Marini & Smith 2015) (Maasalu et al. 2015).

At present, nineteen different genes, connected to OI have been identified ("Switch Gene – Osteogenesis Imperfecta Variant Database – Leiden Open Variation Database"). About 90% of OI cases arise due to mutations in the *COL1A1* and *COL1A2* genes, which code for collagen type I α1 and α2 chains respectively and inherited by autosomal dominent way. The rest of cases are represented by mostly recessive mutations in the genes which are connected to collagen type I post-translational modifications (cartilage associated protein *(CRTAP),* leucine proline-enriched proteoglycan 1 (*LEPRE1),* peptidylprolyl isomerase B *(PPIB),* procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (*PLOD2),* beta-subunit of prolyl 4-hydroxylase (*P4HB*)), folding and transport (serpin family H member 1 (*SERPINH1),* FK506 binding protein 10 (*FKBP10),* SEC24 family member D *(SEC24D))*, osteoblast proliferation (serine protease 7 (*SP7),* cAMP responsive element binding protein 3 like 1 *(CREB3L1))*, osteoclast maintenance (plastin 3 (*PLS3*)), extracellular matrix formation (bone morphogenetic protein 1 (*BMP1),* secreted protein acidic cysteine (*SPARC)*) and mineralization (serpin family F member 1 *(SERPINF1),* interferon-induced transmembrane protein 5 (*IFITM5)*), or cell signaling (wingless-type family member 1(*WNT1),* transmembrane protein 38B *(TMEM38B)*) (Roughley et al. 2003) (Forlino et al. 2011) (Glorieux & Moffatt 2013) (Valadares et al. 2014) (Marini et al. 2014) (Van Dijk & Sillence 2014). Some OI cases remain genetically undiagnosed. Underlying this is the fact that OI pathogenesis and genetics are not completely known. The presence of other OI molecular defects has been hypothesized (Christiansen et al. 2010).

In 1979, David Sillence with his coworkers classified OI into four types (I–IV). Type I is mild, type II is the most severe and prenatally lethal, type III is the most severe OI type within survival groups, and type IV is an intermediate type between I and III. The initial classification, based on clinical and radiographic signs, is still in use today (Sillence et al. 1979) (Clarke et al. 2013).

2.3.1. History of osteogenesis imperfecta

Osteogenesis imperfecta is known under various names: Brittle bone disease; fragilitas ossium; Ekman, Lobstein or Vrolik disease (Seedorrf 1949) (Sillence et al. 1979) (Sillence & Lamandé 2014). The first time OI was described was in an Egyptian mummy dated by paleopathologists to be from around 1000 BC. An OI infant skeleton was identified by its thin long bones, dentinogenesis imperfecta (DI) and skull deformities such as vertical flattening and transverse widening (Lowenstein 2009). In 1674 Melabranche reported a male patient, who suffered from multiple bone fractures throughout his life. In the year 1788, Olaus Jacobus Ekman described this using the name "osteomalacia" in some cases. Edmond (1831) described some of his patients, as people with blue sclerae, hypermobility of the joints, bone brittleness and short stature. In 1883

Lobstein studied and described the nature of genetic "brittle bone disease", so OI was named after him, as Lobstein's syndrome. The term "osteogenesis imperfecta" was introduced by Dutch anatomist Willem Vrolik (1801–1863), who dissected the corpses of OI patients and described skeletal manifestations specific to this condition. Sometimes the disease was mentioned as Vrolik disease (Seedorrf 1949) (Sillence et al. 1979) (Sillence & Lamandé 2014) (Baljet 2002). From 1949 to 1977, there were many reports of pedigrees with autosomal recessive or dominant inheritance (Sillence et al. 1979).

In 1979, Sillence and colleagues published a special classification, based on the severity of OI forms. The Sillence classification is still in use for many diagnostic applications in clinical and research practice. He also supposed, that genetic heterogeneity is the cause of variable OI forms (Sillence et al. 1979). In the 1970s and 1980s, new information about heritable disorders of connective tissues became available, and was applied to study bone metabolic disorders, biologic collagen and in particular OI. There have been many advances in the study of the molecular biology, transcription and translation of collagen proteins, and the OI molecular pathology (Sillence & Lamandé 2014) (Cole 1988) (Pinnell et al. 1972). In the 21st century, after the invention of modern genetic technologies, knowledge about genetic and molecular heterogeneity of the disease expanded significantly. So far, scientists have discovered 17 genes causing osteogenesis imperfecta (Van Dijk & Sillence 2014) (Database 2015).

2.3.2. Incidence

OI occurs worldwide and the precise incidence of the disorder is unknown so far. Sillence et al. reported in 1979 a minimum incidence of 3.5/100,000 live births for OI type I, 1.6/100,000 live births for type II; and 1/68,800 live births for type III in Australia (Shapiro 2014) (Sillence et al. 1979). The frequency of each OI type, according to types of the Sillence classification, are 45% for type I, 10% for type II, 25% and 20% for type III and type IV, respectively (Martin & Shapiro 2009). The incidence of OI type II in Northern Ireland was 1.5/100,000 (Donnelly et al. 2010). The incidence in nine South American countries, from 1983 to 1987, was 0.4/10,000 births, according to the ECLAMC (Latin-American Collaborative Study of Congenital Malformations) database (Orioli et al. 1986) (Martin & Shapiro 2009). The estimated population of OI sufferers across the rest of the world is 500,000, with an incidence of 0.008% and no differences in gender or ethnicity (Shapiro 2014). The prevalence of the disorder is low. OI case reports vary between countries, but the most common reported incidence is about 1/15,000 – 1/20,000 births. The measurement of incidence has been based on the diagnosis of OI infants (Pollitt et al. 2006) (Shapiro 2014).

In the United States, the OI incidence is estimated as 1/10,000 live births, and there are predicted to be approximately 25,000 to 50,000 OI patients (Martin & Shapiro 2009). The population prevalence was 10.6/100,000 in Den-

mark (Andersen & Hauge 1989). About 400 affected people are born each year in USA. In the United Kingdom, the estimated number of patients is 3,400 individuals (coresponding to a prevalence of 6/100,000) (Table 1) (Shapiro 2014).

Table 1. Incidence or prevalance of OI in the countries

Country	Incidence or Prevalance*
American	10/100,000
Denmark	10.6/100,000*
Northern Ireland	1/100,000
Sweden	7.4/100,000*
South American	4/100,000
United Kingdom	6/100,000*

2.4. Osteogenesis imperfecta types

2.4.1. Sillence classification (Type I–IV) and phenotypes

David Sillence and his collegues, from the Department of Medical Genetics, University of Melbourne, presented an OI classification in 1979, which is still in use today. The classification is based on clinical syndromes, X-ray and genetic features of OI patients (Sillence et al. 1979) (Van Dijk et al. 2010) (Clarke et al. 2013). Sillence explored OI cases from Melbourne and satellite hospitals during the period 1955–1977 (Sillence et al. 1979). The Sillence classification divides OI into four basic types (Sillence et al. 1979) (Clarke et al. 2013) (Womack 2014). The type I unites patients with some fractures, variable blue sclerae, and hearing loss. The type II is connected to neonatal fractures and lethality before or after birth. The type III includes those patients who have intrauterine fractures, fractures during delivery or early after birth, severe deformity of rib, limbs and spine, but normal sclerae. The type IV of patients show distinct deformity of long bones, but have normal sclerae (Sillence et al. 1979) (Clarke et al. 2013) (Rimoin 1978) (Womack 2014).

OI type I. This is the most common OI type, caused by autosomal dominant mutations in one of either the *COL1A1* or *COL1A2* genes, usually due to a premature stop codon, which induces quantitative collagen type I defects; the collagen is normal but insufficient (Clarke et al. 2013) (Arundel & Bishop 2010). Due to inheritance patterns, the description of genealogical information, and systematization of phenotype manifestations of affected OI family members should be carefully assessed (Sillence et al. 1979) (Cole 1988). OI type I is the mildest OI form with a birth incidence of 1/25,000 live births (Steiner et al.2013) (Van Dijk & Sillence 2014). Sufferers are of normal birthweight and length, and rarely have multiple fractures at birth (Rimoin 1978). The number

of fractures is usually less than in the case of other types (Glorieux 2008). The first fracture in most patients usually occurs sometime in their preschool period. Incidence of fractures tends to decrease after puberty and a new wave of fractures begins in later adult years in postmenopausal women and elder men, connected to osteoporosis susceptibility circumstance (Sillence et al. 1979) (Cole 1988) (Glorieux 2008) (Riggs 1991). The fractures heal well because of normal modelling of epiphyses, metaphyses and diaphyses of long bones (Cole 1988). Skeletal deformities are usually mild, with minimal bowing of long bones and mild scoliosis due to vertebral fractures (Arundel & Bishop 2010) (Van Dijk & Sillence 2014).

Type I involves hearing loss of varying degrees, that may be detectable in infancy (Womack 2014) (Cole 1988) (Van Dijk & Sillence 2014). The majority of patients suffer from progressive hearing loss; 40% of adults have severe hearing impairment, and it starts when the patient is around 30 years old and tends to get worse (Sillence et al. 1979). Type I can be subdivided into types IA and IB depending on the presence or absence respectively of DI (Paterson et al.1983) (Arundel & Bishop 2010). Patients with DI have a significantly more fractures, as well as fractures at birth, more severe short stature and greater skeletal deformity than patients without DI (Van Dijk & Sillence 2014) (Paterson et.al.1983)..

The presence of blue sclerae or pale blueness is due to either thin sclerae or normal sclera thickness but with abnormal arrangement of the collagen molecular framework (Sillence et al. 1979) (Blumcke et al. 1972). Type I OI is linked with blue sclerae in most cases, and also easy bruising, and joint hypermobility (Sillence et al. 1979) (Van Dijk & Sillence 2014).

OI type II. OI type II is the most severe, lethal form of OI, and occurs in 10% of the OI population (Sillence et al. 1979) (Womack 2014) (Glorieux 2008). Rarely these babies survive; one-fifth are stillborn and 90% die by 4 weeks of age (Van Dijk & Sillence 2014) (Sillence et al. 1984). Type II is caused by autosomal dominantly inherited *de novo* mutations in the collagen genes *COL1A1* and *COL1A2*, or autosomal recessively inherited mutation in non-collagenous genes as *CRTAP, LEPRE1, PPIB* (Arundel & Bishop 2010) (Van Dijk et al. 2010) (Maasalu et al. 2015). Type II can be detected around weeks 18–20 during the gestation period via routine ultrasounds; images show multiple fractures, deformities and bowing of long bones, and extreme under-ossification of the facial and skull bones (Van Dijk & Sillence 2014) (Sillence et al. 1984).

The estimated incidence of type II is 1–6/100 000 live births (Sillence et al. 1979). The patients usually die at prenatal age, normally from respiratory failure due to rib fractures, short thorax, flail chest, or infection (Sillence et al. 1984).

The type II phenotype stands out with its extreme skeletal deformities, which arise from numerous intrauterine fractures of long bones, ribs and the spine (Glorieux 2008) (Arundel & Bishop 2010). The patient has weight and height reduction, because of shortening and bowing of long bones. Also, patients

develop flattened vertebrae, severe osteoporosis, a small face, accordion-like ribs, and dark-blue sclerae bone (Sillence et al. 1979) (Sillence et al. 1984). In cases of OI type II, a soft skull is present due to a lack of mineralization, and the skeletal system shows reduced cortical bone thickness and amount trabecular bone (Sillence et al. 1979) (Glorieux 2008) (Sillence et al. 1984).

OI type III. OI type III is the most severe OI type within survival groups (Arundel & Bishop 2010) (Glorieux 2008). The pattern of inheritance may be both autosomal dominant and recessive. Mutations, causing OI type III may arise in addition to the collagen genes *COL1A1* and *COL1A2,* also in recessive or so called "non-collagenous OI genes" genes (Arundel & Bishop 2010) (Van Dijk et al. 2010). The incidence of this disorder is 1/68,000 live births (Sillence et al. 1979). Radiography at birth can detect generalized osteopenia, multiple fractures, and bowing long bones (Van Dijk & Sillence 2014).

Today, the survival time is improved and patients can survive into adult life if therapeutic options are followed (Glorieux et al. 1998) (Van Dijk & Sillence 2014). OI type III sufferers usually die earlier from complications of skeletal chest wall deformity, pulmonary hypertension, and/or cardio-respiratory failure (Van Dijk & Sillence 2014) (Glorieux et al. 1998). In mild or moderate cases the mean life expectancy is similar to original population.

OI type III is connected to severe bone fragility; an enormous number of fractures arise throughout the life of the patient, beginning in utero, and lasting into the growing period. Scoliosis and kyphosis cause deformation of the rib cage; the wide rib cage overlaps the narrow pelvis (Sillence et al. 1979) (Rimoin 1978) (Arundel & Bishop 2010) (Cole 1988) (Glorieux et al. 1998). Skeletal deformity, short stature, pale blue or grayish sclerae, barrel-shaped chest with a pectus carinatum, and triangular face commonly appear in the patient (Sillence et al. 1979) (Glorieux 2008) (Cole 1988). The phenotypic traits also may include DI, progressive hearing loss, and joint hypermobility (Arundel & Bishop 2010) (Cole 1988) (Glorieux 2008). Most of the patients are immobile (able only to sit or lay down), but some of the patients are able to move with the assistance of walking devices (at a younger age) or wheelchair (Lin et al. 2009).

OI type IV. OI type IV is an intermediate type between types I and III. It has a diverse phenotype, with moderate severity, which may vary from mild to severe forms (Sillence et al. 1979) (Glorieux 2008). It is caused by autosomal dominant mutations in collagen genes *COL1A1* and *COL1A2,* and non-collagenous genes by their function as *CRTAP, LEPRE1, PPIB* (Arundel & Bishop 2010) (Van Dijk et al. 2010).

Patients develop relatively short stature, fractures can happen at birth, and their fracture number is variable. Moderate bone deformities are present (Clarke et al. 2013) (Glorieux 2008). Non-skeletal traits include normal sclerae, but they may be bluish at birth and then fade during childhood (Sillence et al. 1979) (Van Dijk & Sillence 2014), and there is the possible presence of DI and hearing loss (Sillence et al. 1979) (Cole 1988).

2.4.2. New OI classification according to genetic causes

There is a new OI classification according to genetic causes, comprising 13 OI types (Table 2). Every OI type occurs because of a mutated gene. Approximately 90% of OI cases are caused by a mutation in the *COL1A1* and *COL1A2* genes, and are autosomally dominant. These can result in OI types I–IV. The other genes, such as *IFITM5, CRTAP, LEPRE1, PPIB, SERPINH1, FKBP10, SP7, SERPINF1, BMP1,* correspond to recessive mutations, and account for 10% of all OI cases. Type V caused by the *IFITM5* gene, is characterized by hypertrophic callus and ossification of the interosseous membrane between the radius and ulna. Type VI is based on the lack of *SERPINF1,* leading to mineralization deficiencies. Type VII is caused by *CRTAP* mutations, characterized by white sclerae, small heads and short stature. Type VIII is caused by a *LEPRE1* gene mutation, leading to a cruel growth deficiency. Type IX caused by *PPIB* mutation, which affects collagen folding. Types X and XI caused by *SERPINH1* and *FKBP10* gene mutations causing the collagen to become disordered in the folding procedure. Type XII is based on an *SP7* gene mutation, with resultant abnormal osteoblast differentiation. Type XIII is caused by a *BMP1* gene mutation, with hypermobility of the joints and increased bone mineral concentration (Van Dijk et al. 2013) (Van Dijk & Sillence 2014) (Osteogenesis Imperfecta Foundation 2015) (Thomas & DiMeglio 2016).

Table 2. New OI classification according to genetic causes (Thomas & DiMeglio 2016)

Gene	Function	New classification (type)	Sillence phenotype
COL1A1/2	Structural	I–IV	I–IV
IFITM5	Bone mineralization	V	IV, V
SERPINF1	Bone mineralization	VI	III, IV
CRTAP	Collagen type I post-translational modification	VII	III, IV
LEPRE1	Collagen type I post-translational modification	VIII	II, III
PPIB	Collagen type I post-translational modification	IX	II–IV
SERPINH1	Collagen chaperon	X	II, III
FKBP10	Collagen chaperon	XI	III, IV
SP7	Osteoblast transcription factor	XII	III
BMP1	Reduction in peptidase activity	XIII	IV

2.4.3. Genes causing OI

Osteogenesis imperfecta is associated with high genetic heterogeneity. So far, mutations in 17 different genes have been found to cause OI phenotypes of different severity (Database 2015) (Van Dijk & Sillence 2014). About 90% of the mutations are connected to alterations in the *COL1A1* and *COL1A2* genes (Shaker et al. 2015) (Pollitt et al. 2006). However, with the availability of NGS methods, the number of reports about unexpected genetic causes of OI cases has significantly increased. Recent findings have proved connections between mainly recessive OI forms and non-collagenous genes, which perform collagen post-translational modifications, transport, matrix mineralization, cell signaling and development functions (Marini et al. 2014) (Valadares et al. 2014).

COL1A1 and COL1A2 genes. The *COL1A1* and *COL1A2* genes are located at chromosome positions 17q21.33 and 7q21.3, respectively (MGI 2015). These genes code for α1/α2 chains of collagen type I (Arundel 2004)(Van Dijk & Sillence 2014). Collagen type I is one the most abundant proteins in the human body. It is a structural part of the bone, skin, tendons, cornea, blood vessel walls and other connective tissues (Shapiro 2014). At present more than 1500 OI mutations have been revealed in collagen genes. The mutations in *COL1A1* and *COL1A2* genes may cause a whole range of OI severity, from mild to progressive deformity up to lethal (Marini et al. 2007) (Valadares et al. 2014).

Collagen mutations cause OI, but OI type I is caused by a premature stop codon in *COL1A1* leading to a normal collagen sequence but half the normal collagen amount. On the other hand, types II–IV are caused by structural defects in either of the type I collagen chains, usually a glycine substitution (about 80%) of another amino acid (Marini et al. 2007) (Marini & Smith 2015).

Non-collagenous osteogenesis imperfecta genes. The *IFITM5* gene codes for bone restricted interferon induced transmembrane protein-like protein (BRIL) and is located at chromosome position 11p15.5. The protein plays a role in bone matrix mineralization. The mutations in the 5' untranslated (UTR) region of the *IFITM5* gene are associated with moderate, hypertrophic callus and ossification of the interosseous membrane (Marini & Smith 2015) (Balasubramanian et al. 2013).

The *SERPINF1* gene is located at chromosome position 17p13.3 and codes for Pigment Epithelium Derived factor protein (PEDF). This protein plays an important role in structural development of bone, liver, muscle and fat tissue, via inhibition of angiogenesis (Al-Jallad et al. 2014) (Crowe et al. 2009). Mutations in the *SERPINF1* gene have been identified as the cause of moderate to severe OI situations (Crowe et al. 2009) (Valadares et al. 2014).

The *CRTAP* gene is located at chromosome position 3p22.3. It codes for cartilage-associated protein (CRTAP). Missing or a severely reduced amount of the protein causes severe to lethal OI situations (Valadares et al. 2014) (Morello et al. 2006). This protein functions as a collagen chaperone and 3-hydro-

xylates a single proline residue of the α1 chain of collagen type I (I.M. Ben Amor et al. 2011)(Morello et al. 2006). The prolyl 3-hydroxylase group is encoded by *CRTAP, LEPRE1* and *PPIB,* responsible for the hydroxylation of a single proline residue (P986) in the α1 chain (Baldridge et al. 2008) (Duran et al. 2014) (Willaert et al. 2009).

The *LEPRE1* gene is located at chromosome position 1p34.1, and codes for Leucine proline-enriched proteoglycan (leprecan) 1 or Prolyl 3 hydroxylase 1 (P3H1/LEPRE1). P3H1 is needed for proper collagen synthesis and assembly, and is identified as the cause of severe to lethal OI situations (Moul et al. 2013).

The *PPIB* gene is located at chromosome position 15q22.31, codes for Cyclophilin B (CyPB) (MGI 2015). In the formation of a triple helix, CyPB takes the prolyl-containing peptide bonds, identified as having a role in folding, stability and secretion of procollagen (Willaert et al. 2009). The mutations in *PPIB* causes a severe to lethal forms of OI (Valadares et al. 2014).

The *SERPINH1*gene, located at chromosome position 11q13.5, codes for Heat Shock Protein 47 (HSP47) (Christiansen et al. 2010). The collagen chaperone-like protein HSP47 plays an important role in recognizing and maintaining the folded state of the type I procollagen trimer (Duran et al. 2014) (Macdonald 2001). The mutations in *SERPINH1* lead to a severe OI (Valadares et al. 2014).

The *FKBP10* gene, located at chromosome position 17q21.2, codes the type I procollagen chaperone FKBP65 (Duran et al. 2014) (Barnes et al. 2012) (MGI 2015). This protein functions as a type I procollagen chaperone, specialized in folding (Alanay et al. 2010) (Lapunzina et al. 2010). *FKBP10* is identified as the gene causing progressive deformity, and contractures (Valadares et al. 2014) (Barnes et al. 2012) (Alanay et al. 2010).

The *OSX/SP7* gene*, which is* located at chromosome position 12q13.13 (MGI 2015), and encodes an osteoblast-specific transcription factor (SP7/osterix) (Lapunzina et al. 2010) (Shaker et al. 2015). This protein has a special function as a transcription factor in osteoblast differentiation and bone formation (Peng et al. 2013) (Nakashima et al. 2002) (Database 2015). *OSX* is identified as a gene causing moderate OI (Valadares et al. 2014).

The *BMP1* gene, located at chromosomeposition 8p21.3 (Database 2015), codes for bone morphogenetic protein 1 (BMP1). BMP1 has functions in mature collagen type I, the proteolytic processing of the procollagen I C-propeptide (Asharani et al. 2012), and cleavage of the proα(I) C-terminal propeptide (PICP) (Valencia et al. 2014)(Hopkins et al. 2007). The mutation in *BMP1* is the cause of severe OI.

The *TMEM38B* gene*,* is located at chromosome position 9q31.2 (MGI 2015), codes for trimeric intracellular cation channel type B (TRIC-B). TRIC-B conducts calcium flux from intracellular stores and in cell differentiation. Mutations in *TMEM38B* lead to variable OI severity (Rubinato et al. 2014) (Yazawa et al. 2007).

The *WNT1* gene, located at chromosome position 12q13.12, codes for Wingless-type MMTV integration site family member 1 (MGI 2015). WNT1

has crucial functions in bone remodeling processing, osteoblast differentiation and bone formation, and is required for normal bone homeostasis (Keupp et al. 2013) (Laine et al. 2013) (Pyott et al. 2013). *WNT1* is identified as the cause of early onset osteoporosis and causes variable severity of OI (Laine et al. 2013).

The *SPARC* gene, located at chromosome position 5q33.1, codes for secreted protein acidic rich cysteine (SPARC). This is a glycoprotein that binds to collagen type I and other proteins in the extracellular matrix (Valadares et al. 2014) (Mendoza-Londono et al. 2015) (Kos & Wilding 2010). The mutation is the cause of severe OI (Mendoza-Londono et al. 2015).

The *CREB3L1* gene, which is located at chromosome position 11q11 (MGI 2015), codes for old astrocyte specifically indused substance (OASIS) (Symoens et al. 2013). OASIS has an important role in the activation of *COL1A1* transcription, and in facilitating the secretion of matrix proteins (Symoens et al. 2013) (Murakami et al. 2009).

2.5. Diagnosis of osteogenesis imperfecta

OI is a rare disease. Underdiagnosis or delay in diagnosis can be common in newborns or children. Diagnosis is based on detailed history, as well as clinical examination and investigations. However, diagnosis can be difficult in cases without history of OI in the family, and in cases with mild symptoms, and also when affected adults have no fracture history (Biggin & Munns 2014). Depending on the age, we can apply the appropriate diagnostic methods. Intrauterine diagnosis can be reliable in cases with family history of OI, investigations can be conducted after 16th week of gestation using ultrasound and/or DNA analysis. Diagnosis at birth can be made when these are fractures and short curved limbs, blue sclerae, also imagines of osteoporosis and wormian bones in radiography. A prominent symptom for OI in toddlers is long bone fracturing. During childhood and adolescence OI should be suspected when there are symptoms of repeated fractures with minor injury and deformity at long bones (Cole 1988). With early accurate diagnosis an appropriate care plan can be made, which helps to prevent complications, and improve quality of life for OI patients and their families.

2.5.1. Medical History

A precise and detailed history is essential for the diagnosis of OI. The important thing is to identify the relevant aspects of fractures; their timing, number, mechanism, frequency and location. History of dislocation should be explored, for shouder, hip, or radial head. When children start to walk, comparison with normal children helps in assessing the delay in motor development. Information regarding activities and capacity for exercise during school years should be obtained, including how long the children can walk, and periods of any immobi-

lization. OI encompasses a group of heritable disorders, so family history is important to know. Attention should be paid to: short stature in adulthood, fractures, dislocations, chipped teeth, early onset osteoporosis, and hearing loss. As well as any consanguinity, or previous miscarriage should be asked (Sillence et al. 1979) (Arundel & Bishop 2010).

2.5.2. Clinical signs

Typical clinical skeletal signs of OI are bone fragility, long bone deformations and short stature. There are also several extraskeletal features common to OI. These are blue sclerae, DI, and hearing loss.

The long bone fractures obtained during life is the most important clinical symptom. The short bones have smaller load stress so they fracture less than long bones (Zeitlin et al. 2003) (Lin et al. 2009). Such fractures are also caused by osteoporosis, which develops in most OI cases (Van Dijk & Sillence 2014). Long bone fractures can reveal the following potential problems: shortness, broadness, crumpling, angulation, and numerous fractures of long bone (Sillence et al. 1984). The imperfect subsequent healing following repeated fractures and weight-bearing are important causes of deformities in long bone. The pronation and supination of the forearm is limited in some cases, due to calcification of interosseous membranes, which usually happens between two bones – radius and ulna. Calcification of interosseous membranes may cause non-traumatic dislocation of the radial head. In the fracture location there may present hypertrophic callus (Arundel & Bishop 2010) (Glorieux 2008).

Bone deformities are common and most frequently observed in long bones. Bowing and angulated deformities exist to varying degrees in OI sufferers, with frequent over-modeling of the shafts of long bones (Van Dijk & Sillence 2014). In addition to long bone deformities occurring due to imperfect bone development or fracture healing problems, also weight bearing can cause leg bowing (Moriwake & Seino 1997) (Moriwake & Seino 1997).

Short stature is one of the cardinal features of OI. Endocrine evaluation of the growth axis is normal in most patients with collagen defects; however, about 50% of children had a blunted response to the IGF-1 stimulation test. Given the chondroosseus manifestations of recessive OI, it is reasonable to speculate that the short stature of dominant OI may be related to abnormalities at the transition from cartilage to bone. It is caused by abnormalities in the formation of bone from the growth cartilage, while there is no abnormalities in growth hormon secretion (Forlino et al. 2011). Mildly-affected persons tend to achieve a normal height, but severely-affected patients tend to be of short stature (Biggin & Munns 2014). Clinically, there is disproportion between head and body, and between upper and lower limbs. Head circumferences are often bigger than in non-OI-affected people of the same age. Clinically the short stature shows the severity of the disease; shorter is more affected (Sillence et al. 1979).

The short stature of OI patients can be also induced by deformities of the spine and compression fractures (Wekre et al. 2014). Spine deformity is also common. Spinal assessment should be conducted in both a standing and bent forward position, with scoliosis or kyphosis present as clinical evidence (Arundel & Bishop 2010). Mutation of collagen type I causes hypermobility of the spinal ligaments, which in conjunction with the weak bone can create a spinal deformity (Lubicky 2012) (Engelbert et al. 2003). On the other hand, multiple vertebral fractures also cause deformity. In severe cases, the combination of multiple rib fractures, semicontinuous beading from fracture calluses along each rib, and spinal deformity are responsible for respiratory disorders (Van Dijk & Sillence 2014) (Sillence et al. 1984) (Biggin & Munns 2014).

Skull assessment in OI infants may indicate wide cranial sutures. In severe cases, the skull vault can be extremely soft with multiple Wormian bones in the posterior skull, and the head may not be in proportion with the short body stature. About 30% of OI patients appear to have a triangular face shape. This is caused by temporal bossing and a large head (Arundel & Bishop 2010) (Sillence et al. 1979).

Blue sclerae are common in patients under 6 months They tend to last long in types I and III OI, and tend to fade in type IV with age (Arundel & Bishop 2010) (Sillence et al. 1979). The abnormal structure of the sclerae is caused by thinner collagen fibers, with diameters 30% less than normal fibers. The collagen fibers in OI have no typical cross striations, and are more densely packed.The increased transparency in the sclera and diffraction through abnormal sclera causes a blue hue in affected persons (Sillence et al. 1979) (Blumcke et al. 1972).

Dentinogenesis imperfecta (DI) is commonly revealed in association with OI. DI is caused by the Dentin Sialophosphoprotein gene (*DSPP*), which codes for main non-collagenous proteins, and for a growth of hydroxyapatite crystal (Molla et al. 2014) (Surendra 2013). Dentinogenesis imperfecta is a genetic oral disease and it was probably first recognized by Barret in 1882. The term, ‘dentinogenesis imperfecta’ was coined by Robert and Schour in 1939, as a disorder of tooth development problem. This disorder involves discolouration of shell-like teeth; they may be yellow, brown or opalescent gray, and translucent. The enamel is characterized by hypoplastic or hypocalcified defects in one third of cases. The dentin matrix is altered by interglobular calcification (Surendra 2013) (Sapir & Shapira 2001). Malocclusion, which is classified based on the relation between maxillary and mandibular first molars, is also observed in OI patients. It is due to a deficiency in the maxilla and not in the mandible growth. The maxillary bone is less developed antero-posteriorly, which results in midface hypoplasia (Schwartz & Tsipouras 1984).

Hearing loss is a common clinical symptom in OI. There are many reasons for hearing loss in OI patients. It can be caused by abnormal bone formation of the cochlea and surrounding structures. The atrophy of cells, vascular structures, and bone in the cochlea, or skull fractures can also cause of sensorineural hearing loss. Conductive hearing loss is caused by stapes fixation and a mutation in the

COL1A1 gene. There is no relationship between the degree of hearing loss and mutation type or gene (Swinnen et al. 2009) (Pillion et al. 2011).

The hypermobility of joints is as common clincal sign in OI. It is caused by marked ligamentous laxity, mainly in the metacarpophalangeal and interphalangeal joints, hyperflexion of the thumb, and hyperextension of the fingers are also common. Hypermobility can be seen in elbows, knees, and in flexion of the spine. Incidence between the OI types is not significantly different, but it occurs more often in children, than in adults (Sillence et al. 1979) (Brizola et al. 2014).

As type I collagen is a main component of skin, and plays an important role in skin manifestation, and strength of the skin. It is estimated that 70 to 85% of the skin is made up of collagen. Therefore a deficiency in the synthesis of type I collagen will lead to a change in the mechanical properties of skin. Substitutions of amino acids leads to a triple helix that is less stable than normal. The changes of quantitative and qualitative type I collagen lead to a reduction in tensile strength of the skin. This is explained by the reducible hydroxyproline contents of the skin. The thickness of the dermis of affected individuals does not vary from normal skin but is typically stiffer and less stretchy than normal skin (Brizola et al. 2014) (Boot et al. 2006) (Oxlund et al. 1985).

2.5.3. Investigations

X-ray. Radiographs are not pathognomonic features of OI, but raise suspicion for the diagnosis. Plain radiography of the long bones shows the features of cortical bone thinning, bone fractures, fracture healing and deformities. Radiographs reveal features of osteopenia and significant reductions (about 30 to 50%) in calcified bone mass. Radiography of the skull can reveal a prominent occipital region, a flattening of the cranial vault, or multiple Wormian bones (Semler et al. 2010) (Renaud et al. 2013). On the other hand, depending on OI type, radiography can show features of healing fractures, hypertrophic callus, ossifications of the interosseous membrane between tibia and fibula, and ulna and radius, congenital dislocation of the radial head, popcorn calcifications, dense metaphyseal bands and acetabulum protrusion (Renaud et al. 2013) (Anticevic et al. 2002).

Dual-energy x-ray absorptiometry. Dual-energy x-ray absorptiometry (DXA) is a precise technique used in bone density investigation, and in long-term monitoring of patients. It provides quantification of bone mineral content (BMC) at specific skeletal sites, and a bone mineral density (BMD) measurement. DXA is used at the lumbar, the cervical spine and the intertrochanteric regions of the proximal femur. BMD has no significant differences across OI types, but is significant with respect to fracture susceptibility; low BMD parameters prognosticate an increased risk of fracture (Anticevic et al. 2002) (Wekre et al. 2011) (Melton III et al. 1993).

Quantitative computed tomography and Magnetic resonance imaging (MRI) have no regular use in OI diagnosis, but sometimes it can be useful in differential diagnosis (Arundel & Bishop 2010).

Ultrasound. In cases of antenatal diagnosis, ultrasound has a regular role in the diagnosis of OI and it usually done during the second trimester of pregnancy. The features found this way are abnormalities of the skull, the rib cage, the spine and the limbs. The decreased echogenicity is caused by insufficient mineralization. The deformities are related to fractures, callus formation, increased bone plasticity, and micromelia, especially of the femur (King & Bobechko 1971) (Bulas et al. 1994). Although leg length is normal, bowing of the long bones, such as the tibia and femur, can be identified on fetal ultrasound at 22 weeks gestation to diagnose OI type I in the prenatal setting (Chen et al. 2012). Ultrasound is an important tool for both diagnostic and monitoring purposes in OI patients, as it easily identifies disturbed bone mass and sructure in these patients (Cepollaro et al. 1999).

Bone markers. Bone biochemical parameters are unspecific for investigation of OI, but are widely use to monitor on the effect of treatment. Serum levels of 25-hydroxyvitamin D, PTH, and ionized calcium are found to be normal. In OI osteocalcin and alkaline phosphatase are a proteins produced by osteoblasts. The results have higher levels of osteocalcin and alkaline phosphatase in serum in OI sufferers, even though not significantly different from those in a normal group (Cepollaro et al. 1999) (Shapiro & Brennen 2014). The other bone markers do not provide evidence for OI diagnosis (Arundel & Bishop 2010) (Wekre et al. 2011). Clinically, the concentration of the C-terminal telopeptide of type I collagen (PICP) and N-terminal (PINP) propeptides can reflect the synthesis of type I collagen, or osteoblast and osteoclastactivity. During procollagen is secreted outside the cell and PINP and PCIP are cleaved; these cleavages are assessed by measuring PCIP in-plasma concentration and PINP in-urine concentration. If PINP concentration is lower than normal, it suggests type I collagen synthesis is occurring at a lower rate, that PICP concentration is low, and also decreasing osteoblast action. PICP is significantly reduced in OI patients (Cepollaro et al. 1999) (Shapiro & Brennen 2014).

2.5.4. Genetic diagnosis of OI

Genetic testing is the best option to confirm diagnosis for OI. It shows the severity of the disease and gives the reccurrent risks for other family members and helps in prenatal testing. As a rare Mendelian disorder with extreme genetic heterogenity, identification of OI mutations in patients may present some difficulties. In the past, OI was proposed as a defect of collagen type I genes. However, recent discoveries of new OI-associated genes shifted this paradigm. So far, 17 OI-related genes, including *COL1A1* and *COL1A2*, with hetero-

zygous mutations, which are involved in about 90% of OI cases, have been detected (Van Dijk et al. 2013) (Dalgleish 2017). More new OI mutations and genes keep being reported.

There are guidelines for laboratory diagnosis of OI. In 2012, Van Dijk, Dangliesh et al. published a workflow for OI molecular genetic diagnosis based on modern OI genetic discoveries (Van Dijk et al. 2012). The suggested strategy for genetic testing in OI is following: firstly, all exons of collagen type I genes *COL1A1* and *COL1A2* are sequenced. Primers should be designed far away from intron–-exon splice sites in order to allow identification of pathogenic variants in these regions. Then duplication and deletion of the exons, or the whole allele, is tested with Multiplex Ligation-Dependent Probe Amplification (MLPA) or Quantitative Real Time Polymerase Chain Reaction (qPCR) analysis. In cases involving the absence of genetic causes of OI in the collagen type I gene, analysis should be followed by the sequencing of recessive OI-related genes (Van Dijk et al. 2012).

There are many methods for OI mutation discovery. Techniques differ with power, accuracy, cost effectiveness and time consumption, revealing unique advantages and limitations. The following methods can be usued: qPCR, High Resolution Melting, MLPA, linkage analysis, homozygosity analysis, Sanger and Next Generation Squencing. From these last two methods are mostly use in OI genetic testing.

Sanger sequencing In 1977 Sanger presented a method for DNA sequencing by termination with dideoxynucleotides (ddNTPs). The current method is based on usual DNA synthesis by the polymerase enzyme; however, in the reaction, in addition to deoxynuleotides (dNTPS), ddNTPs are added, which lack the 3'OH end and block formation of the phosphodiester bond, and thus stop the synthesis of the DNA strand (Sanger F 1977). The ddNTPs are labelled fluorescently with dyes of different wavelengths. Capillary electrophoresis separates strands according to their length. The fluorescence signal is detected and allows differentiation of base pairs (Darst et al. 2010) (Smith et al. 1986). The due-terminator read represents a fluorescent peak chromatogram. The Sanger method allows sequencing up to 398 bp. The accuracy of this approach in close to 99.9% (Anasagasti et al. 2012).

Next Generation Sequencing. Next Generation Sequencing (NGS), was designed as a rapid method, to enable sequencing of the whole exome. Since NGS became available, more than 100 genes of different Mendelian disorders have been discovered (Rabbani et al. 2012). Exons make up only 1% of the whole genome (Kumar et al. 2009). Like other monogenic diseases, OI mostly alters coding regions, thus exome sequencing is the most appropriate technique for detection of new disease-related genes and mutations. Exome sequencing kits cover not only exons, but also flanking intron and untranslated (UTR) regions, promoters, miRNA genes and non-coding RNAs, which have been proved to influence diseases (Bamshad et al. 2011). A lot of recent OI genes

and mutations have been discovered with exome sequencing (Becker et al. 2011) (Cho et al. 2012) (Pyott et al. 2013) (Maasalu et al. 2015).

Based on availability of patient data, exome sequencing strategies include sequencing and filtering of unrelated affected individuals, affected individuals from a single family, parents-child trio analysis, or an extreme phenotype approach. Filtering minor allele frequency for rare disorders, including OI, minor allele frequency must be less than 1%. Variant pathogenicity and conservation should be also taken into consideration (Ku et al. 2011) (Bamshad et al. 2011).

SOLiD® exome sequencing is an approach which allows sequencing of target genome regions, based on an in-solution hybridization method (Anasagasti et al. 2012). The method combines both high accuracy and throughput. Firstly, the DNA is restricted with various enzymes in order to create a shotgun library. Next, adaptors are flanked to randomly cleaved DNA. To eliminate off-target material, probes are hybridized with biotinylated nucleic acids. Streptavidin coated magnetic beads connect with biotin and are then washed (Bamshad et al. 2011). At the end of the process, massive parallel sequencing takes place. Probes allow sequencing of a multiple number of samples at the same time with bar-coding (Ku et al. 2011).

2.6. Experience of OI research and medical system description in Vietnam

2.6.1. Health care system description in Vietnam

Vietnam's total length from north to south is 1,650 km, lying on the eastern part of the Indochinese peninsula. Vietnam is a strip of land shaped like the letter "S". The mainland territory is 331,212 km^2, comprising 63 cities and provinces, which are divided into 3 regions: North, Central and South. According to World Population Prospects, the Vietnam population has reached over 90 million people. The birth rate has reached 16.56 births/1,000 population, and the sex ratio at birth is currently 1/ 1.12 male/female (United Nations Department of Economic and Social Affairs 2014) (GOV.UK 2015).

The health system is organized on the basis of three levels, from the national to the commune level. Firstly, regarding the national level, the Ministry of Health (MoH) is responsible for executive decisions and management of the entire system. Secondly, there are 63 provincial health bureaus in the country. Each bureau implements MoH policies and manages directly their local medical network. Finally, the primary level of health network includes district health centres, commune health stations and village health workers. There are 10,866 commune health stations covering nearly all communes in the country (Tien et al. 2011) (Oanh et al. 2014) (WHO Asia Pacific Observatory on Health Systems and Policies 2015).

The public system still plays a key role in the health care system, including hospital, primary health care, prevention medicine and population monitoring and family planning. With a mixed public–private hospital system, private hospitals account for 8% of hospitals nationwide (StoxPlus 2012). Health insurance was held by 67% of the population in 2012, and the plan is to make that 100% by 2020. There are full government subsidies (100%) for those who are very poor, disabled, widiws, veterans and children under six years of age (Tien et al. 2011) (WHO Asia Pacific Observatory on Health Systems and Policies 2015). Health services cover emergency care, diagnosis and basic therapy in hospitals, and high-tech medical services are limited. Fracture and low bone mass prevention therapy for OI patient are not been covered by insurance (Tien et al. 2011).

2.6.2. Osteogenesis imperfecta Vietnamese overview

With the incidence of OI estimated to be 1/100,000 to 1/25,000 (Basel & Steiner 2009) (Martin & Shapiro 2009), we could extrapolate the number of OI patients in Vietnam, based on its population of 90 million. Information regarding OI clinical features and gene mutations is lacking in Vietnam. Due to economic barriers, the laboratory system for OI diagnosis has not been developed in Vietnam yet. In keeping with the culture of the Orient, OI patients live with their family, and do not get adequate medical care, sustainment or co-uncelling from the medical community. Vietnam does not yet have an OI patient database. However, we have some initial data from Hanoi and Ho Chi Minh city – the biggest cities in Vietnam – about clinical experience and treatment of OI with bisphosphonates. Patients in Vietnam are often initially diagnosed upon presentation to the hospital after sustaining a fracture.

The clinical diagnosis and management of patients with OI in Vietnam are limited due to lack of diagnostic and therapeutic tools, and due to lack of specialists for early prenatal/neonatal screening. Importantly, Vietnam does not have a comprehensive programme for OI screening (DNA, RNA blood test for family members) or early detection (chorionic villus sampling). It is necessary to develop an OI research project in Vietnam, create a primary patient database, and to begin both prenatal and neonatal screening for earlier patient identification. A good care plan will require the involvement of the patient, family, medical and nursing staff, and the community. Systematic investigations into OI started in Vietnam in 2013. The main aim of this research is to create an OI database in Vietnam, describe genealogical information, systematize phenotype manifestations of affected OI family members, and identify mutations of OI using Sanger sequencing.

The results of this study demonstrate that OI has become a challenge in Vietnam. Vietnamese OI patients still suffer from a high number of fractures and deformations, most likely due to a lack of medical consulting, as well as economic difficulties. During this study, the worldwide OI genetic database has

been broadened with Vietnamese samples. Comparison of OI mutations in the new Vietnamese database with OI database other countries gives us a better understanding of OI genotype differences between populations.

OI early detection and possible prevention should be addressed, particularly in pedigree groups with a high risk of OI. A more dynamic investigation and the availability of an efficient medical service should be implemented in order to improve the quality of life of OI patients and their families in the future.

2.6.3. Previous osteogenesis imperfecta research experience

Previously very little was known about OI in Vietnam.There are some pilot research projects about OI at the Center for Gene and Protein at HaNoi Medical University, but this is limited to the *COL1A1* gene and a few samples (Hanoi Medical University 2016).

There are not so many OI research projects in Vietnam, and most of them only focus on the clinical signs (Dung et al. 2013). In these projects the patients are diagnosed, monitored and treated in the National Hospital of Pediatric, HaNoi City. For OI disease, there is close collaboration between this hospital and the OI club. The OI Club of Hanoi is a volunteer organization of families of OI patients, enabling their sharing of experiences and mutual support, while promoting health education, minimizing OI-related complications and improving quality of life, as well as offering something in the way of rehabilitation and treatment. Their research focuses on evaluating the results of treatment with bisphosphonates.

OI is a rare disease and there is no focused research investment in Vietnam. There are many hospital in the south of Vietnam that receive OI patients, but also to other hospitals focused treatment for patients with fractures. This is especially the case with the OI Booming Diamond Center, Ho Chi Minh City, where nearly 20 affected individuals regularly board at the center. Here they combine traditional medicine with medical treatment and care. They produce a traditional medicine, which is an organic material extracted from crocodile bone, which is used in the confidence that it will help the body increase the production of collagen I. So far, there are many patients following this method. The research conducted on this is also limited, and has focused on evaluating the results of treatment with traditional medicine.

2.7. Summary

The estimated incidence of OI is 1/100,000 to 1/25,000 (Basel & Steiner 2009) (Martin & Shapiro 2009), but there is little information about OI patients in Vietnam, a country with more than 90 million population. Also, basic information regarding clinical features and gene mutations of OI is lacking in Vietnam. In keeping with the culture of the Orient, OI patients in Vietnam live with

their family, and do not get adequate medical care, sustainment or counseling from the medical community. There is no systematic recording of OI patient clinical information. Patients in Vietnam are often initially diagnosed upon presentation to the hospital after sustaining a fracture in the community.

Meeting with OI patients with very different clinical pictures raised some questions: how many OI families do we have in total in Vietnam, and are there any differences in clinical features or gene defects in Vietnamese OI patients compared to those in other countries?

3. AIMS OF THE THESIS

The overall aim of the present thesis was to collect information about patients with OI in Vietnam, to investigate their clinical manifestations and genetic causes in order to improve the diagnosis and treatment of OI this population.

The specific aims of the thesis were:

3.1. To describe the clinical features of patients with OI, and the distribution of different type of OI in Vietnam

3.2. To describe the dental occlusal features in patients with OI

3.3. To describe the mutations of the *COL1A1* and *COL1A2* genes in Vietnamese patients with OI

4. METHODS AND MATERIALS

4.1. Study group

4.1.1 Data collecting

We performed a cross-secetional study beginning in September 2013 and concluding in April 2016 aiming to identify families possibly afflicted with OI. We requested information about OI patients from all hospitals and medical centers in Vietnam and got feedback from these institutions in 34 out of 63 of Vietnam's provinces. Eleven provinces are defined as border zones and due to geographical reasons are not easily accessible; therefore, they were not included in the study.

Lists of OI patients were collected from hospitals and OI clubs. We then connected directly with each OI family to introduce the purpose and ask content of the study. One family declined examination and blood testing and was not included to the study group. A group of experienced Estonian and Vietnamese doctors visited patients' homes and presence of OI clinical features was determined by them. Two families who did not meet OI clinical criteria were excluded.

Finally, in the study group we included 426 individuals from 120 OI families, comprising 146 OI patients and their close relatives.

4.1.2. Registration of genealogical information

The data we collected from the families included genealogical and clinical information which was all registered in our new database. Interviews with OI families were conducted in order to record genealogical information for a minimum of three sequential generations. Genealogical information included OI history and family consanguinity data. We gathered information about both healthy and affected family members. The history of miscarriages and pregnancies was also reported. A pedigree chart for each family was constructed.

4.1.3. Osteogenesis imperfecta phenotype description

To investigate clinical signs of OI patients, all patients underwent clinical and physical examinations. Medical history was confirmed through a review of their medical records. Cases were described according to the Sillence classification (Types I–IV).

Clinical information, medical history, health problems and treatment associated with OI were recorded from patients medical records or as stated by patients. Each patient was examined for skeletal and extraskeletal signs of OI, and radiology records, if available, were reviewed. Diagnosis and OI type were

confirmed based on observed clinical features and radiological findings. Phenotype description was based on:

a. Patient information and/or medical records, *i.e.* birth data (height, weight, intrauterine and birth fractures), fracture history (time and location of the first fracture, total number of fractures and location of fractures). Hearing was not tested, but information regarding hearing problems was collected from patients and their relatives.
b. Examination of the patient includes skeletal deformations, non-skeletal OI features (hearing loss, presence of DI, sclera colour) and degree of physical mobility. Deformities of the skeleton were assessed by observation and palpation. Scoliosis, kyphosis and mobility of the spine were assessed in different positions, including with the patient bending forward. Weight (kg) and height (cm) were measured during examinations.
c. For occlusal features the data were collected from OI persons in five medical centres in Vietnam. One hundred fourteen OI persons participated; among them, 62 persons were under 11 years old, 26 were aged from 12 to 16 years, and the others were in adult age group. In this study, 12–16 years old children (n = 26) were selected because permanent dentition of this age group could establish a stable occlusal relationship and eliminate the bias of the visible missing teeth variable. Focus was only on analyses of malocclusion of OI persons in this study. The control group consisted of 400 participants, including two hundred 12-year-old school children and two hundred 18-year-old University students. School children were randomly selected from five Vietnamese primary schools. Univeristy students were randomly selected from Danang University of Medical Technology and Pharmacy, Vietnam.

4.2. Genetic studies

We collected blood samples from 426 individuals – patients, their parents, siblings, and close relatives for genetic analysis. Genomic DNA was extracted from EDTA-preserved blood according to standard high-salt extraction methods. It was stored at −80°C, and analyzed using Sanger sequencing.

DNA samples were amplified using a polymerase chain reaction (PCR) with 25 specially designed primer pairs covering the following: 5'UTR and 3'UTR regions and 51 exons of the *COL1A1* gene; 36 primer pairs covering the 5'UTR and 3'UTR regions and 52 exons of the *COL1A2* gene. The PCR reaction was performed in a total volume of 20 µl, which included 4 µl of 5x HOT FIREPol® Blend Master Mix Ready to Load with 7.5 mM $MgCl_2$ (Solis BioDyne, Estonia), 1 µl each of forward and reverse primer (5 pmol), and 1 µl of gDNA (50 ng). The PCR reaction was performed with a Thermal Cycler (Applied Biosystems, USA) PCR machine. The PCR *touchdown* program was used as follows for the reaction of amplification.

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, to control the quality of fragments. The PCR products were then purified with Exonuclease I and Shrimp alkaline phosphatase (Thermo Fisher Scientific, USA). Sanger sequencing reactions were performed on the purified PCR fragments using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Reactions were processed using an ABI3730xl instrument.

Sequence reads were analyzed using Applied Biosystems' Sequence Scanner v1.0 and aligned to the Local Genomic Reference sequence (LGR_1) and GR_2 of the human reference genome. Raw sequencing data are available from authors upon request. Sequence analysis and pathogenic variant identification were performed with Mutation Surveyor DNA variant analysis software (Softgenetics, USA). Prediction of mutation pathogenicity was performed using Alamut visual software (Interactive Biosoftware, France). Mutations were absent from databases of normal variations. Variants were checked against the osteogenesis imperfecta mutation database (Dalgliesh 2017). The pathogenicity of the pathogenic variants was predicted as a SIFT score (Kumar et al. 2009).

4.3. Statistical analysis

Statistical analysis was performed using the program R (The R Foundation 2013). The pedigree of each family was constructed using the kinship2 package (Sinnwell & Atkinson 2011). The relationship between family members and generations was displayed in plots. T-tests were used to determine bivariate relationships and a significant difference was considered based on a p value < 0.05. For clinical characteristics, general statistics were calculated, including mean summary, table, and prop table. Percentage differences were employed to evaluate differences in genetic epidemiology of Vietnamese OI patients and the distribution of *COL1A1* and *COL1A2* mutations.

Dental substudy data were analysed using with Version 17.0 of the Statistical Package for the Social Sciences software (SPSS Inc., Chicago, Illinois, USA). Chisquare and Student's t test determined the significance difference of occlusal features between OI and control groups. The confidence level at 95% and a two sided p value of 0.05 were used for the significant difference.

4.4. Ethics

Before the study we applied for study approval from the Ethical Review Committee on Human Research of Hue University Hospital (approval No. 75/CN-BVYD) and the Ethical Review Committee on Human Research of the University of Tartu (permit No. 221/M-34). The study was also conducted in accordance with the Helsinki Declaration. The process by which the information and samples are collected, coded, stored, tested, and the results explained to participating families. Informed written consent from patients or their legal representatives was obtained before they were included in the study. Patients who did not meet OI clinical criteria or did not want to participate were excluded from the study.

Samples or collected medical information do not carry any personal identifiers. All samples are labeled with subject code that can be traced or linked back to the subject only by the primary reseacher.

5. RESULTS

5.1. Clinical features and phenotype manifestations of the patient with osteogenesis imprefecta in Vietnam

Study group consisted 120 Vietnamese OI families from across the country. The families were 30.8%, 44.2% and 25.0% residents of North, Central and South Vietnam respectively. The total number of examined OI patients was 146, and of them 61 were female and 85 male, the ratio 1:1.39 The largest age group (70.55%) of OI patients was 0–15 years. The mean birthweight was 2.7 kg ± 0.53 for males and 2.6 kg ± 0.43 for females. Birthweight less than 2.5 kg was identified in 39 of 146 case which makes 26.7% of the patients. Accroding to the Sillence classification, there were 46 patients with OI type I, 46 patients with type III and 54 patients with OI type IV (Table 3).

Table 3. Characteristics of Vietnamese OI patients

Characteristic	Number of patients	%
Gender		
Male	85	58.22
Female	61	41.78
Age		
0–5	32	21.92%
6–10	40	27.40%
11–15	31	21.23%
16–20	16	10.96%
21–25	9	6.16%
26–30	1	0.68%
31–35	5	3.42%
36–40	5	3.42%
41–45	3	2.05%
46–50	3	2.05%
>50	1	0.68%
Birthweight		
Male < 2.5 kg	21	14.38
Female < 2.5 kg	18	12.33
No information	7	4.79
OI Type by Sillence		
I	46	31.51
II	0	0.00
III	46	31.51
IV	54	36.98

The 146 OI patients were born to 133 mothers. Out of these, 36 mothers reported a previous miscarriage. Preterm delivery (less than 37 weeks of gestation) was detected in 21 mothers. Of the patients, 129 were born to mothers under 35 years old (Table 4).

Table 4. Characteristics of mothers of Vietnamese OI patients.

	Number of mothers	**%**
Miscarriage		
Yes	36	27.07%
No	88	66.17%
No information	9	6.76%
Full-term pregnancy		
Yes	111	76.03%
No (≤ 37 weeks)	21	14.38%
No information	14	9.59%
Mother's age at OI patient's birth		
≤ 35 years old	129	88.36%
> 35 years old	17	11.64%

Genealogical information revealed that 21 families had two or more generations of OI. There were 99 families without previous history of OI. Four families were found to have twin OI patients, with at least one affected twin. In figure 2 shown a pedigree where two generations were affected.

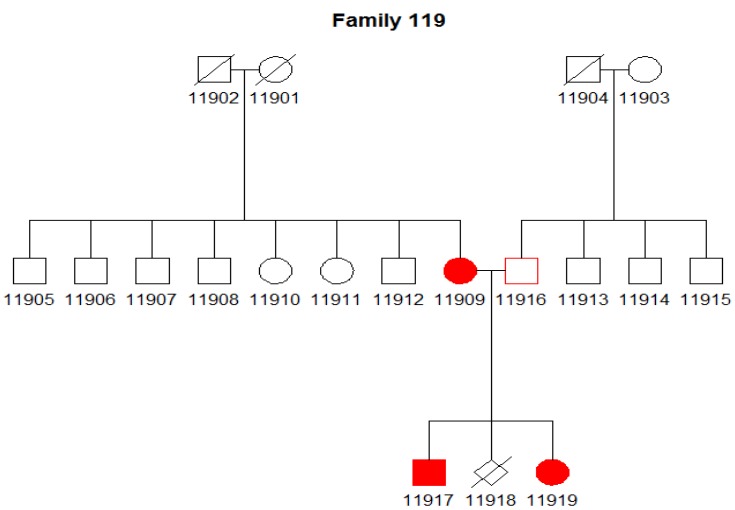

Figure 2. Pedigree tree of a family with history of OI in two generations
OI affected family members (circle – female; square – male) are colored. All family members marked with solid red (11909, 11917, 11919) and father (11916) were subjected to genetic analysis and included into the biobank.

Only 12/146 (8.22%) patients were not expressed non-skeletal OI features; 80.14% patients had blue sclerae, 60.96% patients had DI and 17.81% patients suffered from hearing loss. Although skeletal deformities were present in most patients (134/146, 91.78%), severity and location of the deformities were different. Lower limb deformations were found in 82.88% of the patients and upper limb deformations in 54.8% of the patients. Spine deformations were present in 62.33% and chest deformations in 50.68% of the patients. Most of the patients needed mobility assistance of some level. Only 39.73% patients were capable of ambulating independently. The frequency of signs and symptoms according to different OI types are presented in Table 5.

Table 5. Frequency of signs and clinical symptoms of 146 Vietnamese OI patients

	Total number of patients	**%**	**Number of patients according to OI type**		
			OI Type I	**OI Type III**	**OI Type IV**
Blue sclera					
Yes	117	80.14%	41	34	42
No	29	19.86%	5	12	12
Hearing					
Hearing loss	26	17.81%	5	11	10
Normal	120	82.19%	41	35	44
Teeth					
DI	89	60.96%	21	34	34
Normal	57	39.04%	25	12	20
Mobility					
Lying	17	11.64%	0	14	3
Sitting	49	33.56%	0	26	23
Wheelchair-bound	22	15.07%	3	6	13
Normal	58	39.73%	43	0	15
Deformity					
Upper limb	80	54.80%	11	43	26
Lower limb	121	82.88%	27	45	49
Spine (scoliosis, kyphosis)	91	62.33%	13	44	34
Chest	74	50.68%	7	42	25

Altogether, 142 of the 146 studied (97.26%) patients had fractures. There were four individuals (OI type I) who had not suffered any fractures, but had bone deformities, extraskeletal manifestations, and a positive family history typical to OI. Of the studied individuals, 125 suffered their first fracture during the first six years of life and a total of 34 OI patients had a history of intrauterine fractures. Perinatal fractures had occurred in 9 patients. The first fractured bone for 92 out of 142 patients (64.79%) was the femur. The femur was also the most commonly fractured bone, and was fractured in 132 of the patients (Table 6).

The mean number of fractures in the OI type I group was 6.04; in the type III group 20.76 and in the type IV group 12.94. Among type III and type IV patients, a total of 18 patients each had over 30 fractures during their lifetime.

Table 6. Fracture characteristics of 146 Vietnamese OI patients

	Number of patients	**%**
Time of the first fracture		
Intrauterine	34	23.29%
Perinatal	9	6.16%
Before 6 years old	82	56.17%
≥ 7 years old	14	9.59%
*No information and no fracture**	7	4.79%
First fractured bone		
Upper arm	15	10.29%
Forearm	9	6.16%
Femur	92	63.01%
Lower leg	14	9.59%
Mixed (≥ 2 different bones)	9	6.16%
*No information and no fracture**	7	4.79%

*There were 4 patients without any fractures, and 3 patients could not report the time of the first fracture and first fractured bone

5.2. Dental features

Total number of genotyped pateints was 91 and from them 46 patients had DI. Out of them 36 were diagnosed with DI. *COL1A1* mutations detected on 20 patients with DI, 16 had *COL1A2* mutations and for 10 patients with DI we did not detected collagen type I mutation (Figure 3).

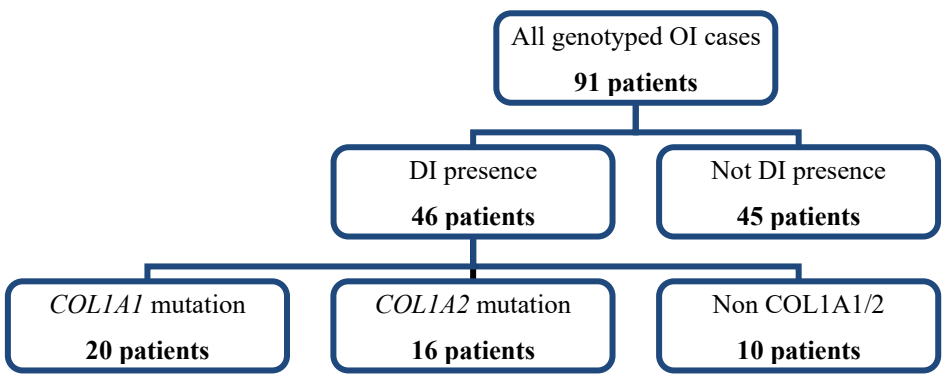

Figure 3: The collagen type I mutations and DI

Significance of associations between genotype and DI presence was tested with Fisher's test. The results clearly show, that among Vietnamese patients, there is a strong correlation between presence of the collagen type I mutation and DI (Table 7).

Table 7. Comparing of genotypes between patients with and without DI. Statistically significant p-value is marked with *****.

	Patients with DI N (%)	Patients without DI N (%)	P-value
No *COL1A1/2* mutations	10 (10.99%)	27 (29.67%)	**0.0002661***
Collagen type I mutations	36 (39.56%)	18 (19.78%)	
COL1A1 mutations	20 (37.04%)	13 (24.07%)	0.3749
COL1A2 mutations	16 (29.63%)	5 (9.26%)	

There were significant differences between OI and control groups respectively in terms of overjet > 3.5 mm (0.0% and 36.2%, $P < 0.001$), open bite > 2 mm (19.2% and 3.5%, $P = 0.004$), reverse overjet > 1 mm (76.9% and 8.5%, $P < 0.001$), posterior crossbite (34.6% and 6.2%, $P < 0.001$), and missing teeth (42.3% and 2.2%, $P < 0.001$) (Table 8).

Table 8. Occlusal features of OI patients compared to the control group

Occlusal feature	**OI group** n=26	**Control group** n=400	**p-value**
Overjet >3.5mm	0.0	36.2	<0.001
Overbite >3.5mm	26.9	26.0	0.917
Openbite >2mm	19.2	3.5	0.004
Contact point displacement >2mm	46.2	54.0	0.437
Posterior crossbite	34.6	6.2	<0.001
Reverse overjet >1mm	76.9	8.5	<0.001
Missing teeth	42.3	2.2	<0.001

Most of the occlusal features were found to differ significantly between OI and control groups. The OI group had a lower score for anterior maxillary overjet (1.44 mm) but a higher score for anterior mandibular overjet (3.94 mm) than the control group (3.13 mm and 2.67 mm respectively, $P < 0.05$). The mean number of missing teeth, including anterior teeth and premolars, was also much higher in the OI group than in the control group ($P < 0.05$). The mean score of the largest mandibular anterior teeth irregularity for the control group (1.43 mm) was statistically higher than that of the OI group (0.89 mm, $P < 0.001$).

5.3. Mutational analysis in the *COL1A1* and *COL1A2* genes

The sample for this analysis consisted of 91 unrelated osteogenesis imperfecta patients. We identified 54 patients (59.4%) with *COL1A1* or *COL1A2* mutations, 33 (36.3%) with mutations in *COL1A1* and 21 (23.1%) in *COL1A2*. There were 34 pathogenic variants in the *COL1A1* gene (missense = 23, nonsense = 4, splice site = 7) and 22 pathogenic variants in the *COL1A2* gene (missense = 21, splice site = 1) identified (Figure 4). Of these, 17 novel *COL1A1* and 10 novel *COL1A2* variants have not been reported before, according to the Dalgliesh database (Tables 9 & 10). *De novo* mutations were observed in 50% (17/34) of *COL1A1* variants and 45.5% (10/22) of *COL1A2* variants. Mutations of the *COL1A1* gene c.2461G>A (p.Gly821Ser) were identified in four unrelated patients. Mutation c.2005G>A (p.Ala669Thr) was also identified in two unrelated patients. Regarding *COL1A1* and *COL1A2* mutations, glyceine substitutions occurred in 64.3% of cases.

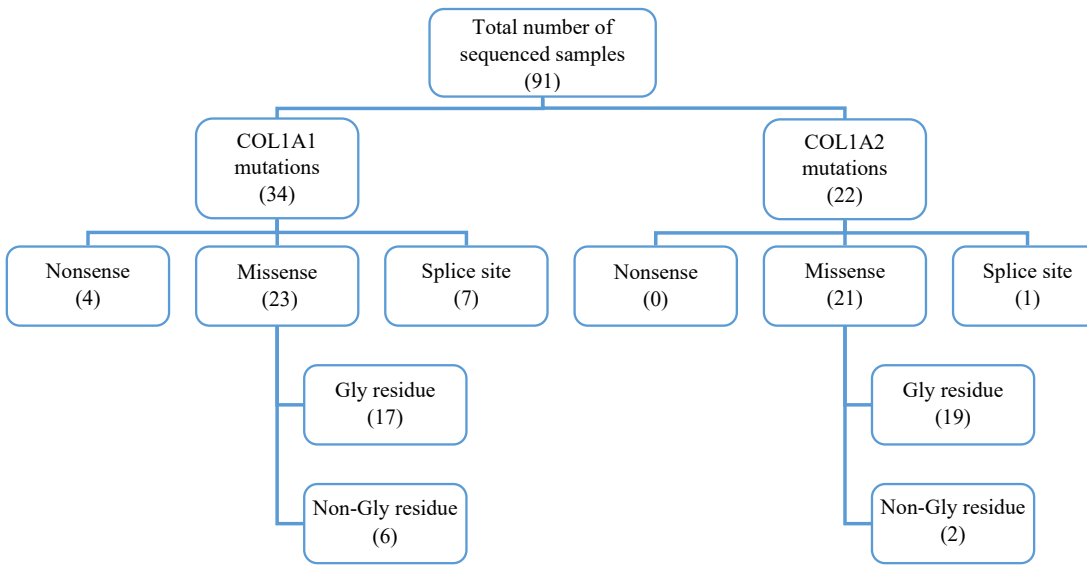

Figure 4. Types of OI mutations in collagen I genes

Table 9. *COL1A1* mutations in unrelated Vietnamese OI patients
Mutations unreported in the Dalgliesh OI database are marked with an *. In the case of heterozygous mutations, both the wild type and mutated alleles are indicated after an arrow (>).

No	Patient ID	*COL1A1* mutation	Exon	Mutation type	Protein alteration
1	VN01	c.2461G>GA	Exon 37	Missense	p.Gly821Ser
		c.2005G>GA*	Exon 30	Missense	p.Ala669Thr,
2	VN02	c.1200+1G>GT*	Intron 18	Splice site	-
3	VN05	c.1072delC, het*	Exon 17	Frameshift	p.Glu358Lysfs*26
4	VN13	c.4391T>C	Exon 52	Missense	p.Leu1464Pro
5	VN18	c.103+2T>TC*	Intron 1	Splice site	-
6	VN21	c.4352dupA, het.*	Exon 52	Nonsense Frameshift	p.Asp1451Glufs*100
7	VN26	c.3226G>GA	Exon 45	Missense	p.Gly1076Ser
8	VN34	c.2461G>GA	Exon 37	Missense	p.Gly821Ser,
9	VN38	c.959G>GA*	Exon 15	Missense	p.Gly320Asp
10	VN39	c.630delG, het*	Exon 8	Frameshift	p.Glu210Aspfs*3
11	VN40	c.2461G>GA	Exon 37	Missense	p.Gly821Ser
12	VN41	c.1102G>GA	Exon 17	Missense	p.Gly368Ser
13	VN49	c.2461G>GA	Exon 37	Missense	p.Gly821Ser
14	VN50	c.932G>GT*	Exon 14	Missense	p.Gly311Val
15	VN51	c.949G>GA*	Exon 14	Missense	p.Gly317Ser
16	VN52	c.2523delT, het.	Exon 37	Frameshift Nonsense	p.Gly842Alafs*266
17	VN58	c.2236-2A>AG*	Intron 32	Splice site	-
18	VN66	c.2596G>AG*	Exon 38	Missense	p.Gly866Ser
19	VN68	c.2299G>GA	Exon 33/34	Missense	p.Gly767Ser
20	VN70	c.2281G>GA*	Exon 33/34	Missense	p.Gly761Ser
21	VN71	c.1002+2T>C	Intron 15	Splice site	-
22	VN72	c.1165G>GT	Exon 18	Missense	p.Gly389Cys
23	VN76	c.1165G>GA	Exon 18	Missense	p.Gly389Ser
24	VN78	c.3766G>GA	Exon 49	Missense	p.Ala1256Thr
25	VN86	c.977G>AG	Exon 15	Missense	p.Gly326Asp
26	VN88	c.2005G>GA*	Exon 30	Missense	p.Ala669Thr
27	VN89	c.2005G>GA*	Exon 30	Missense	p.Ala669Thr
28	VN91	c.1299+1G>C	Intron 19	Splice site	-
29	VN92	c.2299G>GA	Exon 33/34	Missense	p.Gly767Ser
30	VN95	c.590G>GA	Exon 8	Missense	p.Gly197Asp
31	VN99	c.103+2T>TC*	Intron 1	Splice site	-
32	VN104	c.3369+1G>GC*	Intron 46	Splice site	-
33	VN106	c.1350G>GC*	Exon 20	Missense	p.Glu450Asp

Table 10. *COL1A2* mutations in unrelated Vietnamese OI patients
Mutations unreported in the Dalgliesh OI database are marked with an *. In the case of heterozygous mutations, both the wild type and mutated alleles are indicated after an arrow (>).

	Patient ID	***COL1A2* mutation**	**Exon**	**Mutation type**	**Protein alteration**
1	VN09	c.3305G>GT	Exon 49	Missense	p.Gly1102>Val
2	VN23	c.2261G>GT*	Exon 37	Missense	p.Gly754Val
3	VN25	c.1072G>GT	Exon 37	Missense	p.Gly358Ser
4	VN29	c.1630G>GA*	Exon 28	Missense	p.Gly544Ser
5	VN45	c.1090G>GA	Exon 21	Missense	p.Gly364Ser
6	VN47	c.3034G>GA	Exon 46	Missense	p.Gly1012Ser
		c.2569C>CA	Exon 41	Missense	p.Pro857Thr
7	VN48	c.1451G>GA	Exon 25	Missense	p.Gly484Glu
8	VN56	c.1729G>GA*	Exon 30	Missense	p.Gly577Ser
9	VN60	c.1009G>GA	Exon 19	Missense	p.Gly337Ser
10	VN62	c.1378G>GA	Exon 24	Missense	p.Gly460Ser
11	VN64	c.1964G>GT*	Exon 32	Missense	p.Gly655Val
12	VN65	c.1981G>GC*	Exon 33	Missense	p.Gly661Ser
13	VN69	c.874G>GA	Exon 17	Missense	p.Gly292Ser
14	VN81	c.982G>GA	Exon 19	Missense	p.Gly328Ser
15	VN82	c.2503G>GA	Exon 40	Missense	p.Gly835Ser
16	VN83	c.792+1G>GA	Exon 16	Splice site	-
17	VN84	c.2791G>GA*	Exon 43	Missense	p.Gly931Arg
18	VN85	c.838G>GT*	Exon 17	Missense	p.Gly280Cys
19	VN87	c.2791G>GA*	Exon 43	Missense	p.Gly931Arg
20	VN96	c.892G>GT*	Exon 18	Missense	p.Gly298Cys
21	VN97	c.2538G>GT*	Exon 40	Missense	p.Lys846Asp

6. DISCUSSION

6.1. Clinical features and phenotype manifestations of the patient with Osteogenesis Imprefecta in Vietnam

The data was collected from 34 of Vietnam's 63 provinces (total population of those provinces is 60 million, and of Vietnam 90 million) (Figure 5), spread across different geographical areas of Vietnam (Brinkhoff 2014). It is estimated that OI affects approximately 1 person per 25,000–100,000 people, however the incidence worldwide varies (Basel & Steiner 2009) (Martin & Shapiro 2009). Our numbers suggest that OI prevalence is 1/400 000 what is four time less than other countries. That may be due to the most probably underdiagnosed in Vietnam, especially in cases where there is no history of OI in the family or symptoms are mild.

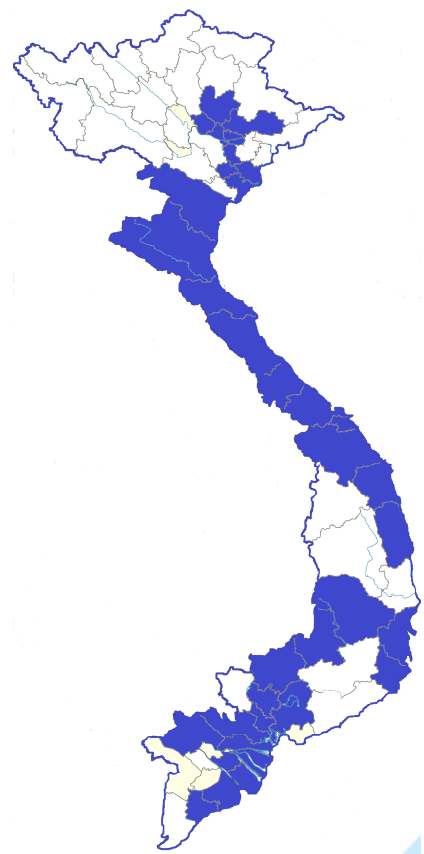

Figure 5. OI patients from 34 provinces (colored) of Vietnam's 63 provinces

There was a total of 61 female (41.78%) and 85 male (58.22%) OI patients in our study, with a 1:1.39 ratio. A study in Taiwan showed a female to male ratio of 2.2:1 (Lin et al. 2009). OI is mostly connected to autosomal dominant inheritance, which is why there is no difference in gender. Most studies have not indicated the gender of affected individuals and OI worldwide usually occurs without gender differences (Sillence et al. 1979) (Martin & Shapiro 2009). The difference in the gender ratio at birth of OI patients in Vietnam could be explained by insufficient diagnosis of OI cases, but could also be related to the slightly higher number of male births in Vietnam (the male to female ratio is 112:100) (Ministry of Planning and Investment 2011).

We discovered birthweight less than 2.5 kg in 39 OI patients. The mean birthweight of healthy Vietnamese boys and girls is 3.1 kg and 3.0 kg, respectively (Nguyen et al. 2013), the mean birthweight of OI patients in our study was 2.7 kg for boys and 2.6 kg for girls, respectively, which is significantly lower than the healthy population. A prior study describes a mean birth weight of 3.2 in OI patients (Ben Amor et al. 2013), although does not comment on gestational age of these patients. It is unclear from prior studies, as well as this study, if and how these birth weights in OI may be related to pregnancy term at delivery. Decreased birthweight and short stature of OI sufferers are caused by abnormal intrauterine development of the foetus. The difference between the OI groups and controls appears to be larger than might be expected from the overall smaller body frame in OI (Rauch et al. 2000). In a study by Sillence and colleagues, the majority of OI patients also had low birthweight (Sillence et al. 1979).

In our research, we observed relatively equal number of patients with different OI types; 46 patients with OI types I, 46 with type III and 54 with type IV. Other studies have shown that the prevalence of type I patients is usually higher compared to types III and IV (Martin & Shapiro 2009). Lin *et al.* reported OI prevalence in Taiwan as follows: 19 OI type I, 10 type III and 19 type IV and shows lower prevalence in tuype III (Lin et al. 2009). We were unable to find reports of OI type II patients, as this form is typically lethal in the perinatal period. The predominance of severe forms of OI in Vietnam suggests underdiagnosis of mild OI types.

We have 99 families (82.5%) without a history of OI and only 21 families (17.5 %) with OI history in previous generations. Other studies reported a positive family history 41–48% (Lin et al. 2009). Seventeen unrelated families had 2 or 3 OI children, but their parents did not reveal any OI clinical symptoms or OI history. This number may increase after the genetic testing is preformed to all family members. Genetic analysis should be done as the next step of our research in order to detect OI in subsequent generations in these families.

Types I and IV have an autosomal dominant inheritance pattern, thus there is a 50% chance that parents transmit the OI mutation to their offspring. Recessive inheritance patterns of OI mutations usually give rise to OI type III. There is also a 1–3% risk of inheriting OI due to gonadal mosaicism in the parents (Sharma et al. 2001).

Osteogenesis imperfecta in twins is a rare phenomenon. Previously a few investigators focused on this topic. According to our best knowledge the only work considering osteofenesis imperfecta in twins is done by Gupta and the coworkers (Gupta et al. 1990). Interestingly, in our sample, we had four twin pairs. Three of the pairs were same sex (2 boys or 2 girls) and one twin pair was a boy–girl pair. Two pairs the both of the cildren wre affected and two pairs olny one child had clinical signs of OI. The cause may be due to a mutation in germ cells. We have found no evidence of OI history in families with twins, nor the presence of consanguineous marriage. Further exome sequencing will be performed on twin pairs in order to examine the zygosity and genotype–phenotype correlations.

A history of miscarriage was found in mothers of 36 OI patients (27.07%). We recorded that 111 mothers (76.03%) had a full-term pregnancy and 21 mothers (14.38%) had a premature delivery. The previous study of premature delivery including 6.600 pregnant in Vietnam, the proportion is 9.58% (Dat et al. 2013). So the premature dilivery occurrence is higher in OI group. The goal of management during the perinatal period is to control an atraumatic delivery for both OI foetus and mother (Sharma et al. 2001). Most mothers and fathers (129, 88.36% and 109, 81.85%) delivered the OI patients when they were under 35 years of age.

We recognized different levels of severity and localization of deformities in patients. Deformities were most frequently observed in long bones. Lower limb deformities were found in 121 patients (82.88%), upper limb deformities in 80 patients (54.80%), spine deformities in 91 patients (62.33%) and chest deformities in 74 patients (50.68%). Bowing and angulated deformities exist to varying degrees in OI sufferers, with frequent over-modeling of the shafts of long bones (Van Dijk & Sillence 2014). In addition to long bone deformities occurring due to imperfect bone development or fracture healing problems, weight bearing alone can cause leg bowing (Moriwake & Seino 1997). In previous studies, bone deformities were reported in 54% of patients (Lin et al. 2009). Lower limb deformities were 28.7%, humerus 14.2% (Patel et al. 2015). Scoliosis, kyphosis, flattened vertebra and compression fractures are common in OI sufferers. Severe vertebral deformity may cause respiratory problems in some patients (Moriwake & Seino 1997). Previous studies reported scoliosis in 74.5% (76/102) (Karbowki et al. 1999) and 50% (157/316) of cases (Anissipour et al. 2014).

In our study, 125 patients suffered their first fracture in the first six years of life. Sillence also found that fractures usually first appear during the preschool period (Sillence et al. 1979). The long bone fractures after minimal trauma were the most common and characterized of OI patient. Fracture rates tend to decrease after adolescence (Thomas & DiMeglio 2016). In both genders and for all OI types, fracture rates diminish during the teenage years; however the reason is not known (Paterson 1997). In our study, 18 patients (12.33%) who had either type III or type IV had suffered over 30 fractures. The mean number of lifetime fractures for OI patients in our study was 13.23. The mean number of lifetime fractures in type I patients was 6.043, in type III patients 20.76 and in type IV patients 12.94. There were statistically signficant differences between the number of total

fractures between type I and III groups ($p = 3.028\times10^{-12}$), type I and IV groups ($p = 2.81\times10^{-6}$) and type III and IV groups ($p = 0.0001254$). In other previous studies, the mean fractures for each OI patient were reported to be 7 (Greeley et al. 2013). Most of patients (86%) had sustained fractures, nearly all the participants had fractures affecting the long bones (Wekre et al. 2011). Fifty-eight unrelated subjects in the study of Zhang and his workers showed 100% patients experienced one or more fractures (Zhang et al. 2012)

Most of the patients in our study required assistance with ambulation: 58 (39.73%) moved independently; 22 (15.07%) were wheelchair-bound; 49 (33.56%) were only able to sit; 17 (11.64%) could only be in a lying position. A previous study reported that almost 100% of OI type I, 63% of type IV and 0% of type III patients were able to walk without aids or supporting devices. However 100% of OI type III and 21% of OI type IV patients were reliant on a wheelchair (Lin et al. 2009). Wekre reported that 20% (19/97) of patients of all OI types, including 100% of OI type III patients, used a wheelchair for ambulation (Wekre et al. 2011). OI type III (Figure 6) is connected to severe bone fragility, and a significantly increased risk of lifetime fractures, starting as early as in the uterus. Scoliosis and kyphosis cause deformations of the rib cage (Glorieux 2008). Such OI type III patients develop relatively short stature, and fractures can happen at birth with moderate bone deformities present (Glorieux 2008) (Clarke et al. 2013).

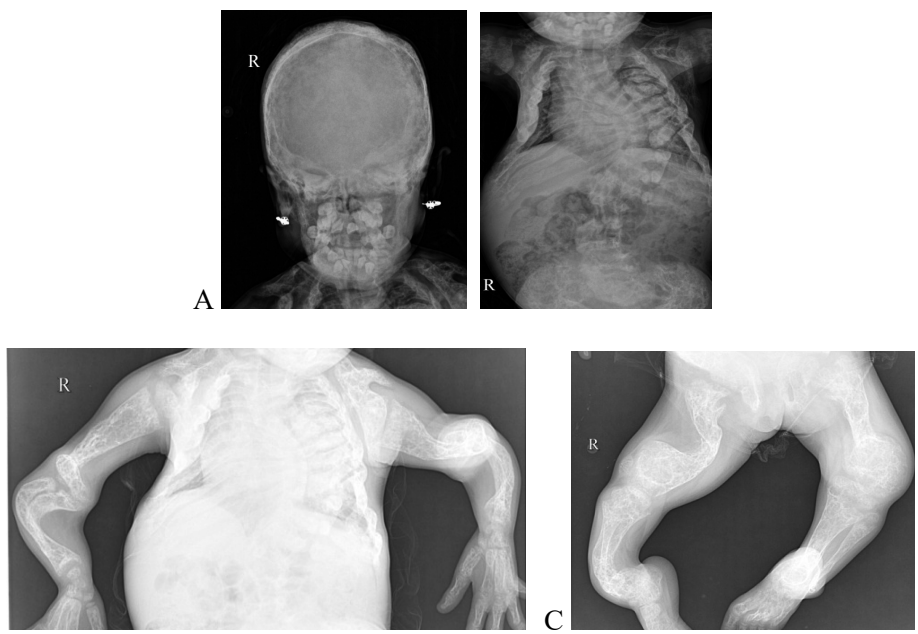

Figure 6. Typical patient with OI type III (severe deforming OI)
X-ray showing: (A) skull, scoliosis and chest deformation; (B) upper limb deformities (C) lower limb deformities.

The presence of blue sclerae or pale blueness is due to either thin sclerae or normal sclera thickness but with abnormal arrangement of the collagen molecular framework (Sillence et al. 1979). We identified 117 patients (80.14%) with blue sclerae; out of them 41 had OI type I, 34 had OI type III and 42 had OI type IV. The presence of blue sclerae was slightly higher than in previous studies, especially for patients with OI type IV. Other OI research studies have shown a 75% incidence of blue sclerae; 89%, 80% and 58% for OI types I, III and IV respectively (Lin et al. 2009), and a 78.1% (425/544 OI-affected individuals) incidence of blue sclerae (Patel et al. 2015).

We found 26 patients (17.81%) with hearing loss of varying levels. Previous studies have shown the incidence worldwide to be variable, *e.g.* 6.7% (Kuurila et al. 2000) or 13.4% (73/544) of cases (Patel et al. 2015). There are many possible reasons for hearing loss in OI patients. It may be caused by the abnormal bone formation of the malleus, incus, or stapes of the middle ear (Pillion et al. 2011); microfractures and incomplete ossification are often found in the otic capsule and the malleus in newborns. However, in older patients otospongiosis lesions in the footplate of the stapes cause hearing loss. Underminerlization imaging of the otic capsule, cochlea, semicircular and auditory canals have been revealed on CT scans (Pillion et al. 2011) (Heimert et al. 2002). As we were not testing about hearing loss, we can not say exactly what type of hearing loss is in Vietnamise patients

6.2 Dental features

In our research DI was identified in 89 patients (60.96%), including 21/46 (45.65%) OI type I patients, 34/46 (73.91%) OI type III patients and 34/54 (62.96%) OI type IV patients. The reported prevalence of DI in OI sufferers has been highly variable. In a study by Majorana and colleagues, DI prevalence was shown to be 62.5% (10/16 OI-affected individuals), with 30% of type III patients affected (Majorana et al. 2010). Other authors have reported the prevalence of DI to be as low as 19% (Sæves et al. 2009) and as high as 80% for OI type III patients (Lin et al. 2009).

Our results showed strong correlation between presence of the collagen type I mutation and DI, and this is in concordance with several previous works (Lindahl et al. 2015) (Rauch et al. 2010) (Amor et al. 2011). In the study of Taiwanese population no correlation between mutation type and DI was found (Lin et al. 2015).

The main occlusion problems of OI patients were associated with reserve overjet. The most obvious difference between OI and control groups in the current study concerned reverse overjet. Moreover, open bite was significantly higher (> 2 mm) than in the control group. For this reason, we also hypothesize that a collagen mutation in the foetal phase might cause structural disorders of collagen in the mandibular and condylar regions, leading to the reverse overjet, and therefore the gonial angle of the mandible of OI patients is larger compared

to those of non-OI individuals. Moreover, spinal curvature and the triangular face of OI patients might result in the mandible resting on their chest and open bite (Chang et al. 2007) (Rizkallah et al. 2013) (Minh Son Nguyen et al. 2017).

The study of Chang et al. 2007 demonstrated the poor growth of the maxilla based on analysing cephalometric radiographs of OI patients (Chang et al. 2007). This finding could explain our results, in that no OI patient had an overjet over 3.5 mm, which was however often observed in the control group (36.2%), while the crossbite of OI patients (34.6%) was higher than those of the control group (6.2%).

We showed that 42.3% of OI individuals had missing teeth, whereas only 2.2% of the control group did. The cause of missing teeth could be tooth extraction, delayed tooth eruption, hypodontia or abnormal odontogenesis. In the current study, missing teeth in the OI group could be referred to genetic disorders that would cause hypodontia. Genetic disorders such as cleft lip and palate, ectodermal dysplasia or Down's syndrome are important causes of hypodontia (AlShahrani et al. 2013) (Cobourne 2007). The candidate genes, therefore, causing missing teeth need to be found in the future.

6.3. Mutational analysis in the *COL1A1* and *COL1A2* genes

Genetic analysis was performed in 91 unrelated patients. We discovered *COL1A1* and *COL1A2* pathogenic variants in 54 (59.4%) patients. Of these, 33 patients harboured 34 mutations in the *COL1A1* gene, and 21 patients had 22 mutations and the *COL1A2* gene (Figure 4). Previous investigations reported the percentage of collagen mutations to be as high as 90% (Pollitt et al. 2006) (Shaker et al. 2015), although different mutation rates up to 96% in *COL1A1* and *COL1A2* genes occur in different populations (Stephen et al. 2014) (Lee et al. 2006) (Zhang et al. 2012). Recently, our research group has finished *COL1A1/2* mutational analysis in Ukrainian and Estonian OI patients using the same methodology of patient recruitment and mutation discovery described in the current study of Vietnamese OI patients. The results revealed 89.66% (26/29) of individuals harbor collagen type I mutations among Estonian OI patients and 65.79% (50/76) among Ukrainian OI patients (unpublished data). These findings lend support to the presence of differences in proportions of *COL1A1/2* mutations between populations, as well as significantly reduced number of collagen type I mutations in Vietnamese OI individuals. These differences come from the diversity of studied cohort sizes. However, such big differences in the Vietnamese population might be also linked to an increased number of non-collagenous variants. In order to clarify this phenomenon, the cohort of Vietnamese patients must be expanded, and exome analysis in those patients who were negative for collagenous mutations must be performed.

Glycine is situated at every third position in the amino acid sequence of type I collagen alfa chains, thus missense OI mutations are usually represented with Gly substitutions. We discovered 17/23 and 19/21 Gly substitutions in the

COL1A1 and *COL1A2* gene missense mutations respectively. The total number of Gly substitutions was 36/56 (64.3%) of all collagen mutations.

In 36 cases Gly was substituted with Serine (Ser) 23 times (63.9%), with Valine (Val) 4 times (11.1%), with Cystine (Cys) 3 times (8,3%) and Aspartic acid (Asp) 3 times (8,3%). According to earlier investigations, Gly substitution with Cys causes the most severe phenotypes, and Gly substitution with Arginine (Arg) is often lethal (Cole et al. 1990). The severity of the phenotype depends not only on the substituted residue, and differences in properties between amino acids, but also on the position of the substitution. Ser was observed as the most common amino acid, with which Gly was substituted in Chinese OI patients (72%). However, 40% of Taiwanese OI patients harboured mutations, that caused Gly substitution with Asp (Zhang et al. 2012) (Lin et al. 2015). The reasons for the high diversity in amino acid substitutions between populations have not been revealed yet.

We have discovered 8 intronic variants that cause pathogenic alterations in splice sites. In a Chinese study, 7/56 patients were reported to harbour pathogenic intron variants (Zhang et al. 2012). Splice site mutations are connected to exon skipping, intron inclusion, and activation of cryptic sites (Kuivaniemi et al. 1997). We have also revealed nonsense variants in exons 52 and 37. Splice site and nonsense mutations cause a quantitative defect of collagen type I due to haploinsufficiency. Quantitative defects are connected to the mild OI phenotype, type I. Sometimes patients develop the moderate phenotype, OI type IV. The reasons behind the diversity between carriers of the same mutations are not yet known.

Interestingly, four unrelated patients harboured the same mutation c.2461G>A (p.Gly821Ser) in exon 37, in the *COL1A1* gene. They may be have a common ancestor although nowadays they are living in different parts of the country.

The *COL1A1* heterozygous mutation c.2005G>A (p.Ala669Thr) was shared by individuals VN88 and VN89, both with clinical OI type IV. Double mutations were described by Zhang *et al.* and Lee *et al.* in both the *COL1A1* and *COL1A2* genes (Lee et al. 2006). There are more than 1500 mutations described in the collagen I genes, supporting the fact, that OI mutations are usually individual and it is rare that different unrelated families harbour the same pathogenic variants (Lee et al. 2006).

We also discovered double mutations in two patients; c.2461G>A (p.Gly821Ser), and c.2005G>A (p.Ala669Thr) mutations were present in the *COL1A1* gene of patient VN01, and the second individual, VN47, was a carrier of two heterozygous mutations in the *COL1A2* gene exon 46 c.3034G>GA (p.Gly1012Ser), and exon 41 c.2569C>CA (p.Pro857Thr). Double mutations are not rare. Previous investigations have discovered double missense mutations in the *COL1A2* gene (p.Gly208Glu and p.Gly235Asp), causing the severe OI phenotype (types II–III) (Takagi et al. 2015). However, patients from our study developed mild and moderate OI with such mutations. Although genotype–phenotype correlations are of great interest for all OI researchers and clinicians,

connections between mutations and OI severity are still not clear and need further investigation.

During our study we discovered 17 and 10 novel mutations in the *COL1A1* and *COL1A2* genes respectively (27/56 pathogenic variants; 48.2%). These novel mutations were absent from the Dalgleish OI mutation database (Dalgleish 2017). Interestingly, in our study there were more new variants compared to studies performed earlier (Stephen et al. 2014) (Lin et al. 2015) (Yang et al. 2011) (Ward et al. 2001). Current findings support the presence of an unusual mutational profile in the Vietnamese OI population. Future exome sequencing is necessary to reveal the rest of the non-collagenous genotypes of Vietnamese OI patients.

OI mutations arose more in the *COL1A1* gene than in the *COL1A2* gene. In the *COL1A1* gene, mutation hotspots included the following positions: intron 1, exons 8, 14–15, 17–20, 30, 33, 34, 37 and 52. The *COL1A2* gene included mutation hotspots in exons 17–49. Collagen type I alfa chains consist of a signal peptide, an N-terminal propeptide, a chain triple helical domain, and a C-terminal propeptide. Exons rich with OI mutations correspond to part of the protein sequence, which tolerates substitutions of amino acids, resulting in a protein with a defective structure, but does not decrease fitness of the fetus, thus allowing it to develop. This is in contrast with regions that are crucially important for collagen functioning and organism survival that stay free of mutations (Marini et al. 2007) (Sweeney et al. 2008).

Design of sequencing primers allowed the covering of splice site mutations. Furthermore, all exons were covered, allowing missense, nonsense and frameshift mutations to be revealed in the coding sequence of the *COL1A1* and *COL1A2* genes. Sanger sequencing accuracy is about 99.9%. The only uncovered changes in the *COL1A1* and *COL1A2* structures were exon or whole gene duplications and deletions (Sanger F 1977). Exon deletions and especially whole gene deletions are rare. However, it means that the number of collagen type I alterations in our study might be slightly underestimated.

7. CONCLUSIONS

1. The results of this study demonstrate that OI has become a challenge in Vietnam. The number of affected individuals and severe phenotypes found indicates that the disease is most probably underdiagnosed, especially in situations with a negative OI family history and in mild cases. Deformities and the mean lifetime fracture were higer in our study group. The results of this study will be of practical use in educating physicians and other medical professionals throughout the Vietnam about the signs and symptoms of OI, helping to increase diagnosis and prevent complications of this rare disorder. More active investigation and implementation of accessible medical services would improve quality of life for OI patients and their families.
2. DI was indentifed in 60.96% patients with OI. Genetic analysis suggest presence of correlation between DI and abnormality of collagen type I. The occlusal features were common in OI patients, especially reverse overjet and missing teeth. Due to associated disability and impact on quality of life, these conditions often necessitate orthodontic treatment for both medical and social purposes.
3. Mutational analysis of OI patients in Vietnam showed a lower number of OI pathogenic variants of collagen in the studied Vietnamese patients compared to reported rates for other (including Asian) OI populations. We discovered *COL1A1* and *2* mutations in 59.4% patients, whereas in most populations the rate is around 90%. The outstanding OI mutational profile of the Vietnamese population is related to the presence of a high number of recessive mutations in non-collagenous OI genes. The reason for unidentified OI genotypes remains obscure and needs further investigation, with focus on analysis of patients negative for collagen OI mutations.

8. MAIN PRACTICAL APPLICATIONS

The results of this study demonstrate that OI has become a challenge in Vietnam. Vietnamese OI patients face a lack of medical consulting, as well as economic difficulties. The creation of an OI database during this study provides a better understanding of OI clinical manifestations and also genotype differences compared to other populations.

Better detection of OI disease and earlier prevention should be addressed, particularly in families with a high risk of OI. Results from this study will help to improve OI detection and investigation, and point out the need for efficient medical service in order to improve the quality of life of OI patients and their families in the future. For a start, already a medical center for OI patients and their families was launched at Hue University Hospital in 2015, and regular follow-up and free treatment is now available for all OI patients. Finally, however, there is still a need for continuous research to detect differences in clinical signs, and the genetic background of OI-affected families in Vietnam.

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10. SUMMARY IN ESTONIAN

Osteogenesis imperfecta Vietnamis

Osteogenesis imperfecta on haruldane geneetiline sidekoe haigus, mida nimetatakse ka habraste luude haiguseks ning mille esinemissagedus on 1/ 25,000–100,000 inimese kohta. *Osteogenesis imperfecta´le* (OI) on iseloomulikud sagedased luumurrud, skeleti deformeerumine ja kasvupeetus. Haigusel on ka palju skeletiväliseid tunnuseid, nagu sinised skleerad, kuulmislangus ja hammaste dentiini häire (*dentinogenesis imperfecta*). Haiguse kliiniline väljendumine võib laialt varieeruda ulatudes kergest vormist kuni letaalseni. Lisaks fenotüübilisele mitmekesisusele esineb haigusel ka suur geneetiline heterogeensus. Aastal 1979 avaldas David Sillence OI klassifikatsiooni, kus eristatakse nelja OI tüüpi (I–IV) kliinilise fenotüübi alusel. OI haiguse põhjuseks on I tüüpi kollageeni sünteesi kvalitatiivne või kvantitatiivne häire. I tüüpi kollageen on väga oluline luude, naha, kõõluste, sarvkesta, veresoonte seinte ning samuti teiste sidekudede ehitusmaterjal. OI geneetiliseks põhjuseks on peamiselt mutatsioonid kollageeni COL1A1 ja COL1A2 geenides ning need võivad põhjustada haigust kergest kuni progresseeruvate deformatsioonide perinataalselt letaalse vormini. Seni on leitud mutatsioone 17-s erinevas geenis, mis kõik võivad põhjustada erineva raskusega haigust. Umbes 90% juhtudest on leitud mutatsioonid COL1A1 ja COL1A2 geenides. Kuigi genotüübi ja fenotüübi vahelisi seoseid on palju uuritud, siis korrelatsiooni OI kliiniliste tunnuste ja geneetiliste mutatsioonide vahel senini leitud ei ole.

OI on haruldane haigus, mistõttu aladiagnoosimine või diagnoosi hilinemine on sagedased. Diagnoosimist aitab lihtsustada perekonna haiguse anamneesi teadmine, kliiniliste tunnuste tundmine ja haigete põhjalik uurimine, kuid diagnoosimine on siiski uutel ja kergete sümptoomidega juhtudel keeruline. Sõltuvalt arengustaadiumist saab rakendada erinevaid diagnostilisi meetodeid. Prenataalne diagnoos on võimalik OI anamneesi korral pärast 16. rasedusnädalat kui teostada ultraheliuuring deformatsioonide ja murdude hindamiseks või vajadusel ka DNA analüüs. Sünnihetkel aitavad diagnoosimisele kaasa luumurdude ja deformatsioonide esinemine, iseloomulikud muutused röntgen ülesvõtetel ning sinise värvusega skleerade esinemine. Lapsepõlve ja noorukieas tuleks haigust kahtlustada kui esinevad sagedased murrud, skeleti deformatsioonid ning abiks on ka silmade siniste skleerade või hammaste dentinogeneesi häirete esinemine.

OI esinemist, selle kliinilist väljendumist või geneetilisi põhjuseid Vietnamis varem uuritud ei ole.

Eesmärgid

Käesoleva töö eesmärgid olid:

1. Kirjeldada OI patsientidel haiguse kliinilisi tunnuseid ja OI esinemist Vietnamis
2. Kirjeldada hammaste ja hambumusega seotud iseärasusi OI patsientidel
3. Kirjeldada *COL1A1* ja *COL1A2* geenide mutatsioone OI patsientidel Vietnamis

Materjal ja meetodid

OI patsientide kaasamine antud uuringusse toimus aastatel 2013–2016. Võimalikke OI perekondi kaasati üle kogu Vietnami. Päring võimalike OI patsientide kohta saadeti kõikidele haiglatele, meditsiinikeskustele ja patsientide organisatsioonidele Vietnamis.

Uuringu andmebaasi registreeriti OI esinemine koos kliiniliste tunnustega ning lisaks ka muude oluliste haiguste esinemine perekonnas, koostati sugupuu vähemalt kolme järjestikuse põlvkonna isikute andmetega. Kõik patsiendid läbisid kliinilise läbivaatuse, mille viis kogu uuringu jooksul läbi sama Eesti-Vietnami arstide meeskond. Samuti uuriti võimalusel radioloogilisi ülesvõtteid, et leida OI tunnuseid ja kirjeldada deformatsioone. Diagnoos ja OI tüüp kinnitati kliiniliste tunnuste ja patoloogilise leiu alusel. OI tüüp määrati vastavalt Sillence´i klassifikatsioonile. Lisaks fenotüübi kirjeldusele koguti ka täiendavat infot nagu sünniandmed (pikkus, kaal, intrauteriinsed ja sündimise käigus esinenud luumurrud/deformatsioonid), luumurdude esinemine elu jooksul (murdude aeg ja lokalisatsioonid, luumurdude arv kokku), skeleti deformatsioonid, skeletivälised OI tunnused (skleerade värvus, *dentinogenesis imperfecta* (DI), kuulmislangus) ja füüsiline aktiivsus ning abivahendite (kargud, ratastool) vajadus.

Hammaste ja hambumuse täiendavaks uurimiseks valiti 12–16-aastaste OI patsientide rühm, kellel esines DI. Andmeid võrreldi kontrollrühmaga, mis koosnes 400 isikust. Analüüsisime hammaste kvaliteeti, hinnati nende puudumist ja hambumuse omadusi.

Kõikidelt patsientidelt ning nende vanematelt koguti vereproovid geneetiliseks analüüsiks. DNA eraldati EDTA-konserveeritud verest vastavalt standardsetele eraldamise meetodile. Proove säilitati temperatuuril -80 ° C ja analüüsiti kasutades Sanger sekveneerimist. Avastatud patogeenseid variante kirjeldati SIFT ja PolyPhen skooridega.

Tulemused

Uuringusse kaasati 120 perekonda, kus esines *osteogenesis imperfecta*. Kokku kanti uuringu andmebaasi 146 OI haiget. Enim OI patsientidest (70.55%) oli vanuserühmast 0-15 aastat. Sillence´i klassifikatsiooni järgi esines 46-l patsiendil I tüüpi, 46-l III tüüpi ja 54-l patsiendil IV tüüpi OI. Letaalse ehk II tüübi OI-ga patsiente ei avastatud. Arvestades Vietnami populatsiooni (ligi 90 mil-

jonit) on 146 OI haiget väga väike haigestunute hulk. Samuti erineb patsientide jagunemine tüüpide vahel oluliselt teistest populatsioonidest, kus on leitud ülekaalukalt rohkem I tüüpi (kerge vormiga) haigeid võrreldes raskemate tüüpidega. Me ei eelda, et OI esinemissagedus Vietnamis on ebaproportsionaalselt madal või, et raskemate tüüpidega patsiente on Vietnamis ülekaalukalt rohkem. Pigem näitavad uuringu tulemused, et OI on Vietnamis oluliselt aladiagnoositud, eriti juhtudel kui OI ei ole perekonnas varem esinenud või sümptomid on kergemad. Väiksemat teadaolevat patsientide hulka võib seostada ka erinevate kultuurilisete ja majanduslike teguritega Vietnamis.

Kuna mõnes perekonnas oli ka mitu OI patsienti, siis erinevaid emasid oli kokku 133, kellest 36-l oli esinenud varem raseduste katkemisi ja 21-l enneaegne sünnitus. Sugupuude uurimisel selgus, et 21-s perekonnas esines OI kahes või enamas põlvkonnas ning 99-s peres ei olnud varem OI diagnoositud. Neljas peres olid kaksikud lapsed, kellest kahel paaril olid OI haiged mõlemad kaksikud ja ülejäänud kahest oli haige üks kaksikutest.

Uuritutest 142-l patsiendil 146-st esinesid luumurrud. Neil OI patsiendil, kelle luumurde ei olnud esinenud, leidsime väljendunud luude deformatsioonid, lisaks esinesid skeletivälised OI tunnused ning samuti positiivne perekonna anamnees. Uuritud patsientidest 34-l oli esinenud luumurd juba intrauteriinselt ning 125 olid saanud oma esimese luumurru esimese kuue eluaasta jooksul. Sündimise käigus olid luumurrud tekkinud 9-l patsiendil. Esimene murdunud luu oli 92-l patsiendil reieluu. Samuti oli reieluu ka üldse kõige sagedamini murdunud luu (132 patsiendil). Keskmine luumurdude arv oli I tüüpi OI grupis 6,04; III tüübi grupis 20,76 ja IV tüübi grupis 12,94. Patsiente, kelle luumurdude üldarv ületas 30 oli kokku 18. Skeleti deformatsioone esines enamikel patsientidest, kuid erinesid nende lokalisatsioonid ja väljendumine. Ootuspäraselt esines enim deformatsioone pikkades toruluudes. Alajäseme deformatsioone leiti 82,88%-l, ülajäseme deformatsioone 54,8%-l, lülisamba deformatsioone 62,33%-l ja rindkere deformatsioone 50,68%-l patsientidest. Enamikul uuritud patsientidest esinesid ka skeletiväliseid OI tunnused: 80,14%-l esinesid sinise värvusega skleerad; 60,96%-l patsientidest DI ja 17,81%-l kuulmislangus. Varasemates uuringutes on näidatud siniste skleerade esinemist 75-78,1%; DI esinemiset 19% -62,5% ja kuulmislangust 6,7-13,4% patsientidest.

Detailsem hammaste ja hambumuse hindamine viidi läbi 26-l OI patsiendil vanuses 12-16 aastat, kellel esines DI. Andmeid võrreldi kontrollrühmaga, kuhu kuulus 400 isikut. Leiti olulisi erinevusi OI ja kontrollrühma vahel: sagitaalne lahi > 3,5 mm (0,0% ja 36,2%, P <0,001), vertikaalne lahihambumus > 2 mm (19,2% ja 3,5%, p = 0,004), eesmine risthambumus > 1 mm (76,9% ja 8,5%, p <0,001), tagumine risthambumus (34,6% ja 6,2%, p <0,001) ja puuduvad hambad (42,3% ja 2,2%, p <0,001). Kõige olulisem erinevus OI ja kontrollrühma vahel oli eesmisse risthambumuse osas. Lisaks esines 42,3% uuritavate grupist puuduvaid hambaid, samas kui kontrollgrupis puudus hambaid vaid 2,2%-l. Genotüpiseeriti kokku 91 OI patsienti, kellest 46-l esines DI. Neist 36-l leiti I tüüpi kollageeni mutatsioone: 16-l juhul esines COL1A2 mutatsioon ja 20-l COL1A1 mutatsioon. Seoste tugevuse hindamisel Fisheri testiga selgus, et

Vietnami patsientidel esineb tugev korrelatsioon I tüüpi kollageeni mutatsioonide ja DI esinemise vahel. Sarnaseid tulemusi on leitud ka varasemates uuringutes.

Kollageeni (COL1A1 ja COL1A2) mutatsioonide analüüs teostati 91 omavahel sugulussidemeid mitteomaval OI patsiendil, kellest 42 oli nais- ja 49 meessoost. COL1A1 või COL1A2 mutatsioone leiti kokku 54 isikul, neist esines 33-l COL1A1 ja 21-l COL1A2 mutatsioon. Seega esines kokku 59,4% uuritud isikutest I tüüpi kollageeni mutatsioone, samas kui varasemad uurimised on näidanud kollageeni mutatsioone koguni kuni 90%-l patsientidest. Esines 34 patogeenset COL1A1 geeni varianti ja 22 patogeenset COL1A2 geeni varianti. Neist 17 oli COL1A1 ja 10 COL1A2 uudset varianti, mida ei ole varem andmebaasidesse kantud. De novo mutatsioone täheldati 50%-l COL1A1 mutatsioonidest ja 45,5% COL1A2 mutatsioonidest. Selles uuringus avastatud suur uudsete patogeensete variantide hulk erineb varasemate uuringute andmetest.

Käesolev uuring näitab, et Vietnami o*steogenesis imperfecta* patsiendid on unikaalsed nii fenotüüpide kui genotüüpide poolest ning täiendavate uuringute läbiviimine võiks anda huvitavaid tulemusi.

Kokkuvõte

Uuringu tulemused näitavad, et OI avastamine on Vietnamis probleemiks. Teadaolevate haigestunud isikute vähesus ja raskete fenotüüpide domineerimine näitab, et haigus on tõenäoliselt aladiagnoositud, eriti olukordades, kus OI esinemine perekonnas ei ole teada või haigus esineb kliiniliselt kergel kujul. OI patsiendel Vietnamis oli deformatsioonide ning luumurdude keskmine arv kõrgem. Antud uurimistöö tulemused on abiks Vietnamis arstidele praktilises töös aidates neil paremini ära tunda OI tunnuseid ja parandades diagnoosimise kvaliteeti. Varasem diagnoosimine ja parem ravi aitavad parandada selle harvaesineva haigusega isikute elukvaliteeti.

Dentinogeneesi häire esines 60,96% OI patsientidest. Geneetilise analüüsi tulemuste hindamisel leiti tugev korrelatsioon DI ja I tüüpi kollageeni häire esinemise vahel. DI patsientidel esines mitmeid hambumuse häireid, millest enim olid väljenudnud eesmine risthambumus ning puuduvad hambad. Need võivad oluliselt mõjutada patsientide elukvaliteeti ning nõuavad sageli ortodontilist ravi meditsiinilisel või sotsiaalsetel põhjustel.

Mutatsioonide analüüs OI patsientidel näitas oluliselt väiksemat I tüüpi kollageeni mutatsioonide hulka võrreldes teiste populatsioonidega. Antud uuringus leidsime COL1A1 ja COL1A 2 mutatsioone 59,4%-l patsientidest, samas kui enamikes uuringutes on leitud kollageeni mutatsioone kuni 90%-l OI haigetest. Oluliselt erinev OI mutatsioonide profiil Vietnamis on seotud suure hulga retsessiivsete mutatsioonidega mitte-kollageeni geenides. Tundmatute OI genotüüpide põhjus jääb hetkel ebaselgeks ja vajab edasist uurimist.

11. SUMMARY IN VIETNAMESE

Bênh tạo xương bất toàn tại Việt Nam

Bệnh tạo xương bất toàn (TXBT), là bệnh lý tổn thương thành phần collagen týp I của mô liên kết. TXBT là bệnh di truyền, hiếm gặp với tỷ lệ xảy ra khoảng 1/25.000-100.000 người. Tuy nhiên tỷ lệ mắc bệnh rất khác nhau ở mỗi nước trên thế giới. Bệnh cảnh lâm sàng không những biểu hiện ở hệ xương như gãy xương tự phát, biến dạng xương, lùn, mà còn có biểu hiện ở các cơ quan khác như tạo răng bất toàn, giảm thính lực, củng mạc mắt xanh, các khớp lỏng lẻo, bất thường kết cấu của da. Năm 1979, Sillence và cộng sự đã dựa vào đặc điểm lâm sàng để phân loại bệnh TXBT thành 4 týp. Phân loại này vẫn được sử dụng rộng rãi cho đến ngày nay. Collagen týp I là thành phần protein chính được tìm thấy trong cấu trúc của xương, da, gân, củng mạc, thành mạch, ngà răng và nhiều tổ chức mô liên kết khác. Các gen mã hoá cho sự tổng hợp hoặc cấu trúc collagen týp 1 bị đột biến sẽ dẫn đến bệnh TXBT thường gặp là gen COL1A1 và COL1A2. Khi đột biến làm giảm số lượng collagen có cấu trúc bình thường hay bất thường sẽ gây nên các triệu chứng của bệnh từ mức độ nhẹ đến nặng. Cho đến nay thế giới đã phát hiện ra 17 loại gen đột biến khác nhau gây nên bệnh TXBT. Tuy nhiên các nghiên cứu cho rằng có đến 90% là do đột biến 2 gen COL1A1 và COL1A2. Ngày nay với sự phát triển của các phương pháp "next generation sequencing", là phương thức giải trình tự đồng thời và lượng lớn các đoạn ngắn nucleotide. Nhiều gen gây ra bệnh TXBT đã được phát hiện. Gần đây các nhà khoa học đã chứng minh có sự liên kết giữa đột biến gen lặn và các gen không phải là collagen.

Mặc dù sự tương quan giữa kiểu hình và kiểu gen là mối quan tâm đặc biệt của các nhà nghiên cứu và lâm sàng, tuy nhiên mối liên quan giữa đột biến gen và mức độ trầm trọng của bệnh vẫn chưa được rõ ràng và cần nghiên cứu hơn nữa. Bệnh TXBT là bệnh hiếm gặp, vì thế đa số các trường hợp bị bỏ sót trong chẩn đoán hoặc chẩn đoán muộn. Chẩn đoán chủ yếu dựa vào tiền sử cá nhân và gia đình, khám lâm sàng và xét nghiệm. Những trường hợp khó chẩn đoán thường gặp do không có tiền sử bệnh TXBT của gia đình, dấu hiệu lâm sàng nhẹ, hoặc cá nhân không có tiền sử gãy xương. Tùy theo từng giai đoạn tuổi mà có thể sử dụng các phương pháp chẩn đoán thích hợp. Trong bào thai thì dựa vào tiền sử gia đình, siêu âm có thể thực hiện để xác định chẩn đoán vào tuần thứ 16 của tuổi thai hoặc phân tích DNA. Chẩn đoán ở giai đoạn sơ sinh thì dựa vào xương bị biến dạng cong hoặc gãy, củng mạc mắt xanh, hoặc hình ảnh loãng xương của các xương dài hoặc khuyết xương sọ trên X quang. Triệu chứng nổi bật để chẩn đoán TXBT ở trẻ em là gãy xương dài, gãy xương lặp lại nhiều lần dù chấn thương rất nhỏ, các xương dài bị biến dạng. Cần phải chẩn đoán sớm, lập kế hoạch chăm sóc và điều trị thích hợp nhằm ngăn ngừa các biến chứng, cải thiện chất lượng cuộc sống của bệnh nhân và gia đình bệnh TXBT.

Với tỷ lệ mắc bệnh chung của thế giới hiện nay, và với dân số Việt Nam hơn 90 triệu, chúng ta có thể ước tính số lượng bệnh nhân TXBT sẽ rất lớn. Những

kiến thức về lâm sàng và đột biến gen của bệnh TXBT chưa được cập nhật ở cộng đồng và các cơ sở y tế. Những nghiên cứu về lâm sàng và phân tích gen bệnh lý TXBT của người Việt Nam vẫn chưa được công bố rộng rãi trên các nghiên cứu quốc tế. Vì vậy, chúng tôi thực hiện đề tài này với mục tiêu:

1. Mô tả các đặc điểm lâm sàng và các thể loại khác nhau của bệnh TXBT tại Việt Nam.
2. Mô tả các đặc điểm về răng và khớp cắn trong bệnh nhân TXBT.
3. Mô tả đặc điểm đột biến gen COL1A1 và COL1A2 của những người Việt Nam bị bệnh TXBT

Đối tượng và phương pháp nghiên cứu

Từ năm 2013 đến 2016, chúng tôi thực hiện một nghiên cứu mô tả cắt ngang về bệnh TXBT. Danh sách bệnh nhân được thu thập từ các bệnh viện, trung tâm và các câu lạc bộ TXBT trên cả nước. Một nhóm bác sĩ của Bệnh viện Trường Đại học Y Dược Huế và Đại học Tartu- Estonia đến thăm khám và thu thập dữ liệu tại nhà các gia đình bệnh TXBT. Những gia đình có bệnh TXBT được chọn với điều kiện được chẩn đoán đúng TXBT và tình nguyện tham gia vào chương trình.

Những dữ liệu được thu thập bao gồm thông tin về lâm sàng và phả hệ. Phỏng vấn trực tiếp các thành viên trong gia đình để thu thập các thông tin xây dựng cây phả hệ, bao gồm 3 thế hệ cho mỗi gia đình. Tất cả bệnh nhân được lựa chọn đều được thăm khám trực tiếp để ghi nhận tiền sử, bệnh sử, triệu chứng lâm sàng, tình trạng chăm sóc, theo dõi, điều trị của bệnh. Thu thập dữ liệu còn căn cứ vào các hồ sơ y tế lưu trữ, X quang của mỗi gia đình. Thăm khám ghi nhận các triệu chứng của hệ cơ xương khớp, cũng như các cơ quan khác liên quan đến bệnh TXBT. Chẩn đoán týp bệnh dựa vào phân độ từ I-IV của Sillence. Thông tin về bệnh nhân bao gồm những thông tin lúc mới sinh (cân nặng, gãy xương trong bào thai hay khi sinh), tiền sử về gãy xương (thời gian và xương gãy lần đầu, ước tính tổng số gãy và xương gãy), biến dạng các xương, các triệu chứng ngoài hệ cơ xương khớp như tạo răng bất toàn, giảm thính lực, củng mạc mắt xanh. Các mức độ vận động bình thường hay giới hạn vận động đi lại. Biến dạng xương cũng được thăm khám và đánh giá.

Nghiên cứu về các bệnh lý liên quan đến răng và khớp cắn trong TXBT được chọn ngẫu nhiên một nhóm bệnh nhân TXBT từ 12 đến 16 tuổi, gồm 26 bệnh nhân. Nhóm chứng được chọn ngẫu nhiên gồm 200 học sinh phổ thông và 200 sinh viên đại học. Chúng tôi phân tích các triệu chứng về khớp cắn như độ cắn chìa, độ cắn phủ, độ cắn hở, cắn chìa răng dưới, cắn chéo răng sau và mất răng.

Mẫu máu có chống đông EDTA được thu thập từ bệnh nhân, cha mẹ, anh chị em, và người thân trong gia đình để phân tích gen. DNA được chiết xuất và phân tích giải trình tự theo phương pháp Sanger. Phát hiện các biến thể đột biến

dựa vào so sánh trình tự gen của bệnh nhân với trình tự gen chuẩn của ngân hàng gen thế giới.

Kết quả

Chúng tôi thăm khám và chọn lựa 146 bệnh nhân TXBT (61 nữ và 85 nam) từ 120 gia đình có bệnh TXBT. Các gia đình cư trú ở miền Bắc là 30.8%, miền Trung 44.2%, và miền Nam 25.0%. Nhóm bệnh dưới 15 tuổi có số lượng nhiều nhất chiếm 70.55%. Theo phân độ của Sillence, týp I có 46 bệnh nhân, týp III 46 bệnh nhân và týp IV 54 bệnh nhân. Tỷ lệ mắc bệnh TXBT trong nghiên cứu của chúng tôi thấp hơn nhiều so với tỷ lệ mắc bệnh hiện hành của thế giới. Chúng tôi giả thuyết rằng, tỷ lệ mắc bệnh TXBT thấp có thể do nhiều trường hợp chưa được chẩn đoán, đặc biệt những trường hợp bệnh nhẹ hoặc không có tiền sử gia đình về bệnh TXBT, đồng thời cũng có thể do đặc điểm về văn hóa và tình trạng kinh tế của gia đình.

146 bệnh nhân TXBT được sinh ra từ 133 bà mẹ, trong đó 36 bà mẹ có tiền sử sẩy thai. 21 bệnh nhân bị sinh non (trước 37 tuần thai). Cây phả hệ phát hiện 21 gia đình có 2 đến 3 thế hệ có bệnh nhân TXBT. 99 gia đình không có tiền sử về bệnh trên lâm sàng. Có 4 cặp bệnh nhân sinh đôi. Trong 146 bệnh nhân có 142 bệnh có tiền sử gãy xương, 4 cá thể chưa gãy xương lần nào nhưng có các biểu hiện của TXBT như biến dạng xương, tạo răng bất toàn, giảm thính lực, củng mạc mắt xanh, hoặc có tiền sử gia đình bị bệnh TXBT. Có 125 bệnh nhân gãy xương lần đầu khi nhỏ hơn 6 tuổi. 34 bệnh nhân được phát hiện gãy xương trong bào thai nhờ siêu âm chẩn đoán ở giai đoạn tiền sản, 9 bệnh nhân bị gãy xương trong giai đoạn sinh. 132 bệnh nhân có gãy xương đùi trong tiền sử và 92 bệnh nhân gãy xương đùi là xương gãy đầu tiên. Tỷ lệ trung bình gãy xương ở týp I, III, IV lần lượt là 6.04; 20.76 và 12.94. Chúng tôi phát hiện có 18 bệnh nhân gãy trên 30 lần trong đời sống của họ.

Biến dạng cũng gặp ở hầu hết bệnh nhân với những mức độ nặng nhẹ và vị trí biến dạng khác nhau. Biến dạng chi dưới chiếm 82.88%, chi trên 54.8%, biến dạng cột sống 62.33%, lồng ngực 50.68%. Nghiên cứu của Lin (2009) với 54% biến dạng.

Nghiên cứu của chúng tôi phát hiện 89 bệnh nhân (60.96%) tạo răng bất toàn. Theo nghiên cứu của Majorana (2010), tỷ lệ tạo răng bất toàn là 62.5%, của Sæves (2009) là 19%. Nghiên cứu cho thấy có 117 bệnh nhân có củng mạc mắt xanh (80.14%), báo cáo của tác giả Patel (2015) là 78.1%. Giảm thính lực gặp ở 26 bệnh nhân (17.81%). Các nghiên cứu khác của Kuurila (2000) và Patel (2005) cho thấy giảm thính lực gặp từ 6.7 đến 13.4%.

Đánh giá về khớp cắn ở 26 bệnh nhân TXBT (30.8% nữ, và 69.2% nam), so sánh với 400 cá thể từ nhóm chứng. Kết quả có sự khác biệt giữa nhóm bệnh và chứng về độ cắn chìa > 3.5 mm (0.0% và 36.2%, $P < 0.001$), độ cắn hở > 2 mm (19.2% và 3.5%, $P = 0.004$), cắn chìa răng dưới > 1 mm (76.9% và 8.5%, $P < 0.001$), độ cắn chéo răng sau (34.6% và 6.2%, $P < 0.001$), mất răng (42.3% và 2.2%, $P < 0.001$). Sự khác biệt có ý nghĩa giữa nhóm bệnh và chứng gặp ở cắn chìa răng dưới và mất răng. Chúng tôi giả thuyết rằng có sự đột biến của

collagen dẫn đến thay đổi cấu trúc các xương hàm trên và hàm dưới, và kết quả dẫn đến cắn chìa răng dưới.

Phân tích đột biến gen COL1A1 và COL1A2 trên 91 bệnh nhân không có liên hệ huyết thống, trong đó 46 bệnh nhân có tạo răng bất toàn. Với kết quả 36/46 bệnh nhân được chẩn đoán đột biến collagen týp I, trong đó 16 bệnh nhân đột biến COL1A2 và 20 bệnh nhân đột biến COL1A1. Nghiên cứu đã thể hiện sự liên quan giữa đột biến kiểu gen và tạo răng bất toàn, điều này cũng tương tự kết quả với các nghiên cứu của tác giả Lindahl (2015), Rauch (2010) và Amor (2011).

Phân tích đột gen trên mẫu máu của 91 bệnh nhân (49 nam và 42 nữ) không có liên hệ huyết thống cho kết quả 54 (59.4%) bệnh nhân có đột biến gen COL1A1 và COL1A2. Trong đó có 33 (36.3%) bệnh nhân đột biến COL1A1 và 21 (23.1%) bệnh nhân với COL1A2. Những báo cáo nghiên cứu của Marini (2007), Valadares (2014), Van Dijk (2014), Shapiro (2014) cho thấy có đến 90% đột biến collagen týp I. Mới đây nhóm chúng tôi cũng phân tích đột biến collagen týp I trên nhóm bệnh nhân Estonia và Ukraina, cùng một phương pháp chọn mẫu và phân tích, cho kết quả đột biến collagen týp I là 89.66% (26/29) ở Estonia và 65.79% (50/76) ở Ukraina. Kết quả này củng cố thêm giả thuyết có sự khác biệt về tỷ lệ đột biến gen COL1A1/2 ở từng nhóm quần thể, chủng tộc khác nhau. Điều này cũng giải thích thêm sự khác biệt có ý nghĩa về tỷ lệ đột biến gen COL1A1/2 giữa Việt Nam và các nước khác trên thế giới.

Trong 34 biến thể đột biến gen COL1A1 và 22 biến thể đột biến gen COL1A2, chúng tôi phát hiện 17 biến thể đột biến mới của COL1A1 và 10 của COL1A2 chưa được các nghiên cứu trước đây đề cập đến. Nghiên cứu đã phát hiện nhiều biến thể đột biến mới hơn các nghiên cứu của Stephen (2014), Lin (2015), Yang (2011) và Ward (2001). Liên quan đến sự thay thế của acide amin trong đột biến COL1A1 và COL1A2, glycine được thay thế nhiều nhất chiếm tỷ lệ 64.3%. Các kết quả nghiên cứu này khẳng định sự cần thiết phải tiếp tục nghiên cứu để phát hiện đột biến gen không phải collagen týp I trên quần thể người Việt Nam.

Kết luận

1. Kết quả nghiên cứu cho thấy tồn tại nhiều thách thức trong bệnh TXBT tại Việt Nam. Tỷ lệ bệnh nhân chưa được phát hiện cao, đặc biệt ở các gia đình không có tiền sử, hoặc bệnh nhân ở thể nhẹ. Các đặc điểm về lâm sàng và thể loại ở Việt Nam cũng giống các nước khác trên thế giới. Tuy nhiên tỷ lệ biến chứng như gãy xương và biến chứng khác gặp cao hơn so với các nghiên cứu đã công bố. Nghiên cứu bệnh TXBT tại Việt Nam này có thể dùng để hướng dẫn các nhân viên y tế phát hiện, chẩn đoán bệnh nhân TXBT qua các dấu hiệu lâm sàng, giúp chẩn đoán sớm và ngăn ngừa các biến chứng cho bệnh nhân. Cần thực thi các điều tra và thực hiện các dịch vụ y tế rộng rãi, để giúp bệnh nhân TXBT cải thiện được chất lượng cuộc sống của họ và gia đình.

2. Tạo răng bất toàn gặp ở 60.96% trường hợp bệnh nhân TXBT. Phân tích gen cho thấy có sự liên quan giữa tạo răng bất toàn và bất bình thường của collagen týp I. Các triệu chứng liên quan đến khớp cắn như độ cắn chìa, độ cắn phủ, độ cắn hở, cắn chìa răng dưới, cắn chéo răng sau và mất răng là rất phổ biến. Do những triệu chứng này có tác động nhiều đến cuộc sống của bệnh nhân, nên cần phải theo dõi và điều trị về chỉnh nha giúp cho bệnh nhân cải thiện được chất lượng cuộc sống.
3. Phân tích đột biến gen cho thấy tỷ lệ đột biến collagen týp I thấp hơn so với các quần thể khác trên thế giới. Nghiên cứu của chúng tôi phát hiện tỷ lệ đột biến của gen COL1A1 và A2 là 59.4%, trong khi các báo cáo của các nghiên cứu khác có thể đến 90%. Điều đó thể hiện kiểu gen đột biến ở người Việt Nam liên quan đến đột biến lặn các gen không thuộc collagen týp I. Cần tiếp tục nghiên cứu và phân tích kiểu gen của những cá thể này trong tương lai.

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Last but not the least, my family, who were always by my side and shared joys and difficulties with me in these four years. I love all of you.

Thank you very much, everyone!

PUBLICATIONS

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EDUCATIONAL

1982–1988	Hue Medical College, student, graduated in 1988
1989–1991	Certificate of the First-level course specializing in Surgery
1998–2000	Certificate of the Master course specializing in Surgery
2000–2001	Training surgery in Herz-Jesus Hospital, Germany
2007–2009	Certificate of the second-level course specializing in Surgery
2013 to present	Tartu University, Estonia, PhD student

PROFESSIONAL EMPLOYMENT

1989- 2001	Hue secondary Medicine School, lecturer
2002 to present	Hue University Hospital, Department of Traumatology and Orthopedics
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PUBLICATIONS

Books

Fundamental nursing, Volume 1,2 The medical publisher, MOH, HaNoi, 2008, Co-author

Surgical Nursing, Volume 1,2 The medical publisher, MOH, HaNoi, 2008, Co-chief-editor

Publications

Early results of reconstruction for lesions of ACL by four-strand semitendinous tendon autograft trans Arthroscopy. Vietnam Practice Medicine Magazine, Health ministry, number 521/2005

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2000–2001	Praktiline ortopeedia väljaõppekursus Herz-Jesus Hospitalis, Saksamaa
2007–2009	Septsialiseerumine (teine aste), ortopeedia
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Töökohad

1989–2001	Hue II Meditsiinikool, lektor
2002–	Hue Ülikooli Haigla, Traumatoloogia ja Ortopeedia osakond, ortopeed
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Raamatud

Fundamental nursing, Volume 1,2 The medical publisher, MOH, HaNoi, 2008, Co-author

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