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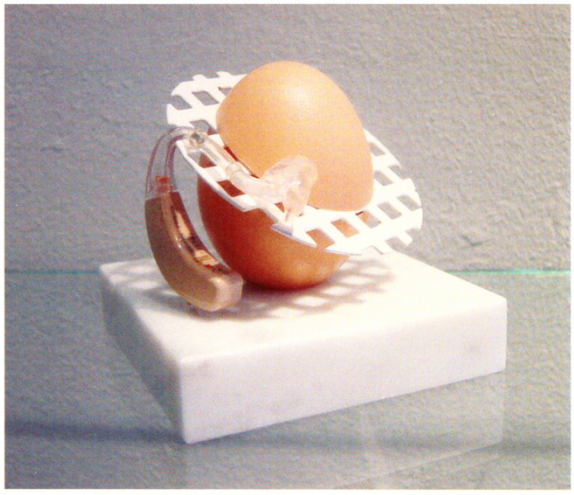
MEDICA - ODONTOLOGICA

HEARING LOSS, BALANCE PROBLEMS AND MOLECULAR DEFECTS IN OSTEOGENESIS IMPERFECTA

- A Nationwide Study in Finland

by Kaija Kuurila



TURUN YLIOPISTO
Turku 2003

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- A NATIONWIDE STUDY IN FINLAND

by

Kaija Kuurila

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Turku 2003

From the Department of Otorhinolaryngology – Head and Neck Surgery,
University of Turku
Turku, Finland

Supervised by

Professor Reidar Grénman, MD
Department of Otorhinolaryngology-Head and neck surgery
University of Turku, Turku, Finland

and

Docent Ilkka Kaitila, MD
Department of Medical Genetics
University of Helsinki, Helsinki, Finland

Reviewed by

Professor Jaakko Ignatius, MD
Department of Genetics
University of Oulu, Oulu, Finland

Professor Ulrik Pedersen, MD
Department of Otorhinolaryngology – Head and Neck Surgery
Aarhus University Hospital, Aarhus, Denmark

and

Docent Hans Ramsay, MD
Department of Otorhinolaryngology – Head and Neck Surgery
University of Helsinki, Helsinki, Finland

Official opponent

Professor David Sillence, MD
Department of Medical Genetics
University of Sydney
Sydney, Australia

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To my family

Cover: " The most visible symptom of OI is the susceptibility to bone fractures. Although the fractures may heal, each new crack injures the support structures of the body. The more you crack, the more scars and flawed posture you are left with. The medication that would patch Humpty Dumpty up again to be as good as new has yet to be discovered. "

Jenni-Juulia Wallinheimo, Humpty Dumpty sat on the wall 2003
photo: Otto Vara

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Abstract

Kaija Kuurila. Hearing loss, balance problems and molecular defects in Osteogenesis imperfecta – a nationwide study in Finland. Department of Otorhinolaryngology - Head and Neck Surgery, University of Turku, Finland. Annales Universitatis Turkuensis, Ser. D. Tom. 572, ISBN 951-29-2526-5, ISSN 0355-9483. Painosalama, Turku, Finland 2003.

Hearing loss, bone fragility and blue sclerae are the principal clinical features in Osteogenesis imperfecta (OI), a genetic disorder mostly caused by mutations in type I collagen genes COL1A1 and COL1A2. Different mutations partially explain the interindividual and intrafamilial variability of the disease.

In this nationwide study on hearing in OI, 299 Finnish patients with OI were ascertained (prevalence 5.74/100 000). Of the 183 patients with audiometric evaluation, 46 were children and 137 were adults.

Hearing loss may already be present in childhood. We found hearing loss in three children. Subjective misjudgment of hearing ability was observed in almost 20% of adult patients. Hearing loss was found in 58% of adults. It affects patients with all types of OI, but tends to be more common in OI types I and III than in OI type IV. Sensorineural hearing loss, especially at an early age, may be more common in OI type I. Because the early detection and treatment of hearing loss is important to avoid aggravation of physical handicap, audiometry should be performed in all OI patients at the age of 10 years and repeated every third year thereafter.

Vestibular dysfunction is common in OI. Inner ear damage appears to be the main reason for vertigo. Occasionally, vertigo is caused by basilar impression (BI), a craniocervical complication affecting up to 25% of OI adults. Still, some OI patients without BI or hearing loss also suffer from vertigo.

Results of 43 middle ear operations were evaluated. The surgical anatomy in OI differs from otosclerosis especially by thick and vascular mucosa with excessive bleeding tendency, and elastic, fractured or atrophic stapes crura. These anatomical peculiarities cause technical problems, and a prerequisite for successful surgery is a correct preoperative differential diagnosis of OI-related hearing loss and otosclerosis. Furthermore, the hearing gain appears to be better after surgery centralized in units with a larger annual number of operations and more experienced surgeons.

Mutations in COL1A1 or COL1A2 were found in 49 unrelated patients, representing the molecular genetic background of 41.1 % of the Finnish OI population. Mutation type is associated with OI type, while null allele mutations most often produce OI type I, and single base substitutions resulting in glycine substitutions in proα2(I) tend to produce OI type I and IV. Neither the mutated gene nor the type of mutation correlated with the presence, type or severity of hearing loss. The hearing loss in OI apparently is a result of multifactorial, yet unknown genetic and environmental effects.

Keywords: Osteogenesis imperfecta, Hearing loss, Vertigo, Basilar impression, Middle ear surgery, Genotype, Phenotype, COL1A1, COL1A2, Molecular defect

List of original publications

This thesis is based on the following original papers, referred to in the text by the Roman numerals I-V:

I **Kuurila K, Grénman R, Johansson R, Kaitila I.** Hearing loss in children with Osteogenesis imperfecta. Eur J Pediatr 2000; 159:515-519

II **Kuurila K, Kaitila I, Johansson R, Grénman R.** Hearing loss in Finnish adults with Osteogenesis imperfecta: A nationwide survey. Ann Otol Rhinol Laryngol 2002;111:939-946

III **Kuurila K, Kentala E, Karjalainen S, Kovero O, Pynnönen S, Kaitila I, Grénman R, Waltimo J.** Vestibular dysfunction in adult patients with Osteogenesis imperfecta. Am J Med Genet 2003; 120A: 350-358

IV **Kuurila K, Pynnönen S, Grénman R.** Stapes surgery in Osteogenesis imperfecta in Finland. Ann Otol Rhinol Laryngol (accepted)

V **Hartikka H, Kuurila K, Körkkö J, Kaitila I, Grénman R, Pynnönen S, Hyland J, Ala-Kokko L.** Osteogenesis imperfecta: Correlation of molecular genetic findings with hearing loss and other clinical features. (Submitted)

In addition, some unpublished data on basilar impression and basilar invagination are presented.

The first two authors made equal contributions (V).

Abbreviations

ABR	auditory brainstem response
BI	basilar impression / basilar invagination
COL1A1	gene encoding the proα1 chains of type I collagen
COL1A2	gene encoding the proα2 chain of type I collagen
bp	base pair(s)
dB	decibel
dNTP	deoxynucleotide triphosphate
CSGE	conformation sensitive gel electrophoresis
DI	dentinogenesis imperfecta
ENG	electronystagmography
HL	hearing level
Hz	hertz
kb	kilobase(s)
OAE	otoacustic emissions
OFI	ocular fixation index
OI	osteogenesis imperfecta
PCR	polymerase chain reaction

1. Introduction

Osteogenesis imperfecta (OI) is a genetic disease of connective tissue. Bone fragility is the main feature of OI, and it may be accompanied by blue sclerae and hearing loss. Short stature, bone deformities, easy bruising and reduced life span are common, and the dentin in teeth may be developmentally abnormal (Byers 2002; Sillence et al. 1979b; Waltimo et al. 1996). The final outcome of OI is in some cases severe physical disability and secondary psychosocial deprivation and, therefore, causes considerable human suffering, while in the majority of cases an almost normal life may be achieved. OI probably affects more than 1/10 000 people. However, precise prevalence calculation is difficult because of unfamiliarity with the disease and poor reporting of some of its forms (Byers 2002; Heiberg 1983; Sillence et al. 1979b; Smårs 1961).

Currently, OI is divided into six different types. Autosomal dominantly inherited type I OI is the mildest and most common form, while the most seriously affected, sporadic patients with type II OI usually die within the first months of life (Glorieux et al. 2000; Glorieux et al. 2002; Hall 2002; Sillence et al. 1979b).

The diagnosis of OI may be obvious at birth, but in mild forms the diagnosis may be delayed or the disease remains undiagnosed (Bischoff et al. 1999; Byers 2002; Paterson et al. 2001; Sillence et al. 1979b). Furthermore, unexplained clinical variability in expression has been observed within many families (Byers 2002). OI is mainly caused by mutations in one of the two genes, COL1A1 and COL1A2, that encode the synthesis of type I collagen (Heller et al. 1975; Penttinen et al. 1975; Sykes 1993; Sykes et al. 1990). Type I collagen forms highly organized fibres and fibrils that provide the structural support for the body in bones, tendons, ligaments, skin, teeth and fasciae (Prockop et al. 1995; Vuorio et al. 1990). As a result of the mutation, the procollagen may be normal, but secreted in smaller amounts than normal, generally leading to milder phenotypes. Moreover, both normal and abnormal procollagen can be secreted, resulting in altered structure and a wide range of phenotypes, from extremely mild to lethal (Byers 2002; Sillence et al. 1979b; Wenstrup et al. 1990). Almost all affected families have their own private mutation (Ala-Kokko et al. 1994). If found, the mutation confirms the diagnosis. In addition, through the mutation, the pathogenesis of the disease may be further studied and understood.

Hearing loss affects about half of the patients with OI (Garretsen et al. 1997; Paterson et al. 2001; Pedersen 1984; Stewart et al. 1989). It is reported to begin in the second to third decade of life, and although there are clinical similarities with otosclerosis, they are distinct entities (Cox et al. 1982; Garretsen et al. 1997; Holdsworth et al. 1973; Pedersen 1984; Quisling et al. 1979; Riedner et al. 1980). Hearing loss has been suggested to be more common in OI type I than IV, and the expression of hearing loss has been reported to vary in a family (Byers 2002; Paterson et al. 2001; Sillence et al. 1979b). Hearing loss in OI may be treated with middle ear surgery similar to that used in otosclerosis. Despite the anatomical peculiarities in the middle ear in OI patients,

the postoperative hearing results have been encouraging, albeit not as satisfactory as in otosclerosis (Armstrong 1984; Cremers et al. 1991; Garretsen et al. 1990; Glasscock et al. 1995; Palva et al. 1977; Pedersen et al. 1983; Shea et al. 1982; Van Der Rijt & Cremers 2003).

Balance problems have been reported as a symptom of basilar impression, a feared craniocervical abnormality sometimes associated with OI (Elies et al. 1980; Hayes et al. 1999; Pozo et al. 1984; Sawin et al. 1997; Sillence 1994). On the other hand, vestibular dysfunction frequently associates with otosclerosis, particularly in patients with sensorineural hearing loss (Cody et al. 1978; Ghorayeb et al. 1978; Igarashi et al. 1982; Morales-Garcia 1972; Thomas et al. 1981; Virolainen 1972).

There are no published studies on the possible association of molecular pathology in OI with different characteristics of hearing loss.

Finland with its homogeneous population and advanced health care registers offers an excellent possibility to collect extensive data on rare disorders, and to study the frequency of certain symptoms in them. The aim of this study was to evaluate the frequency, characteristics and treatment of hearing loss in the Finnish OI population.

2. Review of the literature

2.1. Osteogenesis imperfecta

2.1.1. Historical aspects

OI is a disease known to have existed since antiquity. In the beginning of the nineteenth century, several intact tombs dating from the 21st dynasty (c.1000 B.C.) were found on the east bank of the Nile in Egypt. One small painted coffin in the form of an Osiris figure contained bones that were suggested to be the remains of a mumified monkey. The bones were examined in 1967, revealing the skeleton of an infant with skull and long bones showing the typical appearance of OI, as well as teeth with manifestations of Dentinogenesis imperfecta (DI). So far, this is the oldest known case of OI (Gray 1969). The oldest report of bone fragility dates from the sixteenth century. According to Seedorff, Malbranche reported in 1678, in *Traite de la recherché de la verité* an imbecile, 20-year-old male with bones fractured in as many places as those of a criminal sentenced to be broken on the wheel (Seedorff 1949). Ekman, in his Doctoral thesis in Uppsala in 1788, presented hereditary bone fragility through three generations, a condition he named Osteomalacia congenita (Ekman 1788). Association of bone fragility with blue sclerae was also reported by Axmann, who himself suffered from the disease (Axmann 1831). While the work of Ekman remained unnoticed for a long time, Lobstein received medical acclaim for recognizing the hereditary nature of fragile bones by describing “osteopsathyrosis idiopathica” in 1833 (Lobstein 1835). Vrolik presented the term “Osteogenesis imperfecta” in 1849, by describing an infant with large head and multiple congenital fractures (Figure 1) (Vrolik 1849). The three classical symptoms of OI, bone fragility, blue sclerae and hearing loss, were first described by Adair-Dighton in 1912 in a case report (Adair-Dighton 1912). This triad of OI was confirmed by van der Hoeve and de Kleyn in 1918, and supported by several reports during the following years (Bell 1928; Bronson 1917; Cleminson 1926; Fraser 1919; van der Hoeve et al. 1918).

Seedorff performed the first population study of OI in Denmark in 1949. He personally examined all the living 180 Danish patients travelling by motorcycle around Denmark. All patients had blue sclerae, 84% had bone fragility, and 26% suffered from hearing loss. Dental fragility was also reported. Seedorff also described a historical person with OI, the Danish prince Ivar Boneless, who was said to have legs as soft as cartilage and could not walk, and therefore, he was carried into battle on a shield. His multiple fractures did not prevent him from conquering large parts of England. Unfortunately, William the Conqueror prevented a further study of his bones, when after his conquest he dug up the bones of Ivar Boneless and burned them (Seedorff 1949).

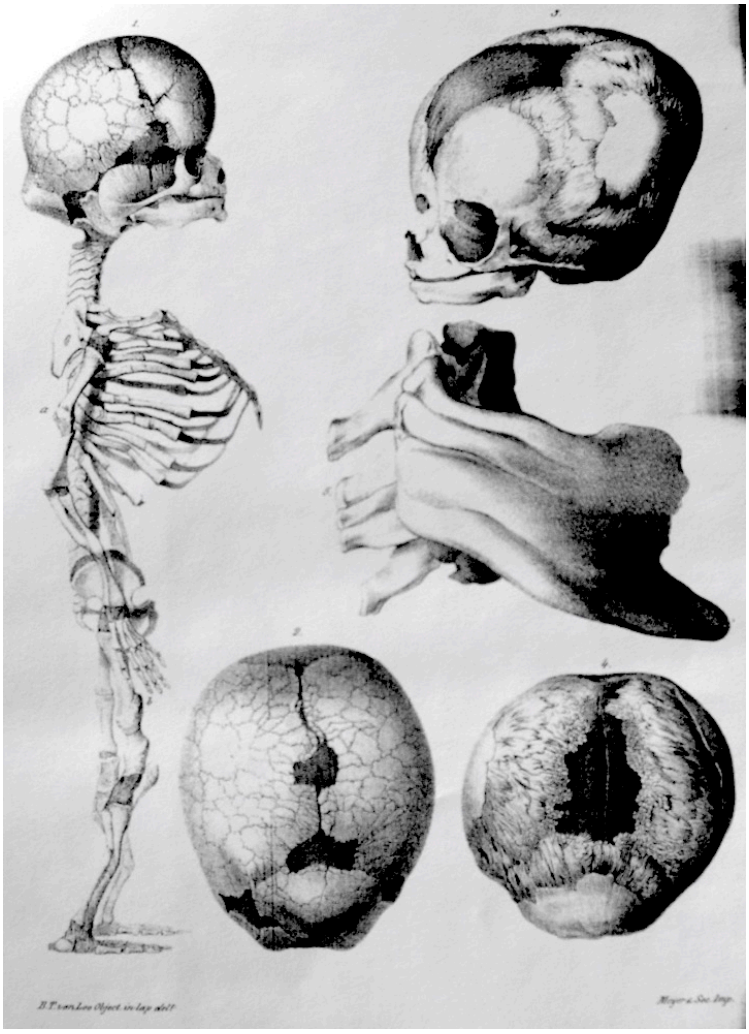

Figure 1. *Vroliks illustration of an infant with imperfect osteogenesis. The child succumbed on the third day of life (Vrolik 1849).*

2.1.2. Epidemiology

OI has been estimated to affect more than 1/10 000 persons, but true whole population studies in hearing loss in OI are scanty (Byers 2002). Prevalence numbers are suspected to be affected by the rarity of and unfamiliarity with the disease, as well as poor reporting of some forms. The perinatally lethal case may remain without diagnosis and the evaluation at birth may also miss the most mildly affected patients (Byers 2002). No racial predisposition has been found (Byers 2002; Seedorff 1949). In population studies on OI in Sweden, Norway and Denmark, prevalence figures between 3.3/100 000 and 5/100 000 have been found (Heiberg 1983; Pedersen 1983; Smårs 1961).

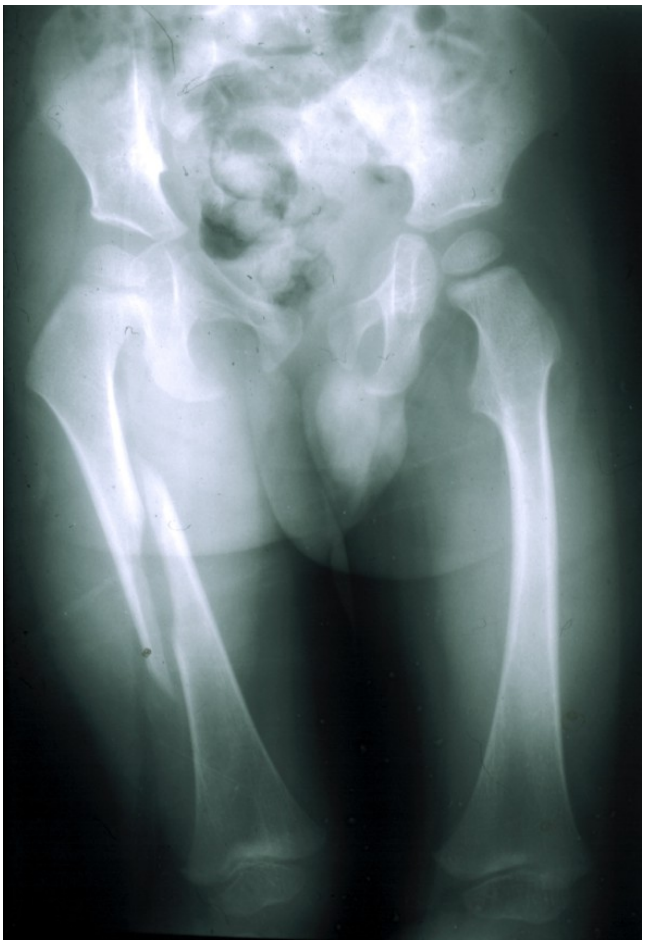

Figure 2. *A skeletal radiograph on the pelvis and femurs of a 2 -year-old boy with a recent diaphyseal fracture of the right femur. The type of the disease was OI type IA.*

In Finland, the epidemiology of OI has not been previously studied. The incidence of OI type I has been estimated to be 1/15 000-20 000 live births, and that of the perinatal lethal type OI II, 1/20 000-1/60 000 (Byers 2002).

2.1.3. Clinical characteristics

OI presents with various manifestations from connective tissue rich in collagen; in bone, tendon, ligament, skin, teeth and fasciae (Prockop et al. 1995; Vuorio et al. 1990). The main feature of OI is bone fragility resulting in fractures caused by minor traumas (Figure 2). Individually, fractures may be rare or frequent, from a few fractures during the whole life time to hundreds already during childhood (Byers 2002; Sillence 1988). Some 10% of the patients do not have any fractures at all (Sillence 1988). Short stature is common, and patients may present with secondary deformities in tubular bones, spine and skull (Byers 2002; Sillence 1988). In severe

forms, bowed limbs and kyphoscoliosis are common (Beighton et al. 1983; Engelbert et al. 1998; Sillence 1988). Sclerae may be intensely blue throughout life (Figure 3), but they may also be blue in infancy, and later pale blue or white (Sillence 1988).

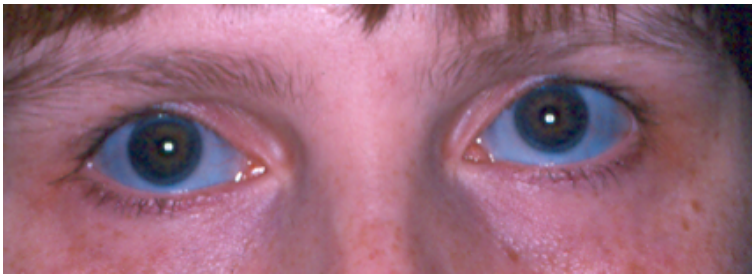

Figure 3. *Blue sclera in a patient with OI type I.*

Hearing loss affects about half of the patients with OI (Garretsen et al. 1991a; Paterson et al. 2001; Pedersen 1984; Quisling et al. 1979; Riedner et al. 1980; Sillence et al. 1979b; Stewart et al. 1989). The organic dentin matrix in teeth can be developmentally aberrant. The affected teeth appear discolored and exhibit fragility due to the poor mineralization of the dentin. This condition has been designated as Dentinogenesis imperfecta (DI type I, Figure 4) (Shields et al. 1973). However, the severity of dentinal manifestations in patients with OI apparently forms a continuum from seemingly normal dentin structure to severe DI. (Waltimo et al. 1996). Joint hypermobility is common, and dislocations and herniae may occur, as well as easy bruising, increased sweating and heat intolerance (Byers 2002; Engelbert et al. 1998; Sillence 1988). Frontal and temporal bossing may contribute to the triangular face typical for OI, and Wormian bones or the radiographic bony islands in the occipital and parietal area contribute to the softness of the skull in childhood (Sillence 1988). Basilar impression may occur (Engelbert et al. 1998; Sillence 1994). In severe forms of OI, the life span is reduced (Byers 2002; Paterson et al. 1996).

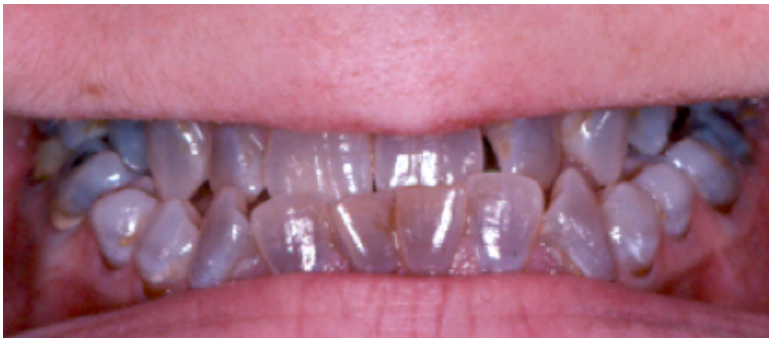

Figure 4. *Visually apparent Dentinogenesis imperfecta in a patient with type IV OI.*

2.1.4. Classification

Until the 1970´s, a classification of OI introduced in 1906 by Looser was used (Looser 1906). He suggested that the hereditary idiopathic bone fragility, Osteogenesis imperfecta, presented by Vrolik in 1849, and the congenital bone fragility named as Osteopsathyrosis idiopathica by Lobstein in 1833, would be one disease entity which may appear with two distinct variants of severity: OI congenita with fractures at birth (Vroliks disease), and OI tarda with fractures first some months or years after the birth (Lobsteins disease) (Lobstein 1835; Looser 1906; Vrolik 1849).

Sillence introduced the classification of OI currently in use in 1979. It was based on clinical and radiographic criteria and the mode of inheritance (Sillence et al. 1978; Sillence et al. 1979a; Sillence et al. 1979b). Later, this classification has been modified on several occasions (Table 1) (Glorieux et al. 2000; Glorieux et al. 2002; Hall 2002; Sillence 1981; Sillence 1988). However, the wide spectrum of the disease, as well as considerable interfamilial and intrafamilial variability often make classifications difficult (Primorac et al. 2001).

In sporadic cases it may be clinically impossible to distinguish severely affected type IV from type III, or type IV cases from types I and III (Sillence 1981).

The catalogue of human genes and genetic disorders, Online Mendelian Inheritance of Man, lists 60 OMIM entries under the diagnosis of Osteogenesis imperfecta. This fact indicates that there is no universally accepted diagnostic classification of OI at the present time. Therefore, in this thesis the numbers of the OMIM entries have been used with the types of OI in the Sillence classification, only (Online Mendelian Inheritance in Man 2000).

2.1.4.1. Osteogenesis imperfecta type I (OMIM 166200)

OI type I is the most common form of OI. Sclerae are distinctly blue and hearing loss is common. Fractures may present already at birth or in the perinatal period, but they usually appear when the child begins to walk. Bone fragility is often quite constant during childhood, decreases after puberty, and increases again after the menopause in women and in the sixth to eighth decade in men. However, some patients with OI type I have no fractures. The fractures mostly affect the long bones, ribs and the small bones of hands and feet. Skeletal deformities may appear, but the long bone deformity is not as severe as in other types of OI. Hyperextensibility of ligaments, bowing and angulation due to fractures and deformities at the knees and in the feet may appear. Kyphosis or scoliosis may occur in some patients. Easy bruising is common. Type I OI is further divided into subgroups A and B, based on visual absence (A) or presence (B) of DI (Byers 2002; Sillence 1988; Sillence & Rimoin 1978; Sillence et al. 1979b). The inheritance is autosomal dominant (Sillence et al. 1979b).

Table 1. *The Sillence classification of Osteogenesis imperfecta, discussed and modified at the 8th International Conference on Osteogenesis imperfecta, Annecy, 2002 (Glorieux et al. 2000; Glorieux et al. 2002; Hall 2002; Sillence 1981) . AD, Autosomal Dominant; AR, Autosomal Recessive*

OI Type	Clinical features	Inheritance
IA	Mild to severe bone fragility; normal or slightly short stature, intensely blue sclerae, presenile hearing loss, normal teeth	AD
IB	Mild to severe bone fragility; normal or slightly short stature, intensely blue sclerae, presenile hearing loss, DI	AD
II	Extremely severe bone fragility, lethal	AR/AD
III	Variable, often severe bone fragility in infancy with progressive skeletal deformity; short stature,bluish sclerae, variable dentin abnormality	AR/AD
IVA	Bone fragility with mild to moderate deformity, often short stature, normal sclerae, normal teeth	AD
IVB	Bone fragility with mild to moderate deformity, often short stature normal sclerae, DI	AD
V	At least one episode of hyperplastic callus formation, moderate to severe fragility of long bones and vertebral bodies, limitations of pronation/supination in forearms, ligamentous laxity, white sclerae, normal teeth	AD
VI	Vertebral compression fractures, moderate to severe bone fragility, white or faintly blue sclerae, normal teeth	?
Other types		
	Cole-Carpenter – OI type	AD
	Bruck - OI type	AR
	North American OI	AR
	Osteoporosis-pseudoglioma syndrome	AR
	Additional undefined types	

2.1.4.2. Osteogenesis imperfecta type II (OMIM 166210)

This perinatally lethal type of OI presents with severe osseous fragility and defective ossification, and most often leads to death in utero or within the first months of life. Survival beyond one year is extremely rare (Sillence et al. 1984; Sillence & Rimoin 1978). Sillence has divided this OI type with almost black sclerae into 3 groups on the basis of differences in clinical and radiographic findings. In group A, the babies are small for gestational age and prematurity is common. Some 20% are stillborn, and the remainers die within hours to days after birth. The skull is large, and skull and face are molded. Limbs are short, broad and crumpled, and ribs are continuously beaded. Group B presents with short, broad, crumpled femora, angulation of tibiae but normal ribs or ribs with incontinuous beading. Respiratory distress is not so severe as in group A, and some children may survive for several years. Group C is rare. The babies are small for gestational age, stillborn or die in the newborn period. Long bones are thin, rectangular and inadequately modelled with multiple fractures, and ribs are thin and bent (Sillence 1988; Sillence et al. 1984).

Most cases are sporadic and due to new dominant mutations (Hall 2002; Sillence 1988; Sillence et al. 1984). The rare recurrences in healthy parents may be caused by recessive forms or parenteral mosaicism (Sillence et al. 1984).

2.1.4.3. Osteogenesis imperfecta type III (OMIM 259420)

The progressive deforming type of OI most often presents with a phenotype recognized at birth by short stature and deformities resulting from in utero fractures. Fractures may also appear first during the first years of life caused by minimal trauma, mostly in long bones and ribs. The fracture frequency in OI type III is highest of all those with OI, and 200 fractures during the lifetime is common. Bowing, angulation and rotatory deformation of an abnormally malleable skeleton lead to deformities of the upper and lower limbs (Figure 5). Sclerae are often pale blue at birth and generally become normal by puberty. Severe kyphoscoliosis is common, as are increased sweating and heat intolerance. In adulthood the stature is short, and DI is common (Sillence 1988; Sillence & Rimoin 1978; Sillence et al. 1979b). Hearing loss is thought to be uncommon (Sillence et al. 1979b). Differentiating between OI type III patients and those with a milder form of perinatally lethal OI type II may sometimes be difficult (Sillence et al. 1986). The inheritance is autosomal dominant or recessive, and parenteral mosaicism occurs (Byers 2002; Hall 2002).

2.1.4.4. Osteogenesis imperfecta type IV (OMIM 166220)

OI type IV is clinically more heterogeneous than the other subtypes of OI, and distinct subtypes have been separated from this entity in recent years (Glorieux et al. 2000; Glorieux et al. 2002). The expression and deformity of long bones are extremely variable (Sillence & Rimoin 1978; Sillence et al. 1979b). The stature is generally short in adulthood, although it may be normal at birth. Sclerae are either normal or bluish at

birth, and become normal in childhood or by adulthood. About 25% of the children have fractures at birth. The fracture frequency and total number of fractures during specific age periods are variable, but maximal in childhood with marked reduction after puberty and into adult life. Scoliosis is seen in one-third of the patients. No marked tendency to nosebleeds or easy bruising appears. Hearing loss is assumed to be less common than in OI type I, and, when presented in a family, it may vary in expression (Sillence et al. 1979b). Joint hypermobility and dislocations are common. Type IV OI is further divided into subgroups A and B, based on absence (A) or presence (B) of DI (Byers 2002; Sillence 1988). The inheritance is autosomal dominant.

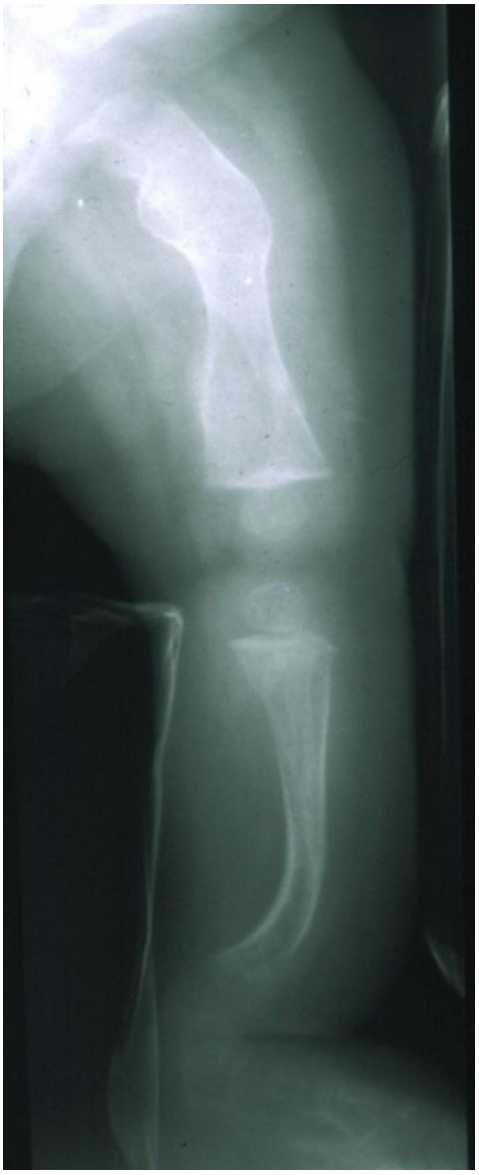

Figure 5. *A skeletal radiograph on the right femur and tibia of a 6-month-old boy with severe OI type III. He was the first child of healthy parents, and had multiple congenital fractures. The fracture of the femur has been healing but left the bone thickened and deformed. In the tibia there was no fracture but the curved anterior deformation developed after birth*

2.1.4.5. Osteogenesis imperfecta type V

Glorieux and co-workers reported in 2000 on a group of patients earlier classified as OI type IV, with a history of moderate to severe increased fragility of long bones and vertebral bodies but at least one episode of hyperplastic callus formation. All patients had limitations in the range of pronation/supination in one or both forearms, associated with a radiologically apparent calcification of the interosseous membrane. None of the type V patients presented with blue sclerae or DI, but ligamentous laxity was similar to that in patients with OI type IV. Autosomal dominant inheritance was reported (Glorieux et al. 2000).

2.1.4.6. Osteogenesis imperfecta type VI

In a study in 2002, another group of patients initially diagnosed as OI type IV was reported by Glorieux and co-workers. Fractures were first documented between 4 and 18 months of age, and they were more frequent than in patients with OI type IV. Sclerae were white or faintly blue and DI was uniformly absent. All patients had vertebral compression fractures. Mutation screening of the coding regions and exon/intron boundaries of both collagen type I genes did not reveal any mutations, type I collagen protein analyses were normal, and, so far, the pattern of inheritance has not been ascertained (Glorieux et al. 2002).

2.1.4.7. Bone fragility related disorders

In addition to the previous classification of OI, other more infrequent dysplasias with decreased bone density have been reported and included under the diagnosis OI (Hall 2002). The molecular pathology of these diseases is unknown. Cole-Carpenter dysplasia (OMIM 112240) is a sporadic condition with bone deformities and multiple fractures as in OI, but it also includes ocular proptosis with orbital craniosynostosis, hydrocephalus, and distinctive facial features (Cole et al. 1987). Bruck reported, in 1897, a patient with multiple joint contractures and OI. Only a few patients with this condition, later named Bruck syndrome (OMIM 259450), have been reported since then, with flexion contractures and recurrent fractures leading to severe deformity, short stature and kyphoscoliosis, white sclerae and normal hearing (Bruck 1897; McPherson et al. 1997). Autosomal dominant inheritence has been found in Bruck dysplasia I, but the inheritance in type II still is unknown (Hall 2002). Osteoporosis-Pseudoglioma dysplasia (OMIM 259770) or the ocular form of OI is an autosomal recessive condition due to mutations in the LRP5 gene (Gong et al. 2001). Singleton-Merton dysplasia, Osteopenia with radiolucent lesions of the mandible (OMIM 166260), Geroderma osteodysplasticum (OMIM 231070) and Idiopathic juvenile osteoporosis (OMIM 259750) are additional rare syndromes sharing similar skeletal symptoms with OI (Hall 2002).

2.1.5. Genetics and molecular pathology

OI is a disease with both autosomal dominant and recessive modes of familial inheritance (Byers 2002; Sillence et al. 1979b). There are sporadic cases caused by new mutations (Byers 2002). There is also growing evidence for parental mosaicism. This situation poses a difficult task for the clinical geneticist in genetic counseling, as a mosaic parent may appear clinically healthy but may carry the mutation in a fraction of the gonadal cells and thus he or she is at a risk of having another affected child (Raghunath et al. 1995).

Since the first evidence of the association between collagen defects and OI in 1975, when skin fibroblasts of a fetus with perinatally lethal OI were found to have decreased synthesis of type I collagen, it has become evident that in at least 90% of the cases OI is caused by mutations in genes that encode the type I procollagen (Heller et al. 1975; Penttinen et al. 1975; Sykes 1993; Sykes et al. 1990). To date, over 20 collagen types have been identified (Myllyharju et al. 2001). The collagens constitute a family of related proteins that are assembled in a variety of supramolecular aggregates with a structural function in the extracellular matrix (Vuorio & de Crombrugghe 1990). On the basis of protein and gene structures, type I collagen is included in the fibril-forming group of collagens, likewise collagens II, III, V and XI. By forming highly organized fibres and fibrils type I collagen provides the structural support for the body in bone, tendon, ligament, skin, teeth and fasciae (Prockop & Kivirikko 1995; Vuorio & de Crombrugghe 1990).

Type I collagen is a helicotrimer of two identical α1(I) chains and one α 1(II) chain. The α -chains are synthetized as precursor-pro α -chains with amino- and carboxy-terminal extensions, referred to as N- and C-propeptides. After a number of posttranslational modifications, three pro α -chains associate through their C-propeptides and fold into a triple-helical procollagen molecule. Extracellularly, specific C- and N-proteases remove the propeptides, and the remaining collagen molecule consists of the triple-helical domain and short N- and C-telopeptid propeptides (Vuorio & de Crombrugghe 1990).

The human COL1A1 gene is located in chromosome 17 (17q21.3-q22), while the location of the COL1A2 gene is in chromosome 7 (7q21.3-q22). The COL1A1 gene is about 18 kb in length with 51 exons, and the COL1A2 gene is about 38 kb in length and consists of 52 exons (Chu et al. 1984; Huerre et al. 1982). Hundreds of mutations in these two genes have been detected in OI patients so far (Kuivaniemi et al. 1997; Prockop et al. 1990). The types of mutations present a wide array: single base-pair mutations resulting in substitution of obligatory glycine residues, deletions and insertions, null allele mutations, splicing mutations, mutations in the carboxyl-terminal propeptide interfering with molecular assembly and multiexon rearrangements (Byers et al. 1991; Kuivaniemi et al. 1997; Prockop et al. 1990).

As a result of the mutation, the procollagen may either be normal, but secreted in smaller amounts than normal, or both normal and abnormal procollagen can be secreted (Byers 2002; Wenstrup et al. 1990). Generally, normal structure of the molecules in reduced amount leads to milder phenotypes, and an altered structure to a wider range of phenotypes, from extremely mild to lethal (Sillence et al. 1979b; Wenstrup et al. 1990). Although most of the mutations are unique for a family with OI, the same mutations have occasionally been found in unrelated individuals (Pruchno et al. 1991).

Most mutations in the mildest variant, type I OI, cause decreased expression of proα1(I)-chains because of either premature-termination codons or RNA-splicing defects in the COL1A1 gene. On the other hand, the most severe variants of OI, OI types II, III and IV, are primarily caused by single-base substitutions that convert a codon for an obligate glycine in the triple helix of the protein into a codon for an amino acid with a bulkier side chain that distorts the conformation of the triple helix (Barsh et al. 1982; Korkko et al. 1997; Redford-Badwal et al. 1996; Willing et al. 1992).

The underlying genetic defect of the new OI types V and VI remains to be elucidated, since no evidence of mutations has been found in type I collagen (Glorieux et al. 2000; Glorieux et al. 2002).

2.1.6. Diagnosis

Medical and family history, clinical examination and skeletal radiographs are still the benchmarks for the diagnosis of OI (Byers 2002).

OI type I is often suspected because of family history or a finding of blue sclerae in a patient with recurrent fractures. In a child with otherwise normal body habitus, OI is an important differential diagnosis for child abuse (Byers 2002; Paterson et al. 1993). Mild forms of type I OI may remain undetected, and in unclear or atypical postmenopausal osteoporosis this diagnosis should be considered (Bischoff et al. 1999).

OI type II is obvious at birth, and may be detectable already during the pregnancy by fetal ultrasonography. The diagnosis can be confirmed by fetal radiographs showing poor calcification and deformities of the skeleton, sometimes with no evidence of skeletal structures (Byers 2002; Heller et al. 1975; Shapiro et al. 1982a).

OI type III is often recognized in the perinatal period, while fractures or deformity may be present at birth. Some patients, however, have only mild femoral bowing and the fracture rate may be variable. Radiographs may reveal undermineralized calvarium with large anterior fontanelle and bony islands of the skull, i.e. so-called Wormian bones, and thin and gracile ribs and long bones with evidence of intrauterine fractures. If no fractures are present at birth, they usually appear during the first year of life caused by minimal trauma (Byers 2002).

OI type IV is in most cases identified in early infancy by fractures in utero or at birth. The diagnosis may be delayed if the fractures are healed, no deformity appears, and no X-ray is taken. The children may also present with only bowing of extremities at birth, with no fractures (Byers 2002). Types V and VI OI may be distinguished from OI type IV by hyperplastic callus or vertebral compression fractures (Glorieux et al. 2000; Glorieux et al. 2002).

DI can be helpful in diagnosis of OI, although a similar-appearing aberration that affects dentin only, DI type II, also exists. (Shields et al. 1973) The marked variation in the severity of the dentinal manifestations of OI makes the diagnosis based on dental findings still more complicated. (Waltimo et al. 1996).

In the absence of family history or obvious diagnosis, a study of collagen synthesis by dermal fibroblasts may be performed (Byers 2002; Holbrook et al. 1989; Wenstrup et al. 1990). Mutations in COL1A1 and COL1A2 genes may also be identified by analysis of genomic DNA, or in expressed sequences using cDNA made from mRNA (Korkko et al. 1998a; Korkko et al. 1997).

2.1.7. Treatment

Until recently, the treatment of OI has been focused on preventing fractures, reducing deformities and promoting normal function, as well as on early supportive physiotherapy to maximize the muscular strength and minimize the inactivity and deformity (Byers 2002; Primorac et al. 2001). Intramedullary rods, especially Bailey-Dubow and Soffield type elongating rods, are commonly used in treatment of children with OI (Primorac et al. 2001). Fractures may also be treated with the usual orthopedic care (Byers 2002). Scoliosis may be treated with a brace, and operative treatment is recommended when the deformity is progressive, pain-causing scoliosis. However, the orthopedic treatment of scoliosis is usually most difficult (Primorac et al. 2001).

The dental treatment largely depends on the degree of tooth involvement. The major task during primary dentition is to ensure favourable conditions for the eruption of the permanent teeth, and later to retain the occlusal function. Therefore, teeth are often treated with composite fillings, and preferably with temporary stainless steel crowns and permanent prosthetic crowns to maintain their vertical height (Ranta et al. 1993).

A number of medical therapies have been attempted for patients with OI. Anabolic steroids have been helpful but because of their adverse side effects they are not in use. Fluoride and magnesium oxide have not been shown to be effective. Calcitonin has been reported to give some subjective relief for pain (Castells 1973). Recently, most encouraging results in the treatment of patients with bisphosphates have been reported by several groups (Adami et al. 2003; Batch et al. 2003; Falk et al. 2003; Glorieux 2000; Glorieux 2001; Glorieux et al. 1998; Primorac et al. 2001; Zeitlin et al. 2003). The bisphosphonates constitute a group of synthetic analogues of pyrophosphate, and they possess a remarkably variable range of effectiveness, e.g. inhibiting bone

resorption. Cyclic intravenous treatment with pamidronate (3-amino-1-hydroxypropylidene-bisphophonate) has been proved to be beneficial in treatment of children with OI, leading to increased bone mineral density and increased physical activity (Falk et al. 2003; Primorac et al. 2001; Zeitlin et al. 2003). In adults, intravenous Neridronate has significantly increased bone mineral density and lowered the risk of clinical fracture(Adami et al. 2003). Therefore, bisphosphonate therapy seems to provide clinical benefits, not only in children with OI, but also in adults. Effects of bisphosphonates on hearing thresholds in OI have not been reported. In otosclerosis, however, there are controversial reports of effects of bisphosphonates: On the one hand, a trend toward stabilization or improvement in air and bone conduction thresholds as well as improvement in neurotological symptoms, and, on the other hand, severe deterioration of pure tone thresholds (Boumans et al. 1991; Brookler et al. 1997; Kennedy et al. 1993; Yasil et al. 1998).

Growth hormone has been reported to cause increased bone formation rate and density, and decreased fracture rates, in children with OI (Marini et al. 2003). Also gene therapy has been studied, in order to reintroduce modified cells capable of homing to bone and participating in new bone formation into the host, and thereby correcting the underlying genetic mutation (Primorac et al. 2001).

2.2. Hearing loss in Osteogenesis imperfecta

2.2.1. Definition

Six population studies on OI have been published so far, with great variation in the definition of the hearing loss (Paterson et al. 2001; Pedersen 1983; Seedorff 1949; Sillence 1981; Smårs 1961; Stewart & O'Reilly 1989). Three of these population studies have focused on hearing loss (Paterson et al. 2001; Pedersen 1984; Stewart & O'Reilly 1989). Definition of the hearing loss has been presented in two of them (Pedersen 1983; Stewart & O'Reilly 1989). In the Danish study by Pedersen a pure tone audiometry with air and bone conduction measurement at frequencies 250, 500, 1000, 2000 and 4000 Hz was used. Sensorineural hearing loss was defined as air conduction thresholds for one or more of these frequencies greater than 15dB(HL) and an air-bone gap of less than 15 dB. Conductive hearing loss was defined as an air-bone gap of 15 dB or greater and a bone conduction threshold of less than 15 dB at one or more of these frequencies, while mixed hearing loss was defined as a bone conduction level greater than 15 dB and an air-bone gap of 15 dB or greater at one or more of the frequencies 250-4000 Hz (Pedersen 1984). In the Scottish study, the sensorineural hearing loss was defined as pure tone hearing thresholds of 30dB(HL)or greater at two or more of the octave frequencies 250-8000 Hz, conductive hearing loss as an air-bone gap of 15 dB or greater at two or more of these frequencies, and mixed hearing loss as a bone conduction level of 30dB or greater and an air-bone gap of 15 dB or greater at two or more of the frequencies 250-8000 Hz (Stewart & O'Reilly 1989). In the recent Scottish study by Paterson, symptomatic hearing loss was recorded (Paterson et al.

2001). In the study by Sillence presenting the classification of OI no definition of hearing loss was given, nor was it given in the Danish study from 1949, while a hearing test with a whispering voice was used in the Swedish study (Seedorff 1949; Sillence et al. 1979b; Smårs 1961).

In addition to the population studies, a major study on hearing loss in OI type I was performed by Garretsen and co-workers in the Netherlands in the 1990´s. Conductive hearing loss was defined as the average air-bone gap for the frequencies 500, 1000 and 2000 Hz, or else the average air-bone gap at 4000 and 8000 Hz greater than 15 dB with a corresponding bone conduction threshold below 15 dB. Sensorineural hearing loss was defined as the average air conduction threshold equal to or greater than 15 dB for the frequencies mentioned above, and a corresponding air-bone gap smaller than 15 dB, and mixed hearing loss as the average air-bone gap equal to or greater than 15 dB with a corresponding bone conduction threshold equal to or greater than 15 dB (Garretsen et al. 1997). These definitions paralleled the definitions used by Pedersen in the population study in Denmark, and the definition given by Shapiro in 1982 in a study of 55 patients with OI. Normal hearing was defined as less than 15 dB drop at frequencies 250 to 8000 Hz, and conductive hearing loss as a 15 dB or greater decrease in air conduction compared to bone conduction thresholds at one or more of these frequencies. Sensorineural loss was defined as a depression in both air and bone conduction threshold without an air-bone gap, and mixed hearing loss as involving features from both conductive and sensorineural hearing loss, usually with a prominent air-bone gap (Shapiro et al. 1982b).

2.2.2. Epidemiology

In population studies, which include systematic audiological investigations on OI, hearing loss has been reported in up to 58% of the patients (Pedersen 1984; Stewart & O'Reilly 1989). In a Danish population study with 201 OI patients, presented by Pedersen in 1984, hearing loss was found in half of the audiologically tested ears (Pedersen 1984). In Scotland, Stewart and O'Reilly found hearing loss in 58% of the 53 patients (Stewart & O'Reilly 1989). Of the previous studies focusing on hearing loss in classified types of OI, Sillence in 1979 and 1988, as well as Paterson in 2001, included all types of OI, while Garretsen in 1991 and 1997 included OI type I, only. Hearing loss has been reported to be most common in OI type I, affecting 35-78% of patients, and it has been thought to be less common in OI type IV. In OI type III, Sillence found only 1 out of 6 patients with hearing loss, whereas Paterson reported hearing loss in 52% of 207 patients (Garretsen et al. 1997; Garretsen & Cremers 1991a; Paterson et al. 2001; Sillence 1988; Sillence et al. 1979b). The definition of hearing loss, however, has varied greatly in the studies referred to, and the study subjects may have been selected, like in Garretsen´s study from 1991, consisting of only type I OI patients, or, as in the study from 1997, of type I OI patients who either were elected for ear surgery or volunteered for the study because of their hearing loss (Garretsen et al. 1997; Garretsen & Cremers 1991a).

2.2.3. General characteristics and etiology

The hearing loss in OI most often begins in early adulthood, in the second to third decade of life, although it may sometimes begin in the first decade of life. The hearing loss is progressive, and often proceeds from conductive hearing loss to a mixed and sensorineural type with increasing age (Cox et al. 1982; Garretsen et al. 1997; Pedersen 1984; Quisling et al. 1979; Riedner et al. 1980). Mixed hearing loss, on the other hand, has been reported as the most frequent form of hearing loss in OI (Garretsen et al. 1997). Additionally, a mild, high-frequency sensorineural hearing loss has been suggested as characteristic of OI (Shapiro et al. 1982b). Sensorineural hearing loss, including high frequency hearing loss, and anacusis has been reported in constant proportions independent of age (Garretsen et al. 1997). The hearing loss in OI is strongly age-related with an estimated annual increase of 1-1.7dB (Garretsen et al. 1997).

In the sixties, otosclerosis was suspected to be a local form of OI (Hall et al. 1961). However, further studies have revealed that hearing impairment in OI and otosclerosis are two histologically, enzymatically and etiologically distinct entities with clinical similarities (Altmann et al. 1967; Bretlau et al. 1969; Holdsworth et al. 1973). Compared with otosclerosis, hearing loss in OI has a tendency to earlier onset, more severe middle ear involvement, and a higher incidence of sensorineural hearing loss (Bergstrom 1981; Bergstrom 1977; Garretsen et al. 1997; Shapiro et al. 1982b; Shea et al. 1982; Shea et al. 1963; Stewart & O'Reilly 1989).

Functional ossicular discontinuity, due either to stapes superstructure fracture or fibrous replacement or thick and fixed stapes footplate, has been suggested as the main etiology for the conductive hearing loss in OI (Armstrong 1984; Bergstrom 1981; Igarashi et al. 1982; Pedersen et al. 1979). Cochlear hair cell loss, stria vascularis atrophy and calcification, tectorial membrane distortion and perilymph hemorrhage, on the other hand, have been reported in autopsy findings, and have been suspected of accounting for sensorineural hearing loss (Bergstrom 1981). In addition, in histological studies of temporal bones, deficient ossification of the otic capsule and ossicles has been described (Bergstrom 1977; Igarashi et al. 1982; Pedersen et al. 1985). In severe OI with intrauterine fractures, intracochlear hemorrhage and persistent cartilage in areas ossified in a normal child of the same age of six weeks have also been reported (Bergstrom 1977).

2.2.4. Treatment

Audiometric examinations have been recommended for the diagnosis of OI, with repeated studies every third year after adolescence, especially during the second to third decade of life (Byers 2002; Cox & Simmons 1982). As in otosclerosis, the hearing loss in OI may be treated with hearing aid or stapes surgery, that has become a safe and reasonable alternative to hearing aids in OI (Pedersen et al. 1983; Riedner et al. 1980; Shea & Postma 1982; Van Der Rijt et al. 2003). In addition, also cochlear

implantation has been reported (Huang et al. 1998). So far, to our knowledge, no reports of a bone-anchored hearing aid (BAHA) in OI have been published.

2.2.4.1. Stapes surgery

2.2.4.1.1. Surgical anatomy of middle ear in OI

The typical surgical findings in the middle ear of an OI-patient with hearing loss are thick and fixed or obliterated footplate, thick and vascular mucosa with excessive bleeding tendency, and brittle atrophic crura (Armstrong 1984; Dieler et al. 1997; Ferekidis et al. 2000; Opheim 1968; Pedersen & Elbrond 1983; Shea & Postma 1982; Shea et al. 1963; Van Der Rijt & Cremers 2003). Crural fractures, closed round window, total bony closure of the oval window, and deficient, short ossicles are also reported (Albahnasawy et al. 2001; Armstrong 1984; Dieler et al. 1997; Pedersen & Elbrond 1983; Shea & Postma 1982; Shea et al. 1963; Van Der Rijt & Cremers 2003). The footplate may be mobile (Albahnasawy et al. 2001; Armstrong 1984). Compared with the dense, hard bone seen in otosclerosis, the footplate area in OI often presents with soft, granular or iceberg type bone, and the footplate may be several times normal thickness (Kosoy et al. 1971; Patterson et al. 1970; Pedersen & Elbrond 1983; Shea et al. 1963; Sooy 1960). The middle ear may also present with massive adhesions (Shea et al. 1963).

2.2.4.1.2. Surgical techniques and results

Despite the challenges in surgery caused by the anatomical peculiarities in the middle ear, the postoperative hearing results have been encouraging, albeit not as satisfactory as in otosclerosis (Armstrong 1984; Cremers et al. 1991; Garretsen et al. 1990; Glasscock et al. 1995; Palva et al. 1977; Pedersen & Elbrond 1983; Shea & Postma 1982; Van Der Rijt & Cremers 2003). Furthermore, it has also been suggested that conductive hearing loss in OI can be surgically relieved with about the same level of predictability as in those with otosclerosis, provided that the surgeon is aware of the particular problems that may occur (Armstrong 1984; Cohen 1984; Dieler et al. 1997). Problems in surgical treatment are different from otosclerosis: the canal skin may be thinner and the scutum bone more brittle, stapes crura may be easily fractured, and troublesome bleeding may occur (Patterson & Stone 1970; Pedersen & Elbrond 1979; Van Der Rijt & Cremers 2003). Slight worsening of the bone conduction thresholds after the surgery has been reported (Garretsen & Cremers 1990; Pedersen & Elbrond 1983). Also postoperative anacusis ($PTA_{500\text{-}2000a}$ more than 90 dB) has been reported in earlier studies in OI, as well as in otosclerosis surgery (Cremers et al. 1991; Garretsen & Cremers 1990; Glasscock et al. 1995; Kosoy & Maddox 1971; Palva et al. 1977; Patterson & Stone 1970; Van Der Rijt & Cremers 2003).

Stapedotomy is today the method of choice in otosclerosis surgery (Glasscock et al. 1995; McGee 1983). Furthermore, laser-assisted stapedotomy has been found to be less traumatic than conventional techniques, and it minimizes the risk of oval window

bleeding (McGee 1983). Laser-assisted stapedotomy might be the method of choice also in middle ear surgery in OI, particularly because of the risk of troublesome bleeding (Patterson & Stone 1970; Pedersen & Elbrond 1979).

Hearing results may be better with centralized surgery (Armstrong 1984; Garretsen & Cremers 1990; Van Der Rijt & Cremers 2003). In general, long-term hearing results have been reported as satisfactory (Ferekidis et al. 2000; Garretsen & Cremers 1990; Pedersen & Elbrond 1979). However, progressive sensorineural hearing loss arising independently of the operation as a result of progression of the disease process in OI, appears to have a severe influence on the final hearing threshold**.** Out of the ten patients with unsuccessful surgical outcome in Garretsen´s study, eight had early sensorineural hearing loss prior to the surgery (Garretsen et al.1991b).

2.3. Vestibular disturbances in Osteogenesis imperfecta

2.3.1. Definition

Dizziness is a symptom, not a disease, resulting in uncertainty of position or motion in space. Clinically it may be divided into four types. Type I dizziness is true vertigo, presenting with a definite rotational component, implying a vestibular abnormality of central or peripheral origin. Type II often accompanies cardiovascular abnormalitites; it is syncope-like and presents with a feeling of loss of consciousness. Type III occurs in patients lacking appropriate sensory input to maintain normal equilibrium, presenting with multisensory difficulties, which produce a lack of balance and coordination. Type IV is a light-headed sensation, diffuse and non-descript, including all diffuse sensations not otherwise included in types I, II and III (Paparella et al. 1991).

2.3.2. Prevalence and characteristics

Very little is written about vertigo in OI, and the prevalence is unknown. Bronson was the first to mention balance problems in a patient with OI in 1917 (Bronson 1917). Although Gottstein, already in 1932, postulated that patients with OI often have balance disturbances, no studies on vertigo in OI have been published (Gottstein 1932). Stoller presented, in 1962, a case report of a 46-year-old female with blue sclerae, recurrent fractures, severe episodic vertigo and gradual hearing loss resulting in anacusis. Right vestibular neurectomy cured the vertigo (Stoller 1962).

In the literature, vertigo has mostly been reported as a symptom of basilar impression, a craniocervical abnormality sometimes associated with OI. Vestibulocerebellar symptoms, giddiness or imbalance, poor balance and gait, and cough headache in the occipital area with brief sensations of postural instability and syncope have been described. Moreover, dizziness and loss of balance while coughing, sneezing, laughing and bending over, as well as neck extention producing vertigo have been described as

symptoms of this craniocervical malformation (Elies et al. 1980; Hayes et al. 1999; Pozo et al. 1984; Sawin et al. 1997; Sillence 1994). Vertigo precipitated by head movements has been reported to be pathognomonic for basilar impression (Elies & Plester 1980; Hayes et al. 1999).

Otosclerosis patients present with vestibular dysfunction with frequencies from 27.7 to 57.7 %, but the etiology of sensorineural hearing loss as well as vertigo in otosclerosis is still unknown (Cody et al. 1978; Ghorayeb et al. 1978; Morales-Garcia 1972; Virolainen 1972). Particularly patients with sensorineural hearing loss due to labyrinthine or cochlear otosclerosis are thought to suffer from vertigo (Cody & Baker 1978; Thomas et al. 1981). Biotoxic effects on the peripheral vestibular nerve, otosclerotic vascular changes due to the disease, and biochemical changes in the inner ear fluids have been suggested as etiological factors of vertigo in otosclerosis (Igarashi et al. 1982; Virolainen 1972).

2.4. Basilar impression and invagination

2.4.1. Definition and prevalence

”Basilar impression” refers to a pathological anatomical condition where the uppermost cervical vertebrae intrude into the foramen magnum, producing variable pressure effects on the medulla and adjacent parts of the central nervous system (Chamberlain 1939; McGregor 1948). ”Basilar invagination” is a radiological designation for a status where the head is positioned abnormally inferiorly in relation to the uppermost cervical vertebrae, and most patients with basilar impression also fill the criteria of basilar invagination (Hayes et al. 1999).

The prevalence of BI (basilar impression or invagination) in population studies of OI has been reported to be 10.5-25% (Charnas et al. 1993; Pozo et al. 1984; Sillence 1994).

2.4.2. General characteristics

In most cases, BI is caused by congenital developmental defects in the cervico-occipital region (McGregor 1948). In OI, it is thought to be secondary to softness of the skull (McGregor 1948; Pozo et al. 1984). BI has been reported to often affect patients with a mild to moderate type of OI (Pozo et al. 1984; Sillence 1994). Most patients seem to develop the symptoms in the second to fourth decades of life (Hayes et al. 1999).

In BI, symptoms of cranial nerve involvement, constriction of the cord in the region of the first cervical segment, and degenerative changes in the lower cervical segments may appear (McGregor 1948). Pure cerebellar disturbances are rare, whereas headache, lower cranial nerve dysfunction, pyramidal tract signs and

vestibulocerebellar symptoms are reported to be common (Elies & Plester 1980; Hayes et al. 1999; Sawin & Menezes 1997). Vertigo precipitated by head movements has been reported to be pathognomonic for BI (Elies & Plester 1980; Hayes et al. 1999). Hearing loss has also been mentioned as a symptom of basilar impression (Elies & Plester 1980).

In most severe cases, spastic paraparesis, transient tetraparesis and acute respiratory failure have been reported, and a disease history from asymptomatic ventricular dilatation, through the foramen magnum compression syndrome, may lead to death from brain-stem compression (Chandy et al. 1991; Harkey et al. 1990).

However, patients with BI may also be asymptomatic (Sawin & Menezes 1997).

2.4.3. Treatment

According to Harkey, previous surgical procedures for BI have involved suboccipital decompression and an upper cervical laminectomy, with operative outcomes from perioperative death to good functional recovery. Neurological abnormalities have often persisted after these operations because of the unchanged distortion of the brain stem. Therefore, extensive removal of the anterior bony compression by a transoral approach has become a recommended treatment. This has been followed by a posterior rigid fixation that transfers the weight of the head to the thoracic spine, in an effort to prevent further basilar invagination. The results of surgery have been relatively good, despite the difficult procedure (Harkey et al. 1990; Hayes et al. 1999).

2.5. Genotype-phenotype correlation

The association between clinical disease and basic molecular genetic defect, i.e. the phenotype and genotype in OI has been searched for, in the hope of better understanding the wide variation in expressivity and heterogeneity that still causes difficulties in clinical classification of both familial and sporadic cases (Cole 1997; Sillence 1981; Sillence et al. 1979b; Ward et al. 2001). The clinical variability is partly explained by the variety of mutations in the COL1A1 and COL1A2 genes leading to phenotypes ranging from mild to severe and lethal conditions (Ward et al. 2001). In general, mutations reducing the amount of type I collagen synthesised by tissues lead to the mildest phenotypes. The wide range of phenotypes from extremely mild to lethal is caused by mutations altering the structure of the proαchains leading to the formation of abnormal molecules (Byers et al. 1991; Cole 1997; Wenstrup et al. 1990). The genotype-phenotype correlation in OI has mainly been focused on the overall severity of the disease, while the correlation between the mutation and hearing loss has not been extensively studied earlier (Byers et al. 1991; Cole 1997; Wenstrup et al. 1990).

3. Aims of the study

The general purpose of the study was to investigate the prevalence of OI in Finland, the hearing loss and balance problems in this rare disorder of connective tissues and skeleton.

The specific aims were:

1. To study the prevalence, age of onset and type of hearing loss in children with OI.
2. To study the prevalence and characteristics of hearing loss in adults with OI.
3. To study the vestibulocochlear disturbances and their possible associations with cervicocranial secondary deformations in adults with OI.
4. To evaluate the results of middle ear surgery for hearing loss in OI in Finland.
5. To analyse the possible correlation between hearing loss and basic molecular defects.

4. Patients and methods

4.1. Ascertainment of patients

In 1995-1996, a nationwide search for OI patients in Finland was made through the patient register of the Department of Clinical Genetics, Helsinki University Central Hospital, through the membership register of the Finnish Osteogenesis imperfecta Association, and the HILMO care register from the Finnish university and central hospitals. In addition, some cases were found through family histories.

Through the sources listed above, 254 Finnish OI patients found by 1996 were included in the present studies. The patients were approached through a questionnaire concerning the history of fractures, colour of sclerae, DI and hearing loss (I, II). During the study period, 1995-2002, an additional 45 OI patients were found. They were not included in the studies on hearing loss, but they were included in the prevalence study. By the end of the year 2002, 205 Finnish OI patients had consented to participate in the studies (I-V). Of these patients, 144 were adults, and 61 were children 16 years of age or younger at the time of the study. A clinical geneticist had determined the type of OI in 200 of the 205 patients using the criteria introduced by Sillence (Sillence et al. 1979b).

The population data obtained from the Population Register Centre (Väestörekisterikeskus) were used to calculate the prevalence of OI in Finland. The point prevalence based on 299 known OI patients and the population in Finland, 5 206 295, was calculated on December 31st 2002.

4.2. Clinical history and confirmation of the diagnosis

The diagnosis was confirmed in all sporadic cases and in most familial patients by clinical and genetic evaluation at the Department of Clinical Genetics, Helsinki University Central Hospital, and in the remaining patients through the hospital records, based on family and fracture history, as well as clinical and radiographic findings. The patients were asked about subjective and objective onset of hearing loss, the clinical course of hearing deficit, the use of hearing aids, presence of vestibular symptoms and surgical treatment.

4.3. Audiometric evaluation (I-V)

Altogether 183 patients with no additional ear disease and age over 4 years were included in the audiometric studies by the end of 2002, with 46 children aged 16 years or younger, and 137 adults over 16 years of age. The frequency and characteristics of hearing loss related to clinical OI classification were studied in the adults, as the hearing loss is most often known to begin in the second to third decade of life (II). As

the hearing loss may begin already in childhood, and no previous reports existed, the prevalence and characteristics of hearing loss were also studied in children (I).

Pure tone audiometry was performed under standard conditions in a soundproof room at a local or university hospital. Hearing measurements in some of the patients also included speech audiometry, tympanometry and measurement of stapedial reflexes (1kHz ipsi- and contralateral stimuli). In the 42 patients included in the vestibular study, Ilo Otodynamics Ltd, Great Britain, was used for analysis of Otoacustic emissions (OAE). Auditory brainstem response (ABR) measurements in these patients were performed with Nicolet Viking IVD (III).

The patients with no audiometric evaluation were excluded from the study, as were the patients with non-OI-related ear disease. Normal hearing was defined as pure tone average at the frequencies 500, 1000 and 2000 Hz ($PTA_{0.5\text{-}2kHz)}$ equal to or better than 15 dB HL in patients over 16 years and under 60 years of age, and equal to or better than 20 dB HL in patients 16 years or younger, as well as 60 years or older. Definitions of different types of hearing loss paralleling the definitions given by Shapiro et al (Shapiro et al. 1982b) and Pedersen (Pedersen 1984) were used:

1. Conductive hearing loss: average air-bone gap at the frequencies 0.5, 1 and 2 kHz greater than 15 dB with corresponding bone conduction threshold smaller than 15 dB.

2. Sensorineural hearing loss: air conduction thresholds at the frequencies 0.5, 1 and 2 kHz equal to or greater than 15 dB with corresponding air-bone gap smaller than 15 dB.

3. Mixed hearing loss: average air-bone gap at the frequencies 0.5, 1 and 2 kHz greater than 15 dB with corresponding bone conduction threshold equal to or greater than 15 dB.

The hearing age of the patients, i.e. the age at the time of audiometry, was registered (I-V). In patients with previous middle ear operation, the hearing age and an audiometry prior to the first operation were used (IV, V). Early sensorineural hearing loss was defined as mean bone conduction thresholds at speech frequencies of more than 20 dB at under 30 years of age (V).

4.4. Vestibular and cephalometric evaluation (III)

Because 44% of the adults in the audiometric study (II) reported vertigo, the frequency, characteristics and etiology of vertigo were further studied in a separate adult population (III). Adults (age over 16 years) living in the Turku or Helsinki region were approached through a questionnaire concerning the subjective and objective onset and the clinical course of eventual hearing loss or vestibular problems. Since BI has been suggested to cause vertigo, and BI has been reported to be most common in OI type IV, all Finnish adults with OI type IV were also contacted. Frequency, etiology, and characteristics of vestibular dysfunction could be studied in 42 of the contacted 73 patients. In addition to

a clinical examination and an X-ray of the cervicocranial region to detect eventual BI, electronystagmography with Hortmann's ENG equipment was performed. In ENG, eye movements and caloric reactions, as well as spontaneous nystagmus were recorded, and directional preponderance was calculated. Abnormal findings in saccadic eye movements and in pursuit and optokinetic tests, indicating central lesions were recorded, and the ocular fixation index (OFI) was calculated.

The cephalometric measurements from standardised lateral radiographs for the analysis of the skull base morphology are presented in III, Figure 1. A line was drawn on tracing paper from the most posterior-caudal point of the clivus (anterior margin of the foramen magnum) to the opisthion point (posterior margin of the foramen magnum). When the contour of the dens projected above this line, the patient was recorded as radiologically fulfilling the criteria of basilar impression. For the evaluation of basilar invagination, we used our own modifications of previously documented criteria (Chamberlain 1939; Hayes et al. 1999). We recorded as abnormal only the situations where the dens projected 10 mm or more above a line drawn from the most posterior point of the hard palate to the opisthion point. The selection of this criterion was based on our radiological survey of a large OI patient material revealing factors that strongly affect the level of the reference plane, that is, an abnormal position and angulation of the hard palate, as well as large structural variations in the posterior skull base in OI. (Waltimo et al, manuscript)

4.5. Evaluation of ear surgery (IV)

Pre- and postoperative data of 40 patients with middle ear surgery were collected, with a total of 62 operations performed between years 1961 and 2002. Seven patients were excluded from the study because of insufficient data, incomplete or revision surgery. The surgical and audiological data of 33 patients (43 operations) could be analysed.

4.6. Molecular genetic analyses (V)

The inclusion criteria for the mutation study were OI, a previous audiometric study and either a previously diagnosed hearing loss or age more than 35 years by 2002. The age limit was used because the hearing loss in OI is known to begin first in the second to third decade of life (Cox et al. 1982; Garretsen et al. 1997; Pedersen 1984; Quisling et al. 1979; Riedner et al. 1980).

Of the total of 82 unrelated patients fulfilling the inclusion criteria, 54 consented to participate the study.

4.6.1. Analyses of the COL1A1 and COL1A2 genes

Blood samples were collected for the analysis of DNA. Conformation sensitive gel electrophoresis (CSGE) and sequencing were used to scan the COL1A1 and COL1A2

genes for mutations in the 54 unrelated patients with OI. Genomic DNA was isolated from blood samples by standard procedures. All 51 exons of *COL1A1* and all 52 exons of *COL1A2* and the flanking sequences were amplified by PCR as described previously. (Korkko et al. 1998a) The PCR products were about 200-450 bp in size and contained at least 60 bp of exon flanking sequences. PCR amplifications were carried out in a reaction volume of 23 µl containing 40 ng of genomic DNA, 200 µM of each dNTP, 0.25 µM of each primer, and 1 unit of Taq polymerase (AmpliTaq Gold, Applied Biosystems). The PCR conditions were an initial denaturation at 95°C for 10 min, followed by 95°C for 40 sec, 54-64°C for 40 sec, and 72°C for 40 sec for 35 cycles. To generate heteroduplexes for CSGE analysis the PCR products were denatured at 98°C for 3 min and annealed at 68°C for 30 min. The PCR products were analyzed on an agarose gel to check the quantity and quality of the products. Scanning of the PCR products was performed by CSGE as previously described with the exception that the gels were stained with SYBR Gold nucleic acid gel stain (Eugene, USA) instead of ethidium bromide (Korkko et al. 1998b). PCR products that contained heteroduplexes were sequenced with PCR primers by an automated instrument (ABI PRISM 377 or 3100 Sequencers and ABI PRISM Dye Terminator Cycle Sequencing Ready Kit, Applied Biosystems). Prior to sequencing, the samples were treated with exonuclease I and shrimp alkaline phosphatase (Hanke et al. 1994; Werle et al. 1994).

4.6.2. Genotype-phenotype correlation analysis

Mutations were classified in two ways. In the first classification, mutations were divided into those that were found in COL1A1, and those localized in COL1A2. In the second classification, the mutations were divided into four subgroups. Subgroups one and two contained single base mutations resulting in glycine substitution in proα1(I) and proα2(I), respectively. The third subgroup consisted of mutations that altered consensus RNA splicing sequences. These mutations usually lead to exon skipping or intron inclusion or the use of a cryptic splice site (Pace et al. 2001a; Pace et al. 2001b). The fourth subgroup included nonsense and frameshift mutations, which typically lead to haploinsufficiency through nonsense-mediated mRNA decay.

Eventual statistical dependencies of these different types of mutations and onset, presence, type and severity of hearing loss, as well as early sensorineural hearing loss or expression of hearing loss in families with several affected persons with OI were sought. Further, an eventual correlation of the mutation to type of OI and clinical features like blue sclerae, fracture rate and length was sought.

4.7. Statistical analyses

In studies II, III and V, statistical analyses of group comparisons were carried out with the two sample t-test. Bivariate dependencies were performed using cross-tabulations (contingency tables) with related chi-square statistics and class-wise relative frequencies. The chi-squared statistic indicates the existence of a possible statistical

dependency between the categorical variables, while percentage distributions in classes of one of the variables enable us to identify the fashion in which the variables are dependent on each other. In the cases of 2 by 2 cross-tabulations, and where the sample size remained small, we utilized the so-called Fisher's exact test for testing the independencies. The advantage of this procedure is that it does not rely on the large sample theory like the usual chi-square analysis, and hence should be more powerful in detecting possible dependencies.

In study IV, statistical analyses of the postoperative changes in hearing were carried out with the paired t-tests. The usual two-sample t-test and the chi-square test were utilized in the analyses of operative results, on one hand between university and central hospitals, and on the other hand, between single surgeon and multiple surgeons. F-test was used in investigation of the homogeneity (equality of variances) of the pre- and post-operative hearing results.

4.8. Ethics

The Joint Ethical Committee of Turku and Helsinki University Central Hospital approved the studies. Permission to acquire the patient data was granted by the Ministry of Health and Social Affairs.

5. Results

5.1. Osteogenesis imperfecta in Finland

5.1.1. Epidemiology

By the end of 2002, 299 living Finnish patients with confirmed diagnosis of OI were ascertained. The prevalence of the known cases of OI in Finland on December 31st, therefore, is 5.74/100 000.

5.1.2. General characteristics and classification

The mean age of the 299 Finnish patients was 35 years at the end of 2002. (SD 20.6 years, range 1-94 years) Of the 166 women and 133 men, 61 were children 16 years of age or younger. Of the 299 patients, 105 had sporadic type of OI, while 194 were familial cases representing 60 different families. Four of the families consisted of seven patients with OI, one family of six patients, four families of five patients, nine families of four patients, and 42 families of three or fewer patients with OI.

A clinical geneticist classified 254 patients. OI type I was most common, 78.4%. The type of OI among the 299 patients is presented in Figure 6.

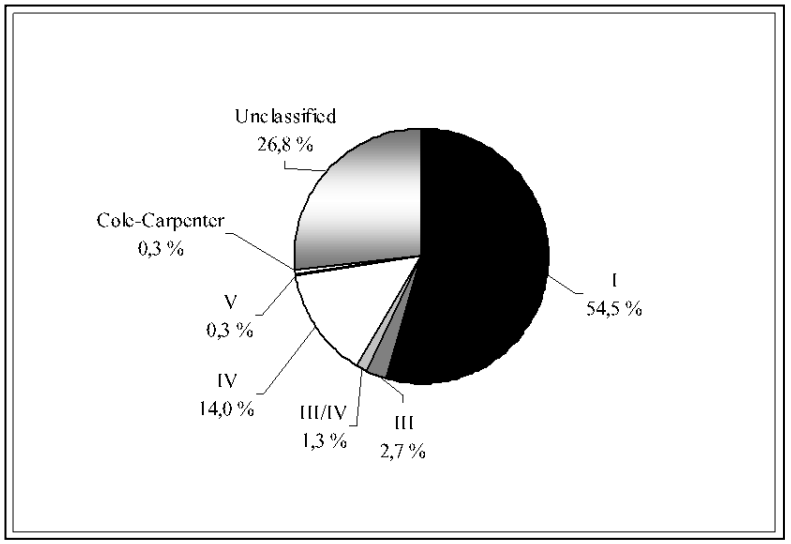

Figure 6. *Type of OI in the 299 Finnish patients.*

Altogether, 205 patients had participated in the nationwide study on hearing in OI by the end of 2002. By the time of the study, the mean age of 118 women and 87 men was 32.0 years (SD 20.0 years, range 1-81 years), and 61 of the 205 patients were

children aged 16 years or younger. Sex and age distributions and the classification of the patients are presented in Table 2. Of the patients, 133 patients had familial type of OI, 178 had blue sclerae, and 81 patients reported DI. The number of fractures in the 205 patients is presented in Figure 7. Of the 205 patients included in this nationwide study, 200 were alive at the end of 2002.

Table 2. *Sex and age distribution of the 205 classified Finnish OI patients included in the nationwide study.*

	Unclassified	I	III	III/IV	IV	V	Cole-Carpenter	Total
Male	2	61	4	1	16	1	0	88
Female	3	82	6	3	25	0	1	116
Mean age (years)	33,4	33,7	25,6	25,5	28,7	43	12	32
SD	16,24	21,3	15,4	25,2	15,9			20,1
Range	12-55	1-81	6-49	1-58	5-65			1-81
Total number of pts	5	143	10	4	41	1	1	205

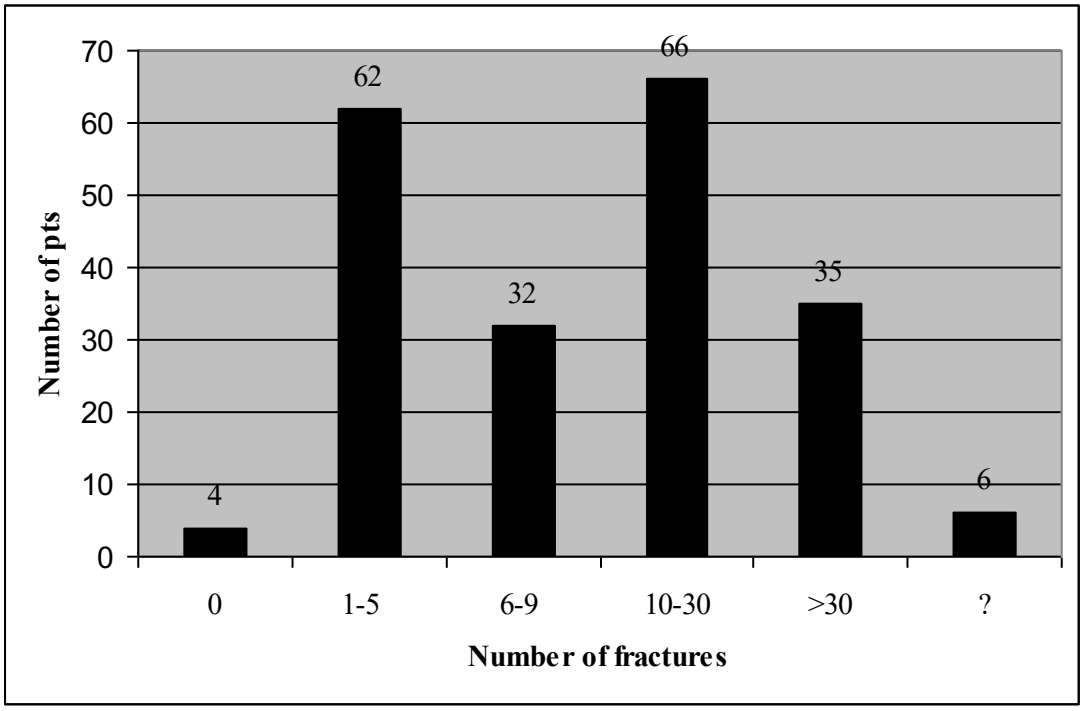

Figure 7. *The number of fractures in the 205 Finnish OI patients included in the study. Four patients had no fractures. The number of fractures was unknown in 6 patients.*

5.2. Hearing loss (I, II)

5.2.1. Patients, prevalence and general characteristics

Of the 183 patients with audiometry, 46 were children aged 16 years or younger, and 137 were adults. OI-related hearing loss in at least one ear was found in 82 of all the patients (44.8%), including one bilaterally operated patient with normal postoperative hearing. Hearing loss was bilateral in 72 of the 82 patients with hearing loss (87.8%). Two patients had unilateral anacusis caused by trauma. One of them also had OI-related hearing loss, while the other patient had normal hearing unilaterally.

The mean age at the onset of hearing loss according to patient history or audiometry was 28 years (SD 15 years, range 1-81 years). The prevalence of hearing loss increased with increasing age. The proportion of hearing loss in different age categories is presented in Table 3. Figure 8 presents the hearing pattern in the 366 ears with audiometry. Mixed hearing loss was the most common type of hearing loss in both operated and non-operated patients. Table 4 presents the type of hearing loss in the 183 patients with audiometry, classified according to Sillence.

Since hearing measurements in only a part of the patients included speech audiometry, tympanometry and measurement of the stapedial reflex (1kHz ipsi- and contralateral stimuli), no analysis of these measurements was performed.

Table 3. *The proportion of hearing loss in different age categories in the 183 patients with audiometry*
** Congenital / early sensorineural hearing loss*
*** Including one patient with unilateral traumatic deafness*
**** Including one patient with normal postoperative hearing*

	<10 years	**10-19 years**	**20-29 years**	**30-39 years**	**40-49 years**	**50-59 years**	**60-69 years**	**>70 years**	**Total**
Normal hearing	19	29	15**	19	9	7	3	0	101
Hearing loss	1* (5%)	4 (12.1%)	12 (44.4%)	19 (50%)	15** (62.5%)	13 (65%)	8 (72.7%)	10 (100%)	82
Total number of pts	20	33	27	38	24	20	11	10	183

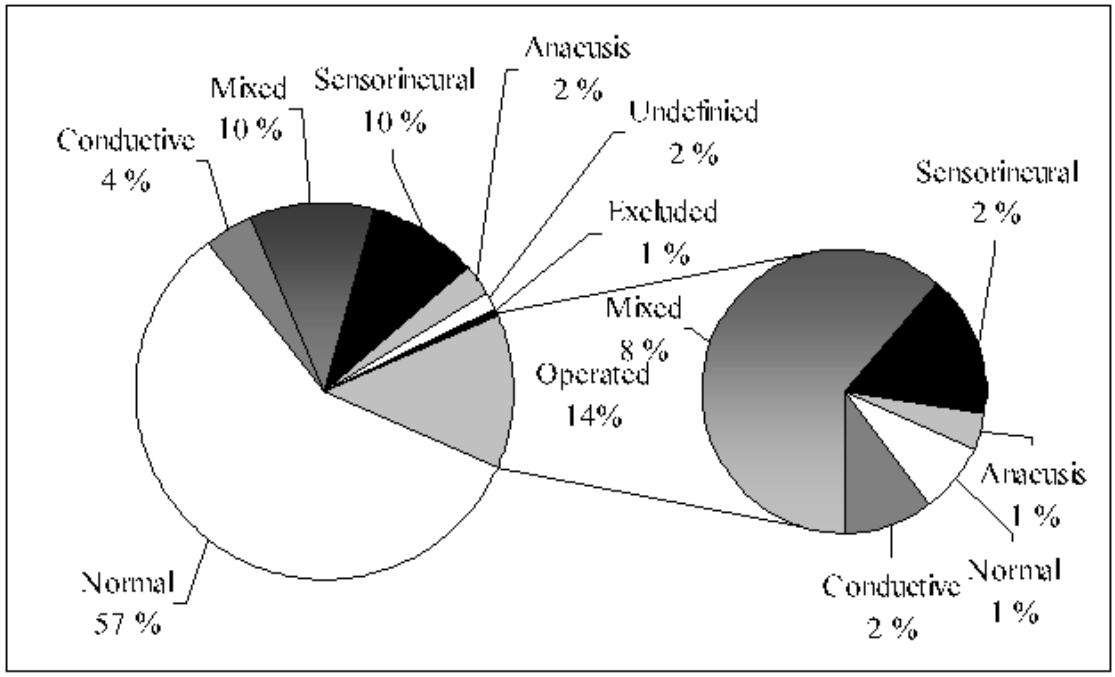

Figure 8. *Hearing pattern in the 366 ears of the 183 patients, with hearing pattern of the patients with previous ear surgery presented separately. For definition of various types of hearing loss, see page 28.*

Table 4. *The type of hearing loss in the 366 ears of the classified 183 patients (Glorieux et al. 2000; Glorieux et al. 2002; Hall 2002; Sillence 1981). Operated patients are presented separately. For definition of various types of hearing loss, see page 28.*

	I	III	III/IV	IV	V	Cole-Carpenter	Unclassified	Total
Normal	140	13	2	48	2	2	4	211
Conductive	6	1	1	8	0	0	0	16
Mixed	29	4	0	4	0	0	1	38
Sensorineural	30	0	2	2	0	0	1	35
Anacusis	9	0	0	1	0	0	0	10
Undefinied	4	1	0	0	0	0	0	5
Excluded	1	0	0	1	0	0	0	2
Operated	37	1	1	10	0	0	0	49
Total	256	20	6	74	2	2	6	366

5.2.2. Hearing loss in children with OI (I)

Hearing loss was found in three of the 46 children with audiometry. An 11-year-old girl and a 15-year-old boy with severe sporadic type IVB OI had earlier undiagnosed conductive hearing loss, with PTA $_{0.5\text{-}2kHz}$ 35/40 dB HL and 27/18 dB HL (right/left ear), respectively (I, Fig. 1 and 2). The third patient, a 7-year-old girl with familial OI

type I had either congenital or severe progressive sensorineural deafness observed at the age of 8 months (I, Fig. 3). According to the parents, the girl behaved like a normal-hearing child up to the age of six months. She had been treated with weight-adapted doses of Tobramycin during four operations for bilateral congenital hip luxation. OI was diagnosed when the left femur was fractured peroperatively at the age of 9 months. Seven relatives had blue sclerae but no bone fractures or hearing loss, and, therefore, no diagnosis of OI. Tympanic membranes were of normal appearance, and the PTA $_{0.5\text{-}2kHz}$ was 97/103dB HL.

5.2.3. Hearing loss in adults with OI (II)

Of the 137 adults with audiometry, 57.7% had hearing loss. Subjective sensation of hearing ability was misjudged by 18.2%, that is, 17% of the patients with normal hearing sensed hearing loss, and 12.7% of the patients with hearing loss did not recognize it. Hearing loss was bilateral in most patients. Mixed and sensorineural hearing loss occurred in all age groups, while conductive hearing loss was predominantly found in younger patients (II, Fig. 4). No clear correlation was found between clinical type of OI and presence of hearing loss. The patients with OI type IV, however, tended to have less hearing loss than the patients with OI type I and III (Fisher p=0.054) (II, Fig. 6). On the other hand, patients with hearing loss tended to have fewer fractures than patients with normal hearing (Fisher p 0.054). No correlation was found between severity, occurrence, or type of hearing loss and clinical features of OI such as blue sclerae, DI, vertigo, or whether the OI was sporadic or familial (II, Fig. 7 and 8).

Four adults had severe deafness ($PTA_{500\text{-}2000} > 90$ dB) not related to surgery. A 49-year-old woman with non-familial OI type I developed rapidly progressing hearing loss resulting in anacusis at the age of 22 years (II, Fig. 9). A 47-year-old man with blue sclerae and DI but no fractures, had anacusis of the right ear reported at the age of seven years. In addition, progressive hearing loss resulted in bilateral anacusis in two patients with mild OI type I: at the age of 71 years in a woman, and at the age of 73 years, in a man. In addition, eight non-operated patients had sensorineural hearing loss before the age of 40 years. Seven of these patients had OI type I, and one patient with blue sclerae and DI was not classified. Indicative statistical dependency between inner-ear-related hearing loss and OI type I was found (p 0.069).

5.3. Vestibular dysfunction and basilar impression (III)

5.3.1. Prevalence and general characteristics

Of the 42 adults consenting to participate in the study 52.4% reported vertigo. The mean age of the 12 men and 30 women was 39.1 years (SD 11.4, range 19-69 years). Twenty-one patients had OI type I, 5 had OI type III and 15 had OI type IV (III, Table II). Nine patients were bound to a wheelchair, while 25 patients walked without aid.

5.3.2. Audiological findings

At audiometry, 59.5% had hearing loss that was mostly bilateral. Hearing loss with a sensorineural component was present in all but two patients who had conductive hearing loss (III, Fig.2). ABR was pathological in 52% of the 25 patients with hearing loss, with a clear statistical association (p=0.0072). ABR pathology, such as abnormal fifth wave latency or side difference of the fifth wave latency, was not associated with vertigo, presence of BI, or with deviant ENG results.

5.3.3. Neurological symptoms and findings

Of the 42 patients, light-headedness was reported by 59.5%. Thirteen patients had sensory defects of the skin, and five of these patients also presented with BI. None of the 42 patients had head-shaking nystagmus. Two patients had spontaneous nystagmus with Frenzel´s glasses, but not at ENG. Both patients had vertigo, hearing loss and tinnitus, but normal skull base.

Subjective symptoms possibly deriving from the central nervous system were reported by 11 patients: two patients had attacks of syncope, one of them also presenting with BI. Seven patients presented with visual blurring or diplopia, three of them also suffering from BI. Three patients reported attacks of dysartria, and three patients suffered from sensory defects of the skin of the face, with one patient in each group also presenting with BI.

5.3.4. Vestibular symptoms and findings

Vertigo mostly presented as short episodes of floating or rotational sensation, often related to rapid head movements or altered position. Head movements or altered position caused vertigo in 16 of the 22 patients with vertigo (72.7%).

Patients with hearing impairment had more vertigo (62.5%) than patients with normal hearing (43.7%), but the difference was not statistically significant (p=0.1). The type of hearing loss was not associated with vertigo. The operated patients did not differ significantly from the non-operated patients regarding frequency, duration, severity or type of vertigo.

5.3.5. Electronystagmographic findings

A generally mild ENG pathology was found in 14 patients (33.3%) (III, Table IV). No correlation between deviant ENG results and vertigo was found. Patients with more severe hearing loss also had more deviant findings at ENG (p=0.049). ENG pathology also tended to be more common in patients with sensorineural or mixed type of hearing loss as compared with patients with normal hearing or conductive hearing loss (p=0.0768). Patients with previous stapedotomy had significantly more ENG

pathology indicating a peripheral vestibular lesion. No correlation between BI and pathology at ENG was found, although a central-type ENG abnormality seemed to be more common in patients with BI (p=0.118).

5.3.6. Radiological findings

Nine patients presented with radiologically defined BI (III, Table III). The mean age of these patients was 38.8 years (SD 15.1, range 20-69 years). Patients with BI had significantly more subjective symptoms deriving from the central nervous system than patients with normal skull base (p=0.036). No correlation between the presence or absence of BI and the type of OI was found, nor between the type of hearing loss and BI. The frequency of vertigo related to altered position or head movements was not significantly more common in patients with BI as compared with other patients, and the characteristics of vertigo were similar in both patient groups.

5.4. Results of stapes surgery (IV)

Surgical and audiological data of 43 middle ear operations performed from 1961 to 2001 were analysed. The mean age of the 33 patients at the onset of subjective hearing loss was 20.8 years (SD 6.1, range 10-35 years), and by the time of the surgery 30.1 years (SD 9.4, range 15-53 years). In 13 of the 43 operations the diagnosis of OI was not made preoperatively, although 12 of these patients had blue sclerae, DI was present in five patients, and all but one patient had had fractures. OI type I was most common in the operated patients (IV, Figure 1).

5.4.1. Surgical methods and anatomy of the middle ear

Of the operations, 75% were stapedotomies (19 ears) or stapedectomies (13 ears) (IV, Table 3). The most frequent surgical findings in the middle ear in the 43 operations were thick, fixated or obliterated footplate, thick and vascular mucosa with excessive bleeding tendency and elastic, fractured or atrophic stapes crura. Fibrosis was also common (IV, Table 4).

5.4.2. Outcome and audiometric results

The pre- and postoperative hearing level in the operated 43 ears is presented in IV, Fig. 2 A-C. These operations were performed by 20 different surgeons in five university hospitals and five central hospitals. One surgeon operated on three ears, four surgeons operated on two ears each, while fifteen surgeons operated on one ear each. In addition, one surgeon performed sixteen operations.

In spite of similar preoperative $PTA_{500\text{-}2000}$ in university and central hospitals, a statistically significant improvement in $PTA_{500\text{-}2000}$ was found in university hospitals compared with central hospitals (p 0.005). In addition, an overall deterioration in the

mean bone conduction thresholds at speech frequencies from 500 to 2000 Hz was found (from 15.7 to 18.6 dB) in the central hospital group, while an overall improvement was noticed (from 21.0/16.2 dB) in the university group. Furthermore, the results of the 16 operations performed by a single surgeon in the university group were more homogenous, and the hearing gain was better, although the difference was not statistically significant.

One patient presented with anacusis at the first postoperative follow-up ($PTA_{500\text{-}2000}$ 100 dB HL; patient number 3 in IV, Fig. 2). This 23-year-old woman with severe form of OI type III/IV had a preoperative bilateral hearing loss ($PTA_{500\text{-}2000}$ 23.3/63.3dB HL dx/sin). In the operated left ear, the facial nerve was reported to be nude, and the thread-like long process of the incus was in the supine position, forcing the prosthesis to be placed in the malleus instead of incus.

5.5. Molecular pathology and genotype-phenotype analysis (V)

Mutations either in COL1A1 or COL1A2 were found in 49 of the 54 patients (V, Table 2). Thirty-two of the 49 patients had familial OI and 16 had sporadic disease. One patient could not be classified as familial or sporadic, since the family members could not be examined. These 45 patients represent the molecular genetic background of 123 of the 299 Finnish OI patients (41.1%) known by the year 2003. The mean age of the 19 men and 30 women at the time of the audiometry was 36.4 years (SD 11.1 years, range 12-59 years). Most of the patients had OI type I (V, Fig. 1). Forty had blue sclerae (81.6 %), and all but one suffered fractures.

5.5.1. Molecular genetic findings

A total of 47 different mutations were identified in 49 patients, as an identical mutation was present in two non-related patients (V, Table 2). CSGE analysis and complete sequencing of the genes did not detect mutations in five patients. Thirty-eight of the mutations were in COL1A1 and eleven in COL1A2. Sixteen patients had a mutation resulting in glycine substitutions in COL1A1 or COL1A2. Six glycine mutations were in COL1A1, and ten in COL1A2. Mutations in the consensus RNA splicing sequences were found in sixteen patients. One OI type III patient with a two nucleotide deletion in exon 33/34 of COL1A1 (c.2268-2269delTC) was included in this group.

Four nonsense mutations and thirteen frameshift mutations, predicted to result in nonsense-mediated mRNA decay, were found in COL1A1. Two unrelated patients had the same c.299insC mutation. Also a nonsense mutation, R848X, appeared in two unrelated patients.

5.5.2. Audiometric findings

Of the 49 patients with a mutation in either COL1A1 or COL1A2, 32 had hearing loss (65.3 %) (V, Table 3). The mean age at the onset of hearing loss was 23.9 years (SD 8.0, range 12-45 years). Hearing loss was found in all OI types (V, Fig. 2).

According to the audiometric studies, 15 of the 32 patients with hearing loss had early sensorineural hearing loss, i.e. bone conduction thresholds at speech frequencies 500-2000 Hz were 20 dB HL or more before the age of thirty years in a previous audiometry. One patient had anacusis. The first audiometry in this woman with OI type I was performed when she was 20 years of age. She was found to have a progressive hearing loss of mixed type. Rapid progression of the hearing loss resulted in total anacusis at the age of 22 years (V, Table 2, patient number 17).

The pattern of familial hearing loss could be studied in 14 cases. No correlation was found between the OI type and expression of hearing loss in families.

5.5.3. Genotype-phenotype correlation

The mutation and clinical data of the 49 patients are presented in V, Table 2. The mutated genes correlated with the OI types. COL1A1 mutations were more frequent in OI types I and III, and COL1A2 mutations were more frequent in OI type IV ($p=0.00086$).

The association between the four different mutation subgroups and clinical OI types was statistically significant ($p=0.0010$), predominantly due to the association of null allele mutations with OI type I and partially due to the association of glycine substitutions in proα2(I) with OI type IV (V, Table 4). Patients with COL1A1 mutations more often had blue sclerae ($p=0.0327$), and they tended to be taller ($p=0.0443$, t-test) than the patients with COL1A2 mutations.

Mutations in COL1A1 were as likely to cause hearing loss as COL1A2 mutations ($p=0.725$). The presence of hearing loss did neither correlate with the different mutation types ($p=0.452$), nor did the type or severity of the hearing loss correlate with the mutated genes or different mutation types. Early sensorineural hearing loss or expression of hearing loss in the families did not correlate with the mutation types.

5.5.4. Patients without detectable mutations in COL1A1 or COL1A2

In five patients, no mutation was detected by CSGE or sequencing of all exons in COL1A1 or COL1A2.

Two patients had sporadic OI type I and moderate bone fragility. A 69-year-old woman (height 153 cm) had normal hearing, and a 41-year-old woman (height 161

cm) had conductive/mixed hearing loss (right/left ear). A 55-year-old woman (height 163 cm) with familial OI type I from a family with twelve affected members in three generations had a moderate fracture rate and mixed hearing loss bilaterally. A 41-year-old, 157 cm tall woman with sporadic OI type IV and moderate bone fragility had white sclerae and normal hearing. A 48-year old, 130 cm tall man with sporadic OI type V had moderate bone fragility and normal hearing.

6. Discussion

6.1. General discussion

This is the first study on OI in Finland, covering all known patients and evaluating hearing problems as a symptom of OI. This is also the first whole population study in which molecular genetic analysis has been applied in OI.

In earlier population studies in Sweden, Norway and Denmark, prevalence figures between 3.3/100 000 and 5/100 000 have been found (Heiberg 1983; Pedersen 1983; Smårs 1961). During this study, a total of 299 cases of OI from a population of 5.2 million was found, corresponding to a prevalence of 5.74/100 000. The true prevalence of OI in Finland is most probably greater, and patients with severe forms compared with mild forms may be over-represented, as the mild forms may still today remain undiagnosed.

Prior to the present study, six population studies on hearing loss including all types of patients with OI have been published. In these studies, hearing loss has been found in 22.6-58% of the OI patients (Paterson et al. 2001; Pedersen 1983; Seedorff 1949; Sillence et al. 1979; Smårs 1961; Stewart et al. 1989). Bilateral, progressive hearing loss often starts in the second to third decade of life, it is predominantly conductive at onset, and later mixed or sensorineural (Bergstrom 1977; Cox et al. 1982; Garretsen et al. 1997; Pedersen 1984; Quisling et al. 1979; Riedner et al. 1980). Hearing loss is reported to be most common in OI type I, and it is thought to be less common in OI type IV(Garretsen et al. 1997; Garretsen et al. 1991; Sillence 1981; Sillence 1988; Stewart & O'Reilly 1989). Comparison of the studies, however, is difficult as the definition of hearing loss may be missing or varies greatly, and the study subjects may be selected (Garretsen et al. 1997; Garretsen & Cremers 1991; Paterson et al. 2001; Pedersen 1984; Sillence 1981; Sillence 1988).

Vertigo in OI patients has not, to the best of our knowledge, been studied earlier, although hearing loss is one of the major symptoms in OI, and vertigo is frequently associated with otosclerosis in which the hearing loss clinically resembles that in OI (Virolainen 1972). In otosclerosis, the prevalence of vertigo has been reported to increase with an increasing sensorineural component in the hearing pattern (Cody et al. 1978; Thomas et al. 1981). Vertigo, as well as sensorineural hearing loss, are also common symptoms in BI, which is reported in up to 25% of OI adults (Elies et al. 1980; Sawin et al. 1997; Sillence 1994). BI has been reported to be most prevalent in OI type IVB, and in more severely affected individuals with OI (Charnas et al. 1993; Elies & Plester 1980; Sawin & Menezes 1997; Sillence 1994).

Although there are clinical similarities with otosclerosis, hearing loss in OI is a distinct entity with earlier onset, more severe middle ear involvement, and higher incidence of sensorineural hearing loss (Bretlau et al. 1969; Garretsen et al. 1997; Holdsworth et al. 1973; Shapiro et al. 1982; Shea et al. 1963; Stewart & O'Reilly

1989). The typical surgical findings in the middle ear of an OI-patient with hearing loss are thick and fixated or obliterated footplate, thick and vascular mucosa with excessive bleeding tendency, and brittle atrophic stapes crura. Crural fractures, closed round window, fragile incus, and deficient, short ossicles have also been reported (Armstrong 1984; Pedersen et al. 1983; Shea et al. 1982; Van Der Rijt & Cremers 2003). Compared with the dense, hard bone seen in otosclerosis, the footplate area in OI often presents with soft, granular or iceberg type bone (Kosoy et al. 1971; Patterson et al. 1970; Pedersen & Elbrond 1983; Sooy 1960). The postoperative hearing results in OI surgery have been encouraging**,** albeit not as satisfactory as in otosclerosis (Armstrong 1984; Cremers et al. 1991; Garretsen et al. 1990; Glasscock et al. 1995; Palva et al. 1977; Pedersen & Elbrond 1983; Shea & Postma 1982; Van Der Rijt & Cremers 2003).

The genotype-phenotype correlation in OI has mainly been focused on the clinical overall severity of the disease. Mutations reducing the amount of type I collagen are reported to produce the mildest phenotypes, while mutations altering the structure of the proα chains produce a wide range of phenotypes (Byers et al. 1991; Cole 1997; Wenstrup et al. 1990). The correlation between the mutation and hearing loss has not been extensively studied earlier.

6.2. Hearing loss in children (I)

The onset of hearing loss in OI most often coincides closely with the time of life when the frequency of fractures is decreasing in the late second decade of life (Bergstrom 1977; Cox & Simmons 1982; Garretsen et al. 1997; Pedersen 1984; Riedner et al. 1980). Although not emphasized in literature, hearing loss in OI may begin already in childhood (Cox & Simmons 1982; Pedersen 1984; Stewart & O'Reilly 1989; Verstreken et al. 1996). Mostly, conductive type of hearing loss has been reported in children (Cox & Simmons 1982; Garretsen et al. 1997; Pedersen 1984). Pedersen (1984) reported conductive hearing loss in 22% of the children in the age group 4-9 years, and in 28 % in the age group 10-19 years (Pedersen 1984). However, hearing loss was explained by secretory otitis media in four of the five children in the younger age group (Pedersen 1984). In a recent Australian study, middle ear effusion was also found to be a main reason for conductive hearing loss in children with OI (Imani et al. 2003). Therefore, tympanometry should be included in the diagnosing of hearing loss in children with OI.

Garretsen´s study (1997) on type I OI patients in the Netherlands revealed the highest frequency of hearing loss among children so far: 31% of the children 4-9 years of age, and 62% of the children 10-19 years of age. The patients, however, were selected for ear surgery or volunteered for the study because of their hearing loss (Garretsen et al. 1997).

In our study on 46 Finnish children with audiometry, prevalence of hearing loss in the youngest age group studied was lower than in previous studies with audiometry (Table

5) (Garretsen & Cremers 1991; Pedersen 1984). This is most probably caused by the inclusion of children with secretory otitis media in previous studies, or the selection of the patients (Garretsen & Cremers 1991; Pedersen 1984). Of the 46 children between 4 and 16 years of age, only two children with sporadic OI type IV had progressive hearing loss of conductive type clearly related to OI (4.4%). In addition, a girl with familial OI type I had anacusis diagnosed during the first year of life. An etiology other than OI was suspected. After a OI type I woman presented with a rapid, progressive hearing loss resulting in total anacusis at the age of 22 years (II), the still unanswered question of the possibility of OI connected anacusis in these two patients arouse. Stewart reported a child in the age group 10-19 years with a flat sensorineural hearing loss of 30 dB HL in one ear and 60 dB HL in the other, with no other explanation than OI (Stewart & O'Reilly 1989).

This study indicates that clinically significant hearing loss in children under 16 years of age with OI is less frequent than suggested by previous studies. The high prevalence of hearing loss in previous studies may be explained by the small or selected study materials, and by secretory otits media, as well as by the different criteria used for the hearing loss (Garretsen et al. 1997; Pedersen 1984). In addition, the age range in our study was between 4 and 16 years of age, and not 19 years, as in previous studies (Table 5).

No audiometry was performed in 15 of the 61 children included in the population study. As almost 20% of the adults were found to misjudge their hearing ability (II), these children may not automatically be considered as normal hearing. Early detection and treatment of hearing loss is of utmost importance to avoid additional disability that aggravates the physical handicap. Therefore, audiometry is recommended in a child with OI if a hearing deficit is suspected, and for an asymptomatic patient a baseline study at the age of 10 years with repeated audiograms every three years is recommended.

6.3. Hearing loss in adults (II)

More than 50% of adults with OI are reported to have hearing loss (Paterson et al. 2001; Pedersen 1984; Sillence et al. 1979; Stewart & O'Reilly 1989). Our study supports this finding, as 57.7% of the 137 adults with audiometry had hearing loss (Table 5). Mixed hearing loss was the most frequent type of hearing loss. Conductive hearing loss was mostly observed in younger patients, while sensorineural and mixed type of hearing loss was seen in all age groups, as reported in earlier studies (Bergstrom 1977; Cox & Simmons 1982; Garretsen et al. 1997; Pedersen 1984; Quisling et al. 1979; Riedner et al. 1980).

Hearing loss has been suggested to be more common in OI type I than in OI type IV (Paterson et al. 2001; Sillence et al. 1979). In Australia, 35% of OI type I patients of all ages had hearing loss. No hearing loss was found in OI type IV (Sillence et al. 1979). In a Scottish study about 30% of patients with OI type IA, IB and IVB reported hearing loss at the age of 30 years, while the proportion for OI type IVA was 9% (Paterson et al. 2001).

***Table 5**. Hearing loss in different age categories in studies on hearing loss in Osteogenesis imperfecta.*

Authors	Number of pts	Patients	Definition of hearing loss	Audiometry	Hearing loss all pts %	4-9 years	10-19 years	20-29 years	30-39 years	40-49 years	50-59 years	>60 years
Pedersen 1984	173	population	yes*	yes + portable	88/173 51%	5/23 22%	10/36 28%	18/31 58%	15/29 52%	12/21 57%	8/12 67%	20/21 95%
Stewart and O´Reilly 1989	53	population	yes*	yes + portable	29/53 50%	0	2/13 15%	6/14 43%	5/7 71%	11/12 92%	7/7 100%	0
Garretsen and Cremers 1991	70	OI type I	yes*	yes	30/70 43%	2/18 11%	13/21 62%	6/13 46%	1/8 13%	2/3 67%	3/4 75%	3/3 100%
Paterson et al 2001	1394	population	no	no	317/1394 23%	20/466 4%	?	?	?	?	?	?
This study 2003	183	population	yes*	yes	82/183 45%	1/20 5%	4/33 12%	12/27 44%	19/38 50%	15/24 63%	13/20 65%	18/21 86%

** Definition of hearing loss varies*

Although no clear statistical dependency was found between the clinical type of OI and presence of hearing loss in our Finnish study, the patients with OI type IV may have less hearing loss than the patients with OI types I and III (Fisher p=0.054). In OI type III, the two previous studies on hearing loss have presented conflicting results (Paterson et al. 2001; Sillence et al. 1979). In our study, four of the five type III patients had hearing loss, supporting Paterson´s finding of hearing loss also being common in OI type III (Paterson et al. 2001). The type of hearing loss also seemed to be slightly different between types I and IV OI, with inner-ear-related hearing loss occurring more often in OI type I (F-test p 0.069). Compared with otosclerosis, a higher incidence of sensorineural hearing loss has been reported in OI (Bergstrom 1977; Holdsworth et al. 1973; Patterson et al. 1970; Shea et al. 1982; Stewart et al. 1989). In our study, sensorineural hearing loss was also found in the youngest age groups, indicating the existence of a group of OI patients with a predominantly sensorineural pattern at the onset of the hearing loss. This early sensorineural hearing loss was found only in patients with OI type I, and the only patient with rapidly progressing hearing loss resulting in anacusis within 8 years at the age of 22 years also had OI type I. However, in the molecular genetic study with retrospective evaluation of early sensorineural hearing loss as one of the characteristics of hearing loss, also patients with other types of OI than OI type I were found to have early sensorineural hearing loss (V, Table 3). In earlier studies, anacusis is reported mostly in older age groups, although sensorineural hearing loss in early age has also been reported (Pedersen 1984; Stewart & O'Reilly 1989). The etiology of early sensorineural hearing loss in OI remains unresolved, and further studies should be carried out.

The misleading subjective assessment of hearing ability found in our study emphasizes the importance of regular audiometric studies in patients with OI, as almost 20% of adults misjudged their hearing ability.

6.4. Vestibular dysfunction (III)

The first study on vestibular dysfunction in OI patients is presented, with 22 of the 42 adults reporting vertigo (52.4%). Vertigo precipitated by head movements is reported as one of the principal neurological symptoms of BI of unspecified etiology, and it has also been reported in otosclerosis (Elies et al. 1980; Sawin et al. 1997; Thomas et al. 1981; Tucker 1979). Also in our study, head movements or altered position was a common trigger, provoking balance problems in 72.7% of the patients with vertigo.

In otosclerosis, the prevalence of vertigo has been reported to increase with an increasing sensorineural component in the hearing pattern (Cody et al. 1978; Thomas & Cody 1981). Sensorineural hearing loss and vertigo were not correlated in our study, but vertigo tended to be more frequent in patients with hearing loss than in patients with normal hearing.

Vertigo was not correlated with deviant ENG results, but as in earlier reports on otosclerotic ears with cochlear involvement as compared with ears with conductive hearing loss, we also found a tendency toward more ENG pathology in the OI patients with sensorineural or mixed-type hearing loss than in those with conductive hearing loss or normal hearing ($p=0.0768$) (Cody & Baker 1978; Morales-Garcia 1972) (III, Table IV). This implies extensive damage to the inner ear.

Nine patients in our study presented with BI (IV, Table III). Sensorineural hearing loss has previously been reported in BI, but no association between BI and presence or type of hearing impairment could be found in our study (Sawin & Menezes 1997). Although BI has been reported to be most prevalent in OI type IVB, no correlation between the type of OI and presence of BI was found in our study (Sillence 1994). BI has also been suggested to occur in more severely affected individuals with OI, but in our study, no correlation between BI and severity of OI was found (Charnas et al. 1993).

Our study confirms that vertigo, in addition to hearing loss, is common in patients with OI. As in otosclerosis, the true etiology for sensorineural hearing loss, as well as for vertigo in OI still remains unknown. Both the fact that the patients with hearing loss tended to have more vertigo, and the co-existence of sensorineural hearing loss and deviant vestibular findings at ENG indicate damage to the inner ear as the main reason for vertigo in these OI patients. A pathology similar to otosclerotic foci in contact with the pars superior of the vestibule or the superior vestibule nerve, as well as otosclerotic vascular changes due to the disease or to biochemical changes in the inner ear fluids are possible (Ghorayeb et al. 1978; Virolainen 1972). Vertigo in OI patients might also be partly explained by BI. Still, some OI patients without hearing loss or basilar impression also suffer from vertigo clinically indistinguishable from the type of vertigo seen in patients with hearing loss or BI. Therefore, patients with OI should be informed about the frequency of vertigo in OI, not only as a symptom of BI, a feared complication of OI.

6.5. Stapes surgery (IV)

Despite the anatomical challenges, satisfying to excellent results of stapes surgery in OI have been presented in former studies (Armstrong 1984; Garretsen et al. 1990; Kosoy et al. 1971; Pedersen et al. 1983; Shea & Postma 1982; Van Der Rijt & Cremers 2003). The outcome of stapes surgery in Finnish patients with OI was evaluated in a study with 43 ears of 33 OI patients operated in Finland between 1961 and 2002. Thirty-six of the operations were performed in university hospitals and seven in central hospitals. Like in previous studies, our study revealed the typical surgical findings in the middle ear to be thick and fixated or obliterated footplate, thick and vascular mucosa with excessive bleeding tendency, and elastic, fractured or atrophic stapes crura (Armstrong 1984; Pedersen & Elbrond 1983; Shea & Postma 1982; Van Der Rijt & Cremers 2003).

In our study, the surgically achieved hearing gain was poorer than in former studies (Armstrong 1984; Garretsen & Cremers 1990; Kosoy & Maddox 1971; Pedersen & Elbrond 1983; Shea & Postma 1982). However, it was better in university hospitals than in central hospitals, and after surgery performed by a single surgeon in a university hospital, the results were comparable with earlier studies. In addition, the mean bone conduction thresholds were unchanged as a result of the operation in university hospitals, while the thresholds were slightly deteriorated after the surgery in central hospitals. The 16 operations performed by one surgeon were performed between 1993 and 2002, when OI-related hearing loss was studied in Finland (I-IV). All these patients were known to suffer from OI, while in thirteen out of the 27 operations in central hospitals and other university hospitals, the patients were treated as patients with the far more common otosclerosis.

The standard of care of patients with such a rare and complicated disease as OI would greatly benefit from centralization to a few national centers. Unfortunately, in Finland, this has only partly been achieved in OI. The same situation has also concerned the treatment of otological problems of OI. The detection of hearing impairment may be delayed. The mild forms of OI may be treated as otosclerosis. Our study indicates that the surgical outcome is better at departments of otology in the university hospitals than at local central hospitals with a smaller annual number of otosclerosis surgery, and even fewer patients with OI-related hearing loss. A correct preoperative differential diagnosis of OI-related hearing loss and otosclerosis is a prerequisite for successful surgery. In addition to more experienced surgeons, different operative results may be explained by the anatomical peculiarities in the middle ear in OI causing technical problems in an operation unit without laser, that facilitates stapes surgery (McGee 1983; Rauch et al. 1992).

6.6. Molecular pathology and genotype-phenotype analysis (V)

The molecular genetic defect was searched for in a selected patient sample of 54. A disease causing mutation was found in 49 patients. Altogether, 47 mutations were

detected. Forty-one of these mutations are presented for the first time. The 49 patients represent the molecular genetic background of 41.1 % of the known Finnish OI population.

Mutations reducing the amount of type I collagen are reported to produce the mildest phenotypes, while mutations altering the structure of type I collagen produce a wide range of phenotypes (Byers 2002; Byers et al. 1991). The association between the mutation type and the OI type was also evident in this study. Null allele mutations most often produced OI type I, while single base substitutions resulting in glycine substitutions in proα2(I) tended to produce OI types I and IV. Furthermore, patients with COL1A1 mutations more often had blue sclerae, whereas patients with COL1A2 mutations tended to be shorter.

Hearing loss has been suggested to be more common in patients with COL1A1 mutations than in patients with mutations in COL1A2 (Sykes et al. 1990). In this study, hearing loss was slightly more common (65.3%) than that previously observed in Finnish OI adults (II). Thirty-eight mutations were found in COL1A1 and eleven mutations in COL1A2. No correlation between the mutated gene and hearing pattern was found.

A variety of mutations causing OI have been identified in COL1A1 and COL1A2 (Byers et al. 1991; Kuivaniemi et al. 1997; Willing et al. 1996). We found 16 single base mutations causing glycine substitution, 16 RNA splicing mutations and 17 null allele mutations. The types of mutations did not correlate with the presence, type, severity or special characteristics of hearing loss. On the contrary, the different mutation types resulted in overlapping hearing phenotypes. Two patients with the same null allele mutation in COL1A1, c.299insC, had different hearing patterns (patients 42 and 45 in V, Table 2). Patient 42 had normal hearing at 56 years, while patient 45 already had a moderate mixed/sensorineural hearing loss (right/left ear) at the age of 17 years. On the other hand, both patients with the R848X COL1A1 mutation had hearing loss that was conductive in patient 41 at the age of 39 years, and mixed in patient 46 at the age of 50 years.

In five patients with clinically confirmed OI, no mutation was found in COL1A1 or COL1A2 genes by CSGE analysis or sequencing of all exons. The sensitivity and specificity of the CSGE mutation search method used in the study are high (Ganguly 2002; Leung et al. 2001). These five patients may have had large gene rearrangements that could not be detected by the mutation detection approach used. On the other hand, they may also demonstrate the heterogeneity of OI.

7. Conclusions

In the present study on the Finnish Osteogenesis imperfecta (OI) population, audiometric findings, results of stapes surgery, and search for molecular defects with genotype-phenotype correlation analyses were investigated. In addition, vestibular problems in a subpopulation of Finnish adults were studied. The following conclusions could be drawn:

- Hearing loss is a common feature in OI, affecting patients with all types of OI. Of the adults, 57.7% had hearing loss. Patients with OI type I or III may have more hearing loss than patients with OI type IV, although the difference was not statistically significant in this study. In addition, sensorineural hearing loss, especially at an early age, may be more common in OI type I.

- Although hearing loss most often presents in adulthood, it may gradually begin already in childhood. Misleading subjective assessment of hearing ability in adults with OI emphasizes the importance of regular audiometric studies in all patients with OI. While early detection and treatment of hearing loss with middle ear surgery and hearing aids are of utmost importance to avoid aggravation of physical handicap, audiometry and tympanometry should be performed in all OI patients as a baseline study at the age of 10 years with repeated audiograms every third year thereafter, even in asymptomatic patients.

- Vertigo is common in OI. Of the adults, 52.4% had vestibular dysfunction. Inner ear damage appears to be the main reason for vertigo, but basilar impression of the skull (BI) may cause vertigo in some patients. Still, some OI patients without hearing loss or BI suffer from vertigo clinically indistinguishable from the type of vertigo seen in patients with hearing loss or BI. In clinical practise, patients with OI should be informed about the frequency of vertigo in OI, which is not only a symptom of BI, a feared complication of OI.

- The surgical anatomy in OI differs from otosclerosis especially in the thick and vascular mucosa with excessive bleeding tendency, a thick, often obliterating stapes footplate formed by abnormal bone and elastic, fractured or atrophic stapes crura. The anatomical peculiarities in the middle ear in OI cause technical problems, and therefore, a prerequisite for successful surgery is a correct preoperative differential diagnosis of OI-related hearing loss and otosclerosis. Furthermore, the hearing gain appears to be better after surgery centralized in units with a larger annual number of otosclerosis surgery and more experienced surgeons.

- COL1A1 mutations are more frequent in OI types I and III, and COL1A2 mutations in OI type IV. Furthermore, null allele mutations most often produce OI type I, while single base substitutions resulting in glycine substitutions in proα2(I) tend to produce OI types I and IV. However, neither the mutated gene nor the type of mutation correlated with the presence, type or severity of hearing loss, the age at onset of the hearing loss, or special features of the hearing loss such as early sensorineural hearing loss or the expression of hearing loss in the family. No mutation in COL1A1 or COL1A2 genes was found in five of the 54 patients with clinically confirmed OI. This may be due to large gene rearrangements not detectableby the mutation detection approach used, or may demonstrate the heterogeneity of OI.

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ORIGINAL PAPER

I

Kaija Kuurila · Reidar Grénman · Reijo Johansson · Ilkka Kaitila

Hearing loss in children with osteogenesis imperfecta

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Abstract Osteogenesis imperfecta (OI) is a genetic disorder of connective tissue. Progressive hearing loss is one of the principal symptoms of OI, affecting about 50% of adult patients. Hearing loss may also occur in childhood and results in additional disability in education and psychosocial adaptation and aggravates the physical handicap. This can be avoided by appropriate otological and audiological treatment. In a nationwide search, 254 Finnish patients with OI were identified indicating a prevalence of 4.9/100 000. Of the 60 children, 45 aged between 4 and 16 years accepting to participate the study on hearing, were evaluated by a questionnaire and clinical audiometry. Hearing loss was defined as pure tone average ($PTA_{0.5–2\ kHz}$) more than 20 dB hearing level (HL). A clinical geneticist determined the type of OI among the 45 patients. Two sporadic OI cases with conductive hearing loss were ascertained (4.4%): An 11-year-old girl with type IV OI with a $PTA_{0.5–2\ kHz}$ of 35/40 dB HL and a 15-year-old boy with type IV OI with a $PTA_{0.5–2\ kHz}$ of 27/18 dB HL. In addition, a 6-year-old girl with familial OI type I had either a congenital sensorineural deafness or early progressive deafness with $PTA_{0.5–2\ kHz}$ of 97/103 dB HL, probably of unrelated aetiology.

Conclusion Hearing loss in children with osteogenesis imperfecta is less frequent than generally suspected. Nevertheless, it is recommended that audiometry is performed in children with osteogenesis imperfecta even without symptoms of hearing loss at the age of 10 years, and repeated every 3 years thereafter.

Key words Osteogenesis imperfecta · Hearing loss · Children

Abbreviations *OI* osteogenesis imperfecta · $PTA_{0.5–2\ kHz}$ pure tone average at frequencies of 0.5, 1 and 2 kHz · *HL* hearing level

Introduction

Osteogenesis imperfecta (OI) is a genetic disorder of connective tissue presenting as bone fragility and deformities, hearing loss, dental fragility and discolouration, blue sclerae, joint hypermobility and easy bruising [4]. OI is most often caused by mutations in one of the two genes COL1A1 and COL1A2 that encode the synthesis of type I collagen [4]. The minimum prevalence of OI is estimated to be 1/10 000–1/30 000 [4, 8, 11]. Variable severity of clinical expression, non-reporting, and lack of patient registers are probably the major reasons for biased prevalence figures [4].

K. Kuurila (✉) · R. Grénman · R. Johansson
Department of Otorhinolaryngology-Head and Neck Surgery, Turku University Central Hospital, Turku, Finland

K. Kuurila
Vaasa Central Hospital, Hietalahdenkatu 2-4, 65100 Vaasa, Finland
e-mail: kaija.kuurila@vshp.vaasa.fi,
Tel.: +358-6-3231111, Fax: +358-6-3231378

I. Kaitila
Department of Clinical Genetics, Helsinki University Central Hospital, Helsinki, Finland

The preliminary results of this study have previously been presented at the 7th International Congress of Paediatric Otorhinolaryngology, Helsinki, 9 June 1998

Table 1 Classification of OI (modified from [16]). (*AD* autosomal dominant, *AR* autosomal recessive)

Type	Clinical features	Inheritance
I	Mild to severe bone fragility; normal or slightly short stature, intensely blue sclerae, presenile hearing loss, normal teeth or dentinogenesis imperfecta	AD
II	Extremely severe bone fragility, intra-uterine or early infantile death	AD/AR
III	Variable, often severe bone fragility in infancy with progressive skeletal deformity; short stature, blueish sclerae, variable dentine abnormality	AD/AR
IV	Bone fragility with mild to moderate deformity, often short stature, normal sclerae, normal teeth or dentinogenesis imperfecta	AD

Since the late 1970s, OI has been divided into four major types using clinical, radiographic and genetic criteria. This classification introduced and later revised by Sillence has replaced the earlier classification into congenita and tarda forms [10, 16, 17] (Table 1). However, the wide variation in expression and heterogeneity probably often causes difficulties in classifying both familial and sporadic cases [15, 18]. Type I is the most common form, presenting with mild to severe bone fragility, normal or mild short stature and distinctly blue sclerae throughout life. Long bone deformation is often less severe than in other types of OI. Perinatal lethal type II is characterised by extreme bone fragility usually leading to intra-uterine or early infantile death. Type III is a progressive deforming form of OI with soft, bowed long bones and spine in addition to severe bone fragility. The post-natal growth failure is marked leading to very short stature. Sclerae are normal or greyish in OI type IV and the stature is generally short with bone fragility of variable severity and mild or moderate deformity. The mode of inheritance is autosomal dominant for types I and IV. Type III may be inherited as a dominant or recessive trait whereas patients with type II are usually sporadic cases, which are therefore due to new mutations in COL1A1 or COL1A2 genes [15–18].

In previous studies, the frequency of hearing loss in patients with OI has been reported to range from 34% to 78%. It is common in adults and usually progressive [5, 7, 11–13]. Presenile hearing loss is most common in OI type I and it appears to be rare in type IV [15, 17]. However, the definition of hearing loss has varied greatly in different studies and the study subjects may have been selected [2, 5–7, 11–13]. Even though the hearing loss in OI resembles that in otosclerosis, they are histologically and biochemically distinct diseases [1, 9] In most cases, the hearing loss is initially conductive and later mixed or sensorineural [2, 5, 7, 11–13]. Sensorineural hearing loss is more frequent in OI than in otosclerosis [2, 9, 14, 19].

The aim of the present study was to evaluate the frequency of clinically significant hearing loss in children with OI. In spite of observations suggesting that the onset of hearing loss may also occur in childhood [2, 3, 5, 13, 14, 20, 21], there are no systematic study reports on children with OI.

Patients and methods

In a nationwide search for patients with OI through the patient register of the Department of Clinical Genetics, Helsinki University Central Hospital, a major referral centre for genetic disorders of the skeleton and connective tissue in Finland, and the membership register of the Finnish Osteogenesis Imperfecta Association, as well as the HILMO care register from Finnish university and central hospitals, a total of 254 patients were identified of whom 204 patients consented to participate in the study. This patient material consisted of 52 families and 129 familial and 70 sporadic cases. Five patients could not be classified either as familial or sporadic. The diagnosis was based on fracture history, clinical and radiological findings and family history. All sporadic cases and the majority of the familial cases had had clinical evaluation at the Department of Clinical Genetics, Helsinki University Central Hospital. The results of the clinical evaluation for the remaining patients were available from other hospitals. Molecular defects in COL1A1 and COL1A2 genes had only rarely been investigated. The number of patients corresponds to a prevalence of 4.9/100 000. It is possible that patients with severe forms are over-represented as compared to mild forms.

The patients were approached via a questionnaire. The history of fractures, blue sclerae, dentinogenesis imperfecta and hearing loss was requested. The patients were asked about subjective and objective onset of the hearing loss, the clinical course of the hearing deficit, the use of hearing devices and surgical treatment. The patients were referred for clinical audiometry including pure tone audiometry (PTA), speech audiometry, tympanometry and measurement of stapedial reflex performed under standard conditions in a sound-proof room at a central or university hospital. Data on patients who did not undergo audiological examination were based upon the subjective sensation of the patients or their parents of the hearing ability. Normal hearing in children was defined as $PTA_{0.5-2\,kHz}$ equal to or better than 20 dB hearing level (HL). A definition for different types of hearing loss paralleling definitions given by Shapiro et al [14] and Pedersen [11] was used. Conductive hearing loss was defined as an average air-bone-gap for the frequencies 0.5, 1 and 2 kHz greater than 15 dB with corresponding bone conduction threshold better than 15 dB. Sensorineural hearing loss was defined as air conduction thresholds for the frequencies 0.5, 1 and 2 kHz equal to or greater than 15 dB with corresponding air-bone-gap smaller than 15 dB and mixed hearing loss as an average air-bone-gap for the frequencies 0.5, 1 and 2 kHz greater than 15 dB with corresponding bone conduction threshold equal to or greater than 15 dB.

There were 60 children aged 16 years or less. Nine children under 4 years of age were excluded from the study on the basis of insufficient co-operation and five children with no audiometry performed were considered to have normal hearing by their parents. Auditory brainstem responses with 2000 Hz click-stimulus at the 20 dB level was normal in a 7-year-old with poor co-operation caused by moderate mental retardation. A total of 45 children were studied by PTA thresholds for the hearing pattern. This study material consisted of 19 boys and 26 girls with a mean age of 10.0 years. A clinical examination of the children with impaired hearing was performed to exclude an aetiology other than OI for the hearing loss. The type of OI was determined by a clinical geneticist using the criteria modified from the Sillence classification [10].

Results

A total of 40 children (88.9%) had blue sclerae, 19 (42.2%) dentinogenesis imperfecta and 23 children (51.1%) had a family history of OI. All children except

Table 2 Classification of OI in Finnish children

Type	I	III	IV	Other	Not classified	Total
N	28	3	10	3	1	45

three could be classified according to Sillence, type I being the most common (62.2%) (Table 2). An 11-year-old boy with multiple fractures, blue sclerae and no dentinogenesis imperfecta had either OI type III or IV, and a 10-year-old girl, born with short and bowed legs as well as a left-sided femur fracture and later of normal stature, dentinogenesis imperfecta, a total of six fractures and blueish sclerae had either OI type I or IV. One child could not be classified. A 12-year-old girl had Cole-Carpenter syndrome-type OI. Audiometry was normal in 42 children (93.3%) with no hearing loss of high frequencies.

Three children had hearing loss (6.7%). The first patient, an 11-year-old girl with OI type IV had severe deforming OI with a history of prenatal and multiple post-natal fractures, very short stature (height 75 cm), dentinogenesis imperfecta and blue sclerae. She had had for some months a sensation of progressive hearing loss and the $PTA_{0.5-2\ kHz}$ was 28/35 dB HL (right ear/left ear), with speech reception thresholds of 35/35 dB at the time of the initial audiometry. Tympanic membranes and tympanograms were normal, no loss of speech discrimination was observed, and the stapedial reflexes were absent both on ipsi- and contralateral stimuli on both sides. Six months later, the $PTA_{0.5-2\ kHz}$ was 35/40 dB HL, and a hearing aid was fitted. The hearing loss was purely conductive except for a Carhardt notch at 2000 Hz (Fig. 1). The girl had no relatives with OI. The second patient, a 15-year-old boy with severe OI type IV, had also very short stature (110 cm) and a history of prenatal and innumerable post-natal fractures with severe skeletal deformation, as well as dentinogenesis imperfecta. The sclerae were white. He had suffered from disturbing low-frequency tinnitus on the left side for some months and the right ear felt blocked. Tympanic membranes were slightly blueish but otherwise normal. The PTA was 27/18 dB HL with purely conductive hearing loss except for a Carhardt notch on the right side. The hearing loss was progressive, with $PTA_{0.5-2\ kHz}$ 40/28 dB HL 2 years later, when, in an attempt to diminish the disturbing tinnitus, a hearing aid was fitted in the left ear (Fig. 2). No improvement was achieved. The third patient, a 7-year-old girl with familial OI type I had either congenital or severe progressive sensorineural deafness observed during the 1st year of life. According to the parents, the girl acted like a normal-hearing child up to the age of 6 months. She had been operated on several times for bilateral congenital hip luxation, and tobramycin had been used in weight-adapted doses during four of the operations. At the age of 9 months the left femur was fractured pre-operatively, after which the diagnosis of OI was confirmed. A right femoral fracture was observed at the age of 1 year and 7 months and a left

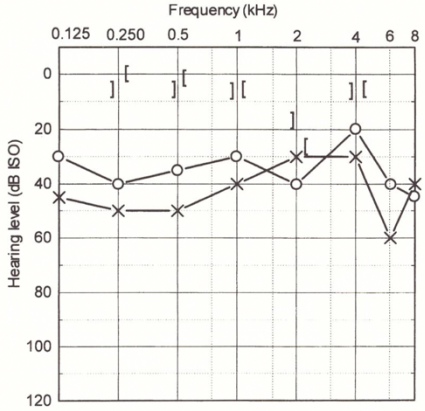

Fig. 1 Audiogram of an 11-year-old girl with OI type IVB and progressive, conductive hearing loss. Air conduction threshold right (*O*) and left (*X*) ear; bone conduction threshold right (*[*) and left (*]*) ear

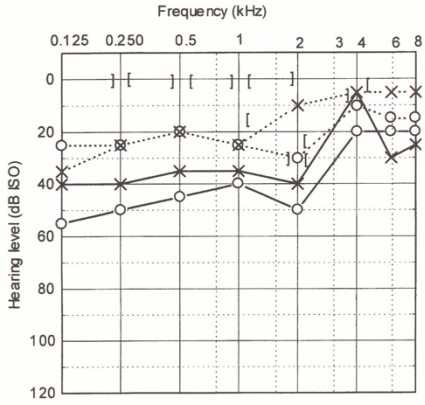

Fig. 2 Progression of hearing loss demonstrated by audiograms of a boy with OI type IV at the age of 15 (*dotted lines*) and 20 years (*solid lines*)

femoral fracture after minor trauma was detected at the age of 2 years and 6 months. The mother, as well as six of her relatives, had blue sclerae but no history of recurrent bone fractures or hearing loss, and therefore, no diagnosis of OI. The girl had normal stature (height 110 cm), hyperlaxity of the joints, and blue sclerae.

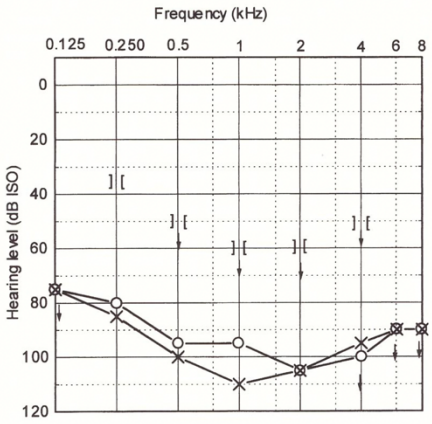

Fig. 3 Audiogram of a 6-year-old girl with severe sensorineural hearing loss, probably unrelated to OI, showing the maximum capacity of the audiometer (*arrows*) indicating that the hearing thresholds are located above this level

Tympanic membranes were of normal appearance and the $PTA_{0.5-2\ kHz}$ was 97/103 dB HL (Fig. 3). A hearing aid had been fitted bilaterally at the age of 11 months.

Discussion

The evaluation of previous studies on hearing in OI is difficult because of variable definitions for hearing loss and selected study materials. The patients may have been recruited because of otological problems, or selected for ear surgery [7]. In some studies, classification of the hearing loss related to age may not have been done, and family studies are more frequent than population studies [5, 12, 13]. The hearing loss in OI is predominantly of the conductive type. The onset coincides closely with the time of life with the frequency of fractures decreasing in the late second decade of life [2, 5, 7, 11, 13]. The onset may also occur in the first decade. Cox and Simmons [5] observed a mild conductive type of hearing loss in a 9-year-old boy with normal tympanograms and Pedersen found five children with conductive hearing loss in the first decade [11]. Sensorineural hearing loss may also occur in children. Stewart and O'Reilly [19] reported one child in the age group 10–19 years with a flat sensorineural hearing loss of 30 dB HL in one ear and 60 dB HL in the other, with no explanation other than OI. Verstreken et al. [21] presented a 14-year-old girl with blue sclerae and slowly progressive hearing loss over a few years. A conductive hearing loss of 43 dB on the right side and a 30 dB mixed hearing loss on the left side were observed. Stapes surgery in the left ear had been performed 1 year previously. Also the right ear was operated on, leading to a conductive hearing loss of 22 dB 1 year post-operatively [21].

The Sillence classification of 1988 is based on a study in 361 Australian and North American families, where hearing loss occurred mostly in OI type I and less frequently in type IV. Hearing loss was strongly age-related and was rarely detected before 10 years of age in OI type I [16]. In a previous study of Sillence et al. [18] among 144 patients in Victoria, Australia, an overall frequency of 35% hearing loss in OI type I was observed and in 20% of those with severe hearing impairment it was evident before 20 years of age. The earliest onset of hearing loss requiring a hearing aid was in a 10-year-old patient. In OI type III, only one of the six patients over 14 years of age had hearing loss. No hearing loss was found in OI type IV [18].

A population study of 201 Danish patients including all types of OI was carried out by Pedersen in 1984 [11]. Conductive hearing loss was observed in 22% of the children in the age group 4–9 years and a mainly conductive hearing loss in 28% in the age group 10–19 years; however, the median age at the onset of hearing loss was 25 years. Thresholds above 15 dB at only one of the frequencies 250–4000 Hz was defined as hearing loss, which may result in sensitive ascertainment [11]. The study of Garretsen et al. [7] of 142 OI type I patients revealed the highest frequency of hearing loss among children so far. Of those children aged 4–9 years, 31% and 62% of those aged 10–19 years had hearing loss using a definition of hearing loss similar to that used in our study. Mixed hearing loss was most frequent and conductive hearing loss was only observed in a few young affected subjects. The patients, however, were designated as a "selected sample" because either they had been previously selected for ear surgery or had volunteered for the study because of their hearing loss [7].

In our study, only two children (4.4%) between 4 and 16 years of age had hearing loss which could be related to OI. Both patients were sporadic cases of OI type IV. In addition, one patient with familial OI type I had an early onset hearing loss probably of unrelated aetiology, an inference also supported by no previous reports of early infant sensorineural deafness in OI in the literature. In sporadic cases of OI, it may be impossible to distinguish type IV cases from types III and I [15]. Ten children in this study had OI type IV, seven of them being sporadic cases. Two of these children had earlier undiagnosed conductive hearing loss, both of them suffering from OI with severe fragility. The hearing loss in both cases showed progression over time and it was clearly related to OI. Stapes surgery was planned at the age of 20 years for the boy who first presented with disturbing tinnitus with a $PTA_{0.5-2\ kHz}$ 45/33 dB HL (Fig. 2). Our results indicate that clinically significant hearing loss in children under 16 years of age with OI is less frequent than suggested by previous studies (Table 3). High prevalence of hearing loss in previous studies may be explained by small or selected study

Table. 3 Studies on hearing loss in children with OI

Reference	*N*	Hearing loss all patients	Hearing loss	
			Age 4–9 years	Age 10–19 years
[2]	32[a]	11/32 (34%)	5/23 (20%)	
[13]	70[a]	29/70 (41%)	0/10 (0)	3/8 (38%)
[5]	30[a]	11/30 (37%)	1/8 (12.5%)	4/10 (40%)
[11]	173	97/173 (56%)	5/23 (22%)	10/36 (28%)
[19]	53	31/53 (58%)		2/13 (15%)
[6]	70[a]	30/70 (43%)	2/18 (11%)	13/21 (62%)
[7]	142[a]	111/142 (78%)	4/13 (31%)	24/38(63%)
This study	45		2/45 (4.4%)[b]	

[a] Selected patients
[b] Children 4–16 years of age

materials as well as different criteria for the hearing loss. In addition, the age range in our study was between 4 and 16 years.

The early detection and treatment of hearing loss is of outmost importance to avoid additional disability in education and psychosocial adaptation, which aggravates the physical handicap. This may be avoided by audiological rehabilitation including hearing devices, as well as careful treatment of eventual inflammatory ear diseases, such as stapedotomy in selected cases. Therefore, audiometry should be performed in a child with OI if a hearing deficit is suspected and as a baseline study at the age of 10 years with repeated audiograms every 3 years, even for an asymptomatic patient. Further genetic, clinical and epidemiological studies in OI are needed to detect risk factors, to understand the pathogenesis of hearing loss and also to develop preventive measures and treatment modalities.

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HEARING LOSS IN FINNISH ADULTS WITH OSTEOGENESIS IMPERFECTA: A NATIONWIDE SURVEY

KAIJA KUURILA, MD
VAASA, FINLAND
ILKKA KAITILA, MD, PHD
HELSINKI, FINLAND
REIJO JOHANSSON, MD, PHD
TURKU, FINLAND
REIDAR GRÉNMAN, MD, PHD
TURKU, FINLAND

II

Hearing loss, bone fragility, and blue sclerae are the principal clinical features in osteogenesis imperfecta (OI), a genetic disorder of connective tissue. In a nationwide search, an audiometric evaluation of 133 adult patients was performed. According to the criteria introduced by Sillence, type I was the most common form of OI. Of the patients with normal hearing on audiometry, 17.1% reported subjective hearing loss, and 19.1% of the patients with impaired hearing did not recognize it. On audiometry, 57.9% of the patients had hearing loss, which was progressive, often of mixed type, and mostly bilateral, and began in the second to fourth decades of life. The frequency or severity of the hearing loss was not correlated with any other clinical features of OI. Hearing loss is common, affecting patients with all types of OI. Subjective misjudgment of hearing ability supports the need for repeated audiometry in all OI patients. A baseline study at the age of 10 years followed by audiograms every third year thereafter is recommended.

KEY WORDS — hearing loss, osteogenesis imperfecta.

INTRODUCTION

Progressive hearing loss is a common additional social and health problem in osteogenesis imperfecta (OI), a genetic disease of the skeleton. The principal clinical feature in OI is remarkable bone fragility that makes the bone susceptible to mild traumas. The propensity for fractures varies from a few fractures during the lifetime to hundreds in childhood. Many patients present with secondary deformities in the tubular bones, spine, and skull and with short stature, which together result in a remarkable physical and secondary psychosocial handicap from childhood onward. Dental discoloration and fragility (dentinogenesis imperfecta; DI) are also common.[1] The prevalence of OI is 1 in 10,000 persons to 1 in 30,000 persons.[1-3] The most common mode of inheritance is autosomal dominant, with large families with several affected generations and multiple affected members. Sporadic cases due to new dominant mutations are common. In addition, there are rare autosomal recessive forms of OI.[4] Osteogenesis imperfecta is mainly caused by mutations in 1 of the 2 genes, COL1A1 and COL1A2, that encode the synthesis of type 1 collagen.[1]

Although the oldest reports of OI date from the 17th century, and the oldest known case that was later diagnosed as OI is in a mummy dating from about 1000 BC, the classic symptoms of OI with bone fragility, blue sclerae, and hearing loss were first described by Adair-Dighton in a 1912 case report.[5-10] The triad of OI, however, was first confirmed by van der Hoeve and de Kleyn in 1918, and was supported by several reports during the following years.[11-14]

Since the late 1970s, OI has been divided into 4 major types on the basis of clinical, radiographic, and genetic criteria (Table 1[4,15,16]). This classification, introduced by Sillence and later modified on several occasions, has replaced the earlier classification into congenita and tarda forms.[4,15-17] The wide variation in expressivity and heterogeneity still today cause difficulties in classifying both familial and sporadic cases.[18,19]

Six population studies on hearing loss in OI have been published.[3,9,19-22] In these studies, hearing loss was found in 22.6% to 58% of OI patients.[3,9,18,20-22] The hearing loss is progressive, often conductive at onset and later mixed or sensorineural, and it usually begins in the second or third decade of life.[3,23-27] Only 4 previous studies have focused on hearing loss in different types of OI: Sillence et al[18] and Paterson et al[22] included all types of OI, and Garretsen et al[27] and Garretsen and Cremers[28] included OI type I only. Hearing loss appears to be most common in OI type I, affecting 35% to 78% of patients, and it has been

From the Department of Otorhinolaryngology–Head and Neck Surgery, Vaasa Central Hospital, Vaasa (Kuurila), the Department of Clinical Genetics, Helsinki University Central Hospital, Helsinki (Kaitila), and the Department of Otorhinolaryngology–Head and Neck Surgery, Turku University Central Hospital, Turku (Johansson, Grénman), Finland. Supported by the Medical Research Fund of the Vaasa Hospital District and the Medical Research Fund of the Vaasa Medical Society.

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CORRESPONDENCE — Kaija Kuurila, MD, Dept of Otorhinolaryngology–Head and Neck Surgery, Vaasa Central Hospital, Hietalahdenk 4-8, 65100 Vaasa, Finland.

TABLE 1. CLASSIFICATION OF OI

*OI Type**	*Clinical Features*	*Inheritance*
IA	Mild to severe bone fragility, normal or slightly short stature, intensely blue sclerae, presenile hearing loss, normal teeth	AD
IB	Mild to severe bone fragility, normal or slightly short stature, intensely blue sclerae, presenile hearing loss, dentinogenesis imperfecta	AD
II	Extremely severe bone fragility	AD or AR
III	Variable, often severe bone fragility in infancy with progressive skeletal deformity, short stature, bluish sclerae, variable dentin abnormality	AD or AR
IVA	Bone fragility with mild to moderate deformity, often short stature, normal sclerae, normal teeth	AD
IVB	Bone fragility with mild to moderate deformity, often short stature, normal sclerae, dentinogenesis	AD
V	Hyperplastic callus OI type	AD
	Other types	
	Cole-Carpenter OI type	AD
	Bruck OI type	AR
	North American OI	AR
	Osteoporosis-pseudoglioma syndrome	AR

OI — osteogenesis imperfecta, AD — autosomal dominant, AR — autosomal recessive.
*Sillence classification, modified at Seventh International Conference on Osteogenesis Imperfecta, Montreal, 1999.[4,15,16]

thought to be less common in OI type IV.[4,19,22,27,28] In OI type III, Sillence[4] found that only 1 of 6 patients had hearing loss, whereas Paterson et al[22] reported that 52% of 207 patients had hearing loss. The definition of hearing loss, however, has varied greatly in the studies referred to, and the study subjects may have been selected.[3,22-28]

The aim of this study was to evaluate the frequency and type of hearing loss in adult Finnish patients with a classified type of OI. We have previously reported on the hearing loss in OI children.[29]

MATERIALS AND METHODS

Altogether, 254 OI patients were ascertained in a nationwide search through the patient register of the Department of Clinical Genetics, Helsinki University Central Hospital, a major referral center for genetic disorders of the skeleton and connective tissue in Finland, and through the membership register of the Finnish Osteogenesis Imperfecta Association, as well as the HILMO care register from Finnish university and local hospitals. The diagnosis was based on fracture history, clinical and radiographic findings, and family history. The number of patients ascertained corresponds to a prevalence of 4.9 per 100,000 persons.

The inclusion criteria for the present audiological study were OI, a previous audiometric study, and age over 16 years. The diagnosis was ascertained in all sporadic cases and in most familial cases by clinical and genetic evaluation at the genetics clinic of Helsinki University Central Hospital, and in the remaining cases through the hospital records. The patients were approached through a questionnaire concerning the history of fractures, the color of the sclerae, DI, and hearing loss. They were asked about the subjective and objective onset of the hearing loss, the clinical course of the hearing deficit, the use of hearing devices, and surgical treatment. Clinical audiometry, including pure tone audiometry, speech audiometry, tympanometry, and measurement of the stapedial reflex, was performed under standard conditions in a soundproof room at a local secondary or tertiary care university hospital. The patients with no audiometric evaluation were excluded from the study, as were patients with non–OI-related ear disease. The questionnaire was returned, indicating consent to participate, by a total of 204 patients (80.3%), of whom 144 were adults. This report focuses on audiometric evaluation of the 133 adult patients, 17 years of age or older, who fulfilled the inclusion criteria. The mean age of the 50 men and 83 women was 40.8 years (SD, 15.98 years; range, 17 to 81 years). Twenty-nine of these patients (21.8%) had undergone a middle ear operation because of hearing loss caused by OI; altogether, 22 right ears and 19 left ears were operated on. A clinical examination of the patients with impaired hearing was performed to exclude causes other than OI for the hearing loss. For 131 patients, the type of OI could be determined by a clinical geneticist using the criteria introduced by Sillence.[4] Subclassification of OI types I and IV into subtypes A and B (Table 1) on the basis of presence or absence of DI was not used in our audiological study, since the dentinal manifestations of OI apparently form a continuum from normal dentin to severe DI, and visual evaluation does not reveal the changes seen on radiography or light and transmission electron microscopy.[30]

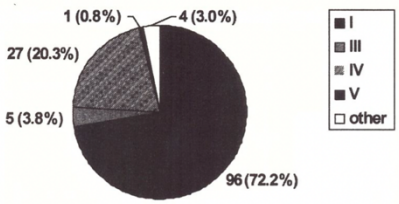

Fig 1. Classification of osteogenesis imperfecta (OI) in 133 Finnish adults.

Normal hearing was defined as a pure tone average at 0.5, 1, and 2 kHz that was equal to or better than 15 dB hearing level (HL) in patients under 60 years of age, and equal to or better than 20 dB HL in patients of 60 years or older. Definitions for different types of hearing loss paralleling the definitions given by Shapiro et al[31] and Pedersen[3] were used, as follows.

1. Conductive hearing loss: an average air-bone gap for the frequencies 0.5, 1, and 2 kHz of greater than 15 dB with a corresponding bone conduction threshold of better than 15 dB.

2. Sensorineural hearing loss: air conduction thresholds for the frequencies 0.5, 1, and 2 kHz of equal to or greater than 15 dB with a corresponding air-bone gap smaller than 15 dB.

3. Mixed hearing loss: an average air-bone gap for the frequencies 0.5, 1, and 2 kHz of greater than 15 dB with a corresponding bone conduction threshold equal to or greater than 15 dB.

RESULTS

Of the total of 133 adult patients in whom audiometry had been performed, 111 patients (83.4%) reported blue sclerae, and all except 1 had had fractures. Fifty-two patients (39.4%) reported DI, and 83 (62.4%) had the familial type of OI. Twenty-eight patients (21.1%) had continuous tinnitus, and 61 (45.9%) had intermittent tinnitus. Fifty-seven patients (43.8%) reported recurrent or continuous vertigo, which could be classified as inner ear–related by case history in 28 patients (21% rotational or swaying vertigo). Thirty-three patients (24.8%) used a hearing aid. All 133 cases except 2 could be classified according to Sillence[4] (Fig 1). Type I was the most common form of OI. The sex and age distribution of the patients is presented in Table 2.

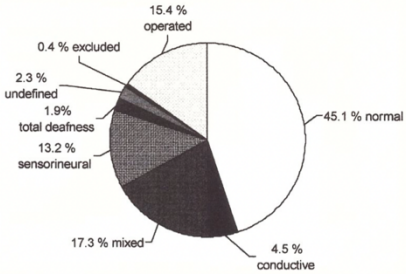

Fig 2. Hearing pattern in 266 OI ears.

The results of audiometric evaluation of 266 OI ears are presented in Fig 2. The subjective sensation of hearing ability was compared with the audiometric findings in the 133 patients studied. Of the patients with normal hearing on audiometry, 17.1% reported hearing loss, and on the other hand, 19.1% of the patients with hearing loss were not aware of any hearing loss. At audiometry, 63 patients (47.4%) had bilateral hearing loss, and 77 (57.9%) had hearing loss in at least one ear (mean age, 45.4 years; range, 17 to 81 years; SD, 16.4 years). In addition, 1 patient had accidental unilateral total deafness caused by a basal skull fracture. The mixed type of hearing loss was most common: 17.3% of the ears. The type of hearing loss could not be defined for 6 ears. In addition, 22 right and 19 left ears of, altogether, 29 patients had been operated on because of conductive or mixed hearing loss caused by OI, with bilateral stapedotomy in 12 of the cases. The hearing loss increased with age. The mean age at the onset of hearing loss was, according to patient history or audiometry, 29.0 years (range, 7 to 81 years; SD, 14.8 years; Fig 3). Both mixed and sensorineural hearing losses occurred in all age groups, while conductive hearing loss was predominantly seen in younger patients (Fig

TABLE 2. CLASSIFICATION OF PATIENTS BY SEX, AGE, AND OI TYPE

	I	*IA*	*IB*	*III*	*IVA*	*IVB*	*III/IV*	*V*	*Unclassifiable*	*Total*
Male	5	23	5	3	5	6	1	1	1	50
Female	5	50	8	2	5	11	1	0	1	83
Total	10	73	13	5	10	17	2	1	2	133
Mean age (y)	52.3	41.3	46.0	36.2	35.2	35.0	42.5	43.0	31.0	40.8
Range (y)	33-81	17-87	19-69	25-47	17-51	20-65	28-57		24-38	17-81
SD (y)	16.5	17.3	16.2	9.4	10.8	14.2				16.0

Types I and IV were divided into A and B by visual evaluation of teeth. Ten patients could not be reliably placed in A or B group of type I.

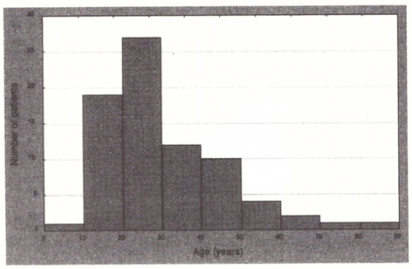

Fig 3. Age at onset of hearing loss in 77 patients. Youngest patient was 7 years old. Twenty-six percent of patients had symptomatic hearing loss before age of 20 years, 61% before age of 30 years, 76.6% before age of 40 years, and 89.6% before age of 50 years.

4). Hearing loss above 30 dB HL in both ears was found in 40 of the 133 patients (30.1%; Fig 5).

The frequency of hearing loss in classified types of OI is presented in Fig 6. The hearing pattern in different types of OI is presented in Fig 7, and the severity of hearing loss in classified types of OI, with operated patients excluded, is presented in Fig 8. No correlation was found between the frequency or severity of hearing loss and the type of OI. The types of hearing loss for the right ear were significantly different between OI types I and IV ($p = .028$), indicating that inner ear–related hearing loss is more common in OI type I. Although a significant difference was not found for the left ear, the tendency was similar ($p = .09$).

A strong correlation was found between the hearing levels of the right and left ears of individual patients, indicating that hearing loss eventually affects both ears (Pearson correlation coefficient, 0.71). The type of hearing loss was usually the same for both ears. The type of hearing loss, especially the mixed type, was similar within the families studied, but no statistical significance could be shown because of

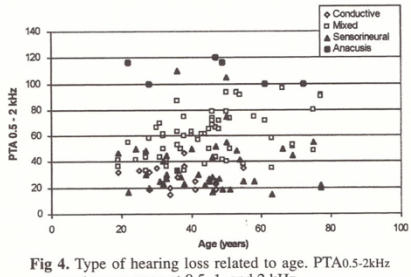

Fig 4. Type of hearing loss related to age. PTA0.5-2kHz — pure tone average at 0.5, 1, and 2 kHz.

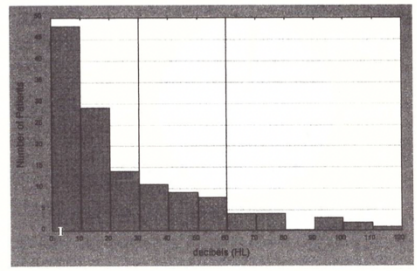

Fig 5. PTA0.5-2kHz of better ear in 133 OI patients. Hearing loss was socially disturbing in 30.1% of patients (40/133).

the small family size: only 1 family had as many as 6 members with OI, and most of the families included 3 or fewer OI patients. No correlation was found between occurrence of hearing loss and blue sclerae, number of fractures, DI, vertigo, or whether the OI was sporadic or familial; nor between the different types of hearing loss and DI, blue sclerae, vertigo, number of fractures, or whether the OI was sporadic or familial. Tinnitus was most frequent in patients with sensorineural or mixed hearing loss, and the frequency of tinnitus increased with age.

Three patients were found to have total deafness not related to surgery. A woman with mild OI type I developed total deafness at the age of 71 years after progressive, OI-related hearing loss. In addition, a 47-year-old man with blue sclerae and DI, but no fractures, had total deafness of the right ear. The objective onset of hearing loss remained unclear, but subjective hearing loss was reported at the age of 7 years. However, in this patient, audiometry was first performed at the age of 20 years. Another woman, 49 years of age, had rapidly progressive hearing loss that resulted in total deafness in early adulthood. She had nonfamilial OI type I. The sclerae were blue, the teeth were normal by visual evaluation, and she had had multiple fractures. Since the age of 15 years, she

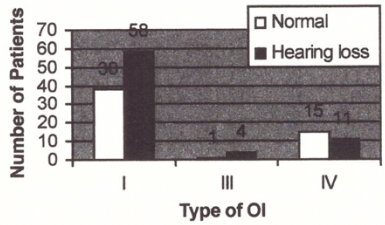

Fig 6. Frequency of hearing loss in classified types of OI (N = 131, including operated patients).

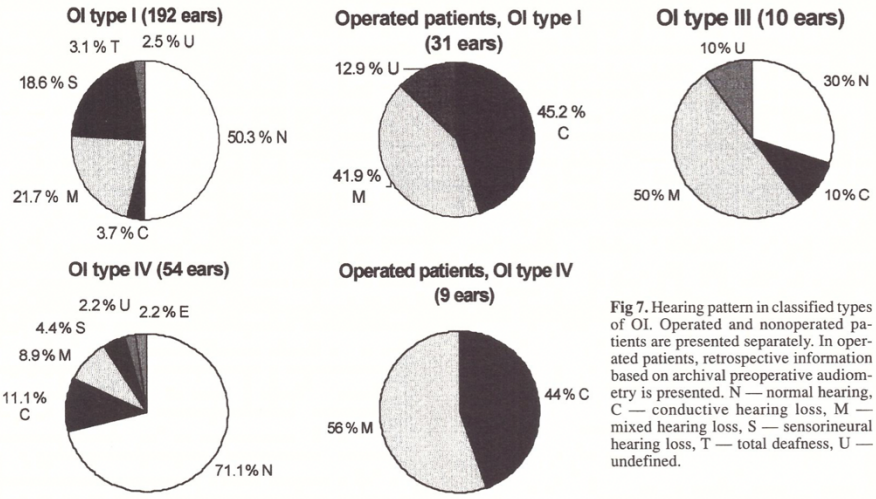

Fig 7. Hearing pattern in classified types of OI. Operated and nonoperated patients are presented separately. In operated patients, retrospective information based on archival preoperative audiometry is presented. N — normal hearing, C — conductive hearing loss, M — mixed hearing loss, S — sensorineural hearing loss, T — total deafness, U — undefined.

had had slowly progressive hearing loss that, at the time of the first audiometry at the age of 20 years, was of mixed type. The hearing loss then progressed rapidly, resulting in total deafness at the age of 22 years (Fig 9). In addition, 8 nonoperated patients were found to have sensorineural hearing loss before the age of 40 years. Seven of these patients had OI type I, and 1 patient with blue sclerae and DI was not classified. The mean age of these patients was 26 years at the time of the study (SD, 26.0 years; range, 17 to 37 years), and the mean age at the onset of the hearing loss was 22.1 years (SD, 7.0 years; range, 15 to 36 years). The mean pure tone average at 0.5, 1, and 2 kHz was 39.2 dB HL in the right ear and 32.9 dB HL in the left ear (SD, 13.4 dB HL in the right ear and 17.1 dB HL in the left ear; range, 20 to 55 dB HL in the right ear and 15 to 66.7 dB HL in the left ear).

DISCUSSION

This study is a population-based survey on a national level. We examined hearing loss in OI patients classified according to Sillence[4] and performed audiometric evaluation in all patients. The low prevalence of OI in Finland found in our study, 4.9 per 100,000 persons, may be explained by the variable severity of clinical expression, nonreporting, and lack of patient registers, as in former studies on OI.[1] It is a general impression that about half of OI patients have progressive hearing loss (Table 3[3,21,22,25-28]). In most studies, the hearing loss is found to be bilateral and progressive. Hearing loss starts in the second or third decade of life, and it is often conductive at the onset and later mixed or sensorineural. However, the definition of hearing loss has varied greatly, and the study subjects may have been selected in previous studies.[3,22-28] The first population study on OI, by Seedorff[9] in Denmark in 1949, showed a 28% incidence of hearing loss, and Smårs[20] in Sweden in 1961 found a 22.6% incidence of hearing loss. More recently, Pedersen[3] in Denmark found a 50% incidence of hearing loss among 173 patients with OI classified into congenita and tarda forms. The hearing loss was predominantly of mixed type and started in the second or third decade of life. The median age at onset was 25 years. In the 1989 study of Stewart and O'Reilly,[21] 46% of the Scottish OI patients had hearing loss, progressing from the conductive type in the

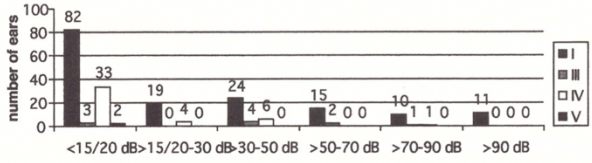

Fig 8. Severity of hearing loss in OI types I, III, IV, and V. In addition to 41 operated ears not presented in this Figure, significant hearing loss was observed in 104 of 224 ears studied.

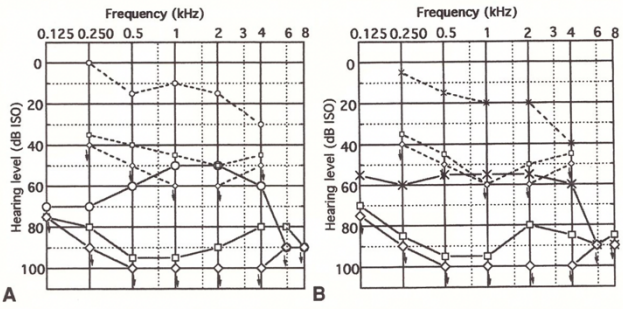

Fig 9. Conductive hearing loss resulting in total deafness at age of 22 years. Pure tone audiometry of **A)** left and **B)** right ears at age of 20 years (crosses), at age of 22 years (squares), and at age of 28 years (diamonds). Solid lines — air conduction threshold; dotted lines — bone conduction threshold. ISO — International Standards Organization.

second or third decade of life to a mixed type later in life. In a 2001 population study by Paterson et al,[22] subjective hearing loss was reported by 22.7% of the British OI patients, starting during the first 4 decades of life. Also in other, non-population studies, the hearing loss has been seen to start in early adulthood, proceeding from conductive hearing loss to a mixed and sensorineural type with increasing age.[24,25] Sensorineural hearing loss beginning as a mild high-frequency hearing loss and expanding to involve the lower frequencies with time has also been reported.[31] In some studies, a mixed hearing loss has been found to be the most frequent, with conductive hearing loss only in some younger patients, and sensorineural hearing loss and deafness in constant proportions independent of age.[27,28]

In our study, hearing loss was found to be progressive and bilateral, beginning predominantly in the second to fourth decades of life, with a mean age of 29.0 years at the onset of the hearing loss. Hearing loss, however, may begin in childhood, as indicated in earlier studies.[3,17,21,27-29] Of the 133 patients we studied, 57.9% (n = 77) had a hearing loss, which was bilateral in 47.4% of them (n = 63). This finding supports the general impression that 50% of OI patients have a hearing impairment.[3,9,18,20,21] Mixed hearing loss was the most frequent type of hearing loss, occurring in 17.3% of the 266 ears. In addition, the preoperative hearing loss was of mixed type in 19 of the 41 operated ears (46.3%). The small amount of conductive hearing loss in this series, only 4.5% of the ears, is explained by previous stapedotomy; 18 of these operations were performed for conductive hearing loss (43.9%). Conductive hearing loss was mostly observed in the younger patients, while sensorineural and mixed types of hearing loss were seen in all age groups, as reported in earlier studies.[3,23-27]

No correlation was found between the hearing loss and other OI features such as blue sclerae, frequency of fractures, and DI. Even though no correlation analysis could be done between hearing loss and familiality of OI because of the small size of the families, the earlier indication of deafness as a familial feature is supported by our study.[22]

In the few previous studies using the present classification of OI, hearing loss is suggested to be more

TABLE 3. HEARING LOSS IN ADULTS WITH OI

Authors	*Year*	*No. of Patients*	*Selected Patients*	*Definition of Hearing Loss**	*Audiometry*	*Hearing Loss*	
						No.	*%*
Riedner et al[25]	1980	70	13 Families	Yes	Yes + portable	26/52	50.0
Cox and Simmons[26]	1982	30	5 Families	Yes	Portable	6/12	50.0
Pedersen[3]	1984	173	Population	Yes	Yes + portable	73/114	64.0
Stewart and O'Reilly[21]	1989	56	Population	Yes	Yes + portable	29/40	72.5
Garretsen and Cremers[28]	1991	70	OI type I	Yes	Yes	15/31	48.4
Garretsen et al[27]	1997	142	OI type I, selected patients	Yes	Yes	83/91	91.2
Paterson et al[22]	2001	1,394	Population	No	No	297/928	32.0
This study		133	Population	Yes	Yes	77/133	57.9

In previous studies, adult patients have been defined as patients ≥20 years of age, with exception of study of Paterson et al,[22] in which patients were ≥10 years of age. In present study, adults are defined as patients ≥17 years of age. Percentage figures are influenced by age structure of population in question, which is not reported in these studies.

Portable — portable audiometry, in addition to clinic audiometry.

*Definition varies among different studies.

common in OI type I than in OI type IV. In the 1979 Sillence et al[18] study from Victoria, Australia, hearing loss was found in 35% of OI type I patients of all ages. No hearing loss was found in OI type IV; there were, however, only 8 patients with OI type IV in the study material. In the 1983 study of Paterson et al,[32] subjective hearing loss was reported by 50% of type I and by 29.4% of type IV patients over 30 years of age, but there was no significant difference (69 versus 48 patients). In a later study by Paterson et al,[22] about 30% of type IA, IB, and IVB patients reported hearing loss at the age of 30 years, while the proportion was significantly lower in OI type IVA (9%). Garretsen and Cremers[28] found that 43% of 70 patients with OI type I had hearing loss. In a later study by Garretsen et al,[27] hearing loss was found in 79% of type I OI patients. The high rate of hearing loss is explained by selection of the patients, who either had been scheduled for ear surgery or volunteered for the study because of hearing loss. Only 2 studies focusing on hearing loss in OI type III have been published, with different outcomes: 52% with hearing loss at the age of 30 years in a study by Paterson et al[22] on 206 type III OI patients, and only 1 of 6 type III OI patients with hearing loss in a study by Sillence et al.[18] In the former study, hearing loss was based on the subjective experience of the patients (personal communication), and in the latter, no definition for hearing loss was given.

In our study, no correlation could be found between the types of OI and the frequency or severity of the hearing loss: 60.4% of the type I OI patients and 42.3% of the type IV OI patients had hearing loss. The types of hearing loss, however, seemed to be slightly different between types I and IV: inner ear–related hearing loss occurred more often in OI type I. Of the 5 type III OI patients, 4 had hearing loss, supporting the finding by Paterson et al[22] that hearing loss is common in OI type III.

Although the hearing loss in OI is clinically otosclerosis-like, OI and otosclerosis are two separate diseases with clinical similarities.[3,33-37] Compared with otosclerosis, the hearing loss in OI has a tendency toward earlier onset, more severe middle ear involvement, and a higher incidence of sensorineural hearing loss.[21,23,32,35] In our study, sensorineural hearing loss was observed also in the youngest age groups, indicating in OI a group of patients with a predominantly sensorineural pattern at the onset of hearing loss, which is rare in otosclerosis. Early sensorineural hearing loss was found only in patients with OI type I, and the only patient with rapidly progressing hearing loss, resulting in total deafness within 8 years at the age of 22 years, also had OI type I. In earlier studies, total deafness is reported mostly in older age groups, but sensorineural hearing loss at an early age is also reported.[3,21,29] The cause of early sensorineural hearing loss in OI remains unresolved, and to date, it has not been possible to establish genotype-phenotype correlations.

Hearing loss is a common feature in OI, affecting patients with all types of OI. The subjective assessment of hearing ability may be misleading: almost 40% of the patients in this study misjudged their hearing ability. This finding supports the need for repeated audiometry in all patients with OI. Because early detection and treatment of hearing loss is of utmost importance to avoid aggravation of the physical handicap, audiometry should be performed in all OI patients as a baseline study at the age of 10 years, with repeated audiograms every third year thereafter, even for asymptomatic patients. Further studies based on molecular genetic classification of OI are needed to reveal the pathogenesis of hearing loss.

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Vestibular Dysfunction in Adult Patients With Osteogenesis Imperfecta

K. Kuurila,[1]* E. Kentala,[2] S. Karjalainen,[3] S. Pynnönen,[4] O. Kovero,[5] I. Kaitila,[6] R. Grénman,[7] and J. Waltimo[8]

[1]*Department of Otorhinolaryngology-Head and Neck Surgery, Vaasa Central Hospital, Vaasa, Finland*
[2]*Department of Otorhinolaryngology-Head and Neck Surgery, Helsinki University Hospital, Helsinki, Finland*
[3]*Department of Audiology, Turku University Central Hospital, Turku, Finland*
[4]*Department of Mathematics and Statistics, University of Vaasa, Vaasa, Finland*
[5]*Institute of Dentistry, University of Helsinki, Helsinki, Finland*
[6]*Department of Clinical Genetics, Helsinki University Hospital, Helsinki, Finland*
[7]*Department of Otorhinolaryngology-Head and Neck Surgery, Turku University Central Hospital, Turku, Finland*
[8]*Institute of Dentistry, University of Helsinki, Institute of Biotechnology, University of Helsinki, Helsinki, Finland*

III

Progressive hearing loss is a major symptom in osteogenesis imperfecta (OI), a genetic brittle bone disease. Vertigo is frequently associated with otosclerosis in which the hearing loss clinically resembles that in OI. Vertigo is also common in basilar impression (BI) found in up to 25% of adult OI patients. In order to evaluate the cause, frequency, and characteristics of vertigo in OI, 42 patients were studied by interview, clinical examination, and audiological examination supplemented with electronystagmography (ENG) and lateral skull radiography. Audiometry showed hearing loss in 25 patients (59.5%). Nine patients (21%) displayed abnormal skull base anatomy in the forms of basilar impression, basilar invagination, or both, all designated here as BI. Twenty-two patients (52.4%) reported vertigo, mostly of floating or rotational sensation of short duration. Patients with hearing loss tended to have more vertigo than patients with normal hearing. Vertigo was not correlated with type of hearing loss or auditory brain-stem response (ABR) pathology. ENG was abnormal in 14 patients (33.3%). No dependency was found between vertigo and deviant ENG results. Patients with BI tended to have more vertigo than patients with normal skull base but the difference was not statistically significant. Neither ENG pathology, nor the presence or type of hearing loss showed correlation with BI. In conclusion, vertigo is common in patients with OI. In most cases, it may be secondary to inner ear pathology, and in only some patients does BI explain it. Since some OI patients without BI or hearing loss also suffer from vertigo, further clinical and neurological studies are needed to define the pathogenesis of vertigo in OI.

KEY WORDS: osteogenesis imperfecta; hearing loss; vertigo; basilar impression

INTRODUCTION

Osteogenesis imperfecta (OI), a genetic disorder of the skeleton, is characterized by variable propensity to fractures caused by mild traumas. The secondary deformities in extremities, spine, and skull, and short stature often result in remarkable physical and psychosocial handicap [Byers, 1993]. Progressive hearing loss affects about 50% of patients with OI [Seedorff, 1949; Smårs, 1961; Sillence et al., 1979b; Pedersen, 1984; Stewart and O'Reilly, 1989; Paterson et al., 2001; Kuurila et al., 2002]. In our recent studies on the prevalence and type of hearing loss in Finnish adult patients, we found that 57 patients (43.8%) reported recurrent or continuous vertigo. This could be classified as inner-ear-related by case history in 28 patients [Kuurila et al., 2002].

Patients suffering from otosclerosis frequently present with vestibular dysfunction with frequencies from

Abbreviations: OI, osteogenesis imperfecta; BI, basilar impression/basilar invagination; DI, dentinogenesis imperfecta; ENG, electronystagmography; OFI, ocular fixation index; ABR, auditory brain-stem response; PTA 0.5–2 kHz, pure tone average at frequencies of 0.5, 1, and 2 kHz; HL, hearing level; OAE, otoacoustic emissions; CT, computer tomography; MRI, magnetic resonance image.

*Correspondence to: Dr. Kaija Kuurila, M.D., Vaasa Central Hospital, Hietalahdenk 2-4, 65100 Vaasa, Finland.
E-mail: kaija.kuurila@vshp.fi

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27.7 to 57.7%, but the cause of sensorineural hearing loss as well as vertigo in otosclerosis is still unknown [Morales-Garcia, 1972; Virolainen, 1972; Cody and Baker, 1978; Ghorayeb and Linthicum, 1978; Thomas and Cody, 1981; Igarashi et al., 1982]. Particularly patients with sensorineural hearing loss due to labyrinthine or cochlear otosclerosis are thought to suffer from vertigo [Cody and Baker, 1978; Thomas and Cody, 1981]. Biotoxic effects on the peripheral vestibular nerve, otosclerotic vascular changes due to the disease, and biochemical changes in the inner ear fluids have been suggested as causal factors of vertigo in otosclerosis [Virolainen, 1972; Igarashi et al., 1982]. Vestibular function generally consists of unilateral depression of labyrinthine activity, manifested as directional preponderance in electronystagmography (ENG) [Virolainen, 1972; Thomas and Cody, 1981]. Abnormal caloric tests, however, have been reported in higher proportions than occurrence of the symptom of vertigo [Cody and Baker, 1978; Thomas and Cody, 1981]. Despite the clinical similarities, hearing loss in OI and otosclerosis are two distinct entities. Compared with otosclerosis, hearing loss in OI has a tendency to earlier onset, more severe middle ear involvement, and a higher incidence of sensorineural hearing loss [Holdsworth et al., 1973; Shapiro et al., 1982; Pedersen, 1984; Stewart and O'Reilly, 1989; Garretsen et al., 1997; Berger et al., 2001; Kuurila et al., 2002].

"Basilar impression" refers to a pathological anatomic condition where the uppermost cervical vertebrae intrude into the foramen magnum, possibly producing marked pressure effects on the medulla and adjacent parts of the central nervous system [Chamberlain, 1939; McGregor, 1948]. "Basilar invagination" is a radiological designation for a status where the head is positioned abnormally inferiorly in relation to the uppermost cervical vertebrae, and most patients with basilar impression also fill the criteria of basilar invagination [Hayes et al., 1999]. In BI (basilar impression/basilar invagination) symptoms of cranial nerve involvement, constriction of the cord in the region of the first cervical segment and degenerative changes in lower cervical segments may appear [McGregor, 1948]. Pure cerebellar disturbances are rare, whereas headache, lower cranial nerve dysfunction, pyramidal tract signs, and vestibulocerebellar symptoms are common [Elies and Plester, 1980; Sawin and Menezes, 1997; Hayes et al., 1999]. Vertigo precipitated by head movements has been reported to be pathognomonic for BI [Elies and Plester, 1980; Hayes et al., 1999]. However, the patients may also be asymptomatic [Sawin and Menezes, 1997]. In most cases, BI is caused by congenital developmental defects in the cervico-occipital region [McGregor, 1948]. In OI, it is thought to be secondary to softness of the skull [McGregor, 1948; Pozo et al., 1984]. BI has been reported to often affect patients with mild to moderate type of the disease [Sillence, 1994]. The reported prevalence of BI in population studies of OI has been 10.5–25% [Charnas and Marini, 1993; Sillence, 1994].

The minimum prevalence of OI is 1/10,000–1/30,000 [Pedersen, 1984; Byers, 1993]. The condition is inherited mostly as an autosomal dominant trait. In addition, there are autosomal recessive forms of OI [Sillence, 1988]. OI is caused by mutations in one of the two genes, *COL1A1* and *COL1A2*, which encode type 1 collagen [Byers, 1993]. The classification of OI, introduced by David Sillence in the late 1970s, has since been modified on several occasions, and it has replaced the earlier classification into congenita and tarda forms. Currently, OI is divided into five major types on the basis of clinical, radiographic and genetic criteria (Table I) [Sillence et al., 1979a; Sillence, 1988; Glorieux et al., 2000; Hall, 2002].

The aims of the present study were to examine vestibular dysfunction in adult patients with OI, to evaluate whether vertigo results from peripheral or central lesions, and to analyze the association of vertigo with hearing loss and BI.

MATERIALS AND METHODS

Patient Population

In a nationwide search through the patient register of the Department of Clinical Genetics, Helsinki

TABLE I. Sillence Classification of Osteogenesis Imperfecta (OI), Modified at the 7th International Conference on Osteogenesis Imperfecta, Montréal, 1999 [Sillence, 1988; Glorieux et al., 2000; Hall, 2002]

OI type	Clinical features	Inheritance
IA	Mild to severe bone fragility; normal or slightly short stature, intensely blue sclerae, presenile hearing loss, normal teeth	AD
IB	Mild to severe bone fragility; normal or slightly short stature, intensely blue sclerae, presenile hearing loss, DI	AD
II	Extremely severe bone fragility	AD/AR
III	Variable, often severe bone fragility in infancy with progressive skeletal deformity; short stature, bluish sclerae, variable dentin abnormality	AD/AR
IVA	Bone fragility with mild to moderate deformity, often short stature, normal sclerae, normal teeth	AD
IVB	Bone fragility with mild to moderate deformity, often short stature normal sclerae, DI	AD
V	Hyperplastic callus—OI type	AD
	Other types	
	Cole-carpenter—OI type	AD
	Bruck—OI type	AR
	North American OI (OI-TB)	AR
	Osteoporosis-pseudoglioma syndrome (OPG)	AR

AD, autosomal dominant; AR, autosomal recessive; DI, dentinogenesis imperfecta.

University Central Hospital, through the membership register of the Finnish OI association, and the HILMO care register from Finnish University and central hospitals, altogether 254 Finnish patients with OI were obtained. This number of patients corresponds to a prevalence of 4.9/100,000. Of these patients, all 61 adults (age over 16 years) living in the Helsinki or Turku area were approached through a questionnaire concerning the subjective and objective onset and the clinical course of the hearing loss as well as vestibular problems, and history of fractures. The OI patients in the Turku and Helsinki area mostly represented OI type I. Since a former study implies that BI is most common in OI type IV [Sillence, 1994], the 12 adults with OI type IV living in other parts of Finland were included as well. The study was approved by the Joint Ethical Committee of Helsinki University Central Hospital. Permission to acquire the patient data was granted by the Ministry of Health and Social Affairs.

Of the 61 patients contacted, 42 (68.8%) consented to participate in the study. All these patients underwent a clinical evaluation in Helsinki or Turku University Hospital. Cause other than OI for the eventual hearing loss was excluded.

Audiometric Evaluation

Pure tone audiometry was performed under standard conditions in a soundproof room. Hearing measurements also included tympanometry and measurement of stapedial reflex (1 kHz ipsi and contralateral stimuli), auditory brain-stem response (ABR) and otoacoustic emissions (OAE). OB822 and OB922 Clinical Audiometer were used for audiometry and Ilo Otodynamics Ltd. (Herts, UK) for OAE. ABR measurements were performed with Nicolet Viking IVD.

Normal hearing was defined as pure tone average (PTA) 0.5–2 kHz equal to or better than 15 dB HL in patients under 60 years of age, and equal to or better than 20 dB HL in patients 60 years old or older. The following definition for different types of hearing loss paralleling definitions given by Shapiro et al. [1982] and Pedersen [1984] was used.

1. Conductive hearing loss: average air-bone-gap for the frequencies 0.5, 1, and 2 kHz greater than 15 dB, with corresponding bone conduction threshold better than 15 dB.
2. Sensorineural hearing loss: air conduction thresholds for the frequencies 0.5, 1, and 2 kHz greater than 15 dB, with corresponding air-bone-gap smaller than 15 dB.
3. Mixed hearing loss: average air-bone-gap for the frequencies 0.5, 1, and 2 kHz greater than 15 dB, with corresponding bone conduction threshold greater than 15 dB.

In ABR, the latency of the fifth wave was considered to be abnormal when smaller than 5.6 msec or greater than 6.2 msec. Side difference of the latency of the fifth wave was considered as abnormal if more than 0.2 msec, under the condition that the difference between the PTA 0.5–2 kHz between the ears was equal to or less than 10 dB HL.

Electronystagmographic Evaluation

Eye movements and caloric reactions were recorded with ENG. Canal paresis and directional preponderance were calculated using the maximum slow phase velocity, according to the formula of Jongkees and Philipszoon [1964]. The upper limit of normal for canal paresis and directional preponderance was considered to be 20%. Spontaneous nystagmus was considered abnormal if equal to or more than 7°/sec [Jongkees and Philipszoon, 1964]. In addition to these abnormalities in ENG pointing to a peripheral lesion, the central type of abnormalities in ENG were also recorded. Abnormal findings in saccadic eye movements and in pursuit and optokinetic tests might indicate central lesions [Kayan, 1987]. The ocular fixation index (OFI) was calculated from the slow phase velocity in caloric testing before and after fixation as a percentage, using the formula of Demanez and Ledoux [1970]. An OFI of 50% or greater was regarded as pathological. Hortmann's ENG equipment was used in ENG measurements.

Cephalometric Measurements

The cephalometric measurements from standardized lateral radiographs for the analysis of the skull base morphology are depicted in Figure 1. A line was drawn on tracing paper from the most posterior-caudal point of the clivus (anterior margin of the foramen magnum) to the opisthion point (posterior margin of the foramen magnum). When the contour of the dens projected above this line, the patient was recorded as radiologically fulfilling the criteria of basilar impression. For the evaluation of basilar invagination, we used our own modifications of previously documented criteria [Chamberlain, 1939; Hayes et al., 1999]. Here we recorded as abnormal only the situations where the dens projected 10 mm or more above a line drawn from the most posterior point of the hard palate to the opisthion point. The selection of this criterion was based on our radiological survey of a large OI patient material which revealed an abnormal position and angulation of the hard palate and large structural variations in the posterior skull base in OI, factors that strongly affect the level of the reference plane (Waltimo et al., manuscript in preparation).

Statistical Analysis

Statistical analyses of bivariate dependencies were carried out using cross-tabulations (contingency tables) with related chi-square statistics and class wise relative frequencies. The chi-squared statistic indicates the existence of a possible statistical dependency between the categorical variables while percentage distributions in classes of one of the variables enable us to identify the fashion in which the variables are dependent on each other. Most of the tables are 2 by 2. In all these cases, we utilized the so-called Fisher's exact test for testing the independencies. The advantage of this procedure is that it does not rely on the large sample theory like the usual

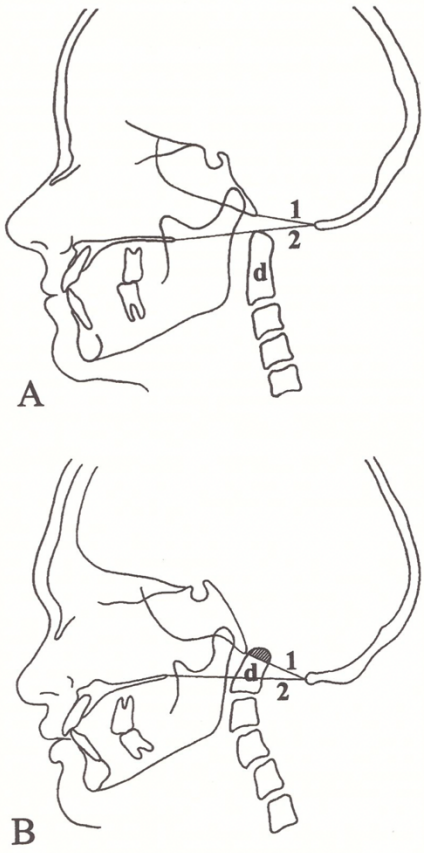

Fig. 1. Schematic drawings from standardized lateral skull radiographs of two 39-year-old female patients, both with type IVA OI. **Patient A**: Normal skull base anatomy, with dens (d) situated below the level of the foramen magnum (line 1) and not exceeding the reference line 2 by 10 mm or more. **Patient B**: Simultaneous presence of basilar impression (BI) (tip of dens 5 mm above line 1; dashed area) and basilar invagination (tip of dens 16 mm above line 2).

chi-square analysis, and hence should be more powerful in detecting possible dependencies.

RESULTS

General Characteristics

The mean age of the present patient sample of 12 men and 30 women was 39.1 years (SD 11.4, range 19–69 years). For all patients except one, the Sillence type of OI could be determined by a clinical geneticist (IK). Type I and type IV OI were divided into subtypes A and B, based on the presence or absence of dentinogenesis imperfecta (DI) by visual evaluation (Table II). Of the patients, 13 had undergone middle-ear operation (stapedotomy) due to hearing loss caused by OI (31%). Thirteen patients had a hearing aid, of whom seven had previously been operated on. DI was present in 17 patients, and 31 patients had blue sclerae. The reported number of fractures varied from 2 to 300. Nine patients were bound to a wheel-chair, while 25 patients walked without aid.

Audiological Symptoms

When asked about the first event of a vestibulocochlear symptom, 16 patients reported hearing loss (38.1%), and six patients tinnitus (14.3%). Before audiological measurements out of 42 patients subjective hearing loss was reported by 26 (61.9%). Continuous or occasional tinnitus was reported by 29 patients.

Audiological Findings—Pure Tone Audiometry

Altogether 25 patients had hearing loss at audiometry (59.5%). It was bilateral in 21 of these patients. The type of hearing loss is presented in Figure 2. All the ear-operated 13 patients had postoperative hearing loss.

Audiological Findings—ABR

ABR was pathological in 13 of the 25 patients with hearing loss (52%), with a clear statistical association ($P = 0.0072$). Hearing loss with a sensorineural component was present in all but two patients who had conductive hearing loss. Of the patients with deviant ABR, 65% had vertigo, whereas this was reported by 50% of the patients with normal ABR. This difference was not statistically significant. ABR pathology was not associated with presence of BI or with deviant ENG results. ABR was also abnormal in two of the 17 patients with normal hearing (11.8%). The wheel-chair-bound patients had OI type III. A severely deformed 20-year-old male with vertigo and BI, blue sclerae, normal teeth, and 50 previous fractures reported vertigo with moderate floating sensation of short duration, less than 1 min. The second patient, a 120-cm long 49-year-old male with over 200 previous fractures, reported mild rotational vertigo precipitated by head movements. The vertigo occurred in both cases less often than once a month.

Audiological Findings—OAE

OAE was pathological in 27 patients. It was deviant in seven patients (10 ears) without hearing loss with PTA 0.5–2 kHz between 5 and 13.33 dB HL. Two of these patients with pathological OAE bilaterally had unilateral hearing loss. Three patients with normal hearing had pathological OAE bilaterally, while two normal hearing patients had unilaterally deviant OAE. On the other hand, OAE was normal in six ears with sensorineural type of hearing loss and bone conduction PTA 0.5–2 kHz between 13.3 and 25 dB HL.

TABLE II. Sex and Age Distribution of the Classified Patients

	IA	IB	III	III/IV	IVA	IVB	Unclassified	All patients
Number of patients	18	3	4	1	7	8	1	42
Mean age (years)	44.3	38.3	34.5	32	32.6	36.0	43	39.1
SD	11.6	13.9	11.8		8.2	10.6		11.4
Range	21–69	23–50	20–49		19–39	26–55		19–69
Male	4	0	3	1	3	1	1	12
Female	14	3	1	0	4	7	0	30

One patient could not reliably be placed in either type III or IV OI.

Vestibular Findings

When asked about the first event of a vestibulocochlear symptom, four patients reported vertigo (9.5%) and three patients both tinnitus and vertigo (7.1%). Out of 42 subjects, 22 had vertigo (52.4%). In 19 of them the duration of the vertigo attacks was less than 1 min, in one patient less than 4 hr, and two patients could not specify the duration of the vertigo. The intensity of the vertigo was slight in 13 patients (59.1%) and moderate in nine patients (40.9%). The vertigo was described as floating by nine patients (40.9%), rotational by six patients (27.2%), and both rotational and floating by five patients (22.7% of all the patients with vertigo). Three patients with floating type vertigo also presented with nausea during the attacks. Head movements or altered position caused vertigo in 16 of the 22 patients with vertigo (72.7%). Nine patients reported unsteadiness (21.4%).

Patients with hearing loss had more vertigo (62.5%) than the patients with normal hearing (43.7%), but the difference was not statistically significant ($P = 0.1$). The type or severity of hearing loss was not associated with vertigo. The proportion of the patients with vertigo and BI or hearing loss is presented in Figure 3.

The operated patients did not differ significantly from the non-operated patients regarding frequency, duration, severity or type of vertigo.

Electronystagmographic Findings

ENG was pathological in 14 patients (33.3%) (Table IV). The pathology was, however, generally mild. No correlation between deviant ENG-results and vertigo was found. Patients with more severe hearing loss also had more deviant findings at ENG ($P = 0.049$). ENG pathology was also significantly more common in patients with sensorineural or mixed type of hearing loss as compared with patients with normal hearing or conductive hearing loss ($P = 0.395$). Patients with previous stapedotomy had significantly more pathology at ENG, both in directional preponderance ($P = 0.0361$) and in caloric asymmetry ($P = 0.0196$), indicating peripheral vestibular lesion. No correlation between BI and pathology at ENG was found, although central-type ENG abnormality seemed to be more common in patients with BI ($P = 0.118$).

Radiological Findings

Nine patients presented with radiologically defined BI (Table III). The mean age of these patients was 38.8 years (SD 15.1, range 20–69 years). No statistical correlation between the type of hearing loss and BI was found; neither in speech frequencies nor in the high frequencies. Six of the nine patients with BI had vertigo (66.7%), while 16 patients with normal skull base (51.6%) suffered from vertigo. This difference was not statistically significant ($P = 0.22$). The frequency of vertigo related to altered position or head movements was not significantly more common in patients with BI as compared with other patients, and the characteristics of vertigo were similar in both patient groups. However, patients with BI had significantly more subjective symptoms deriving from the central nervous system than patients with normal skull base ($P = 0.036$). These symptoms consisted of attacks of syncope, blurred vision, or diplopia, dysartria, sensory defects in the facial skin or abnormalities in cranial nerve function. No correlation between the presence or absence of BI and the type of OI was found.

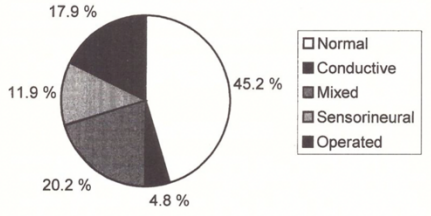

Fig. 2. Hearing pattern in the 42 patients (84 ears). Of the 15 operated ears, the type of hearing loss was sensorineural in nine, mixed in four, and conductive in two.

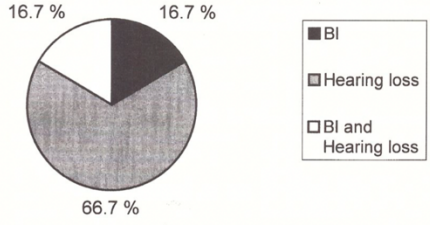

Fig. 3. Hearing loss and basilar impression/invagination (BI) in the 22 patients with vertigo.

TABLE III. Clinical Data of the Nine Patients With Radiologically Defined Basilar Impression, Basilar Invagination, or Both

Pt no.	Age (years)	Sex (male/female)	Class of OI	Length (cm), weight (kg)	Vertigo	Tinnitus	Headache	Fractures number	Hearing loss	Middle ear surgery	Dens above level 1 (mm)	Dens above level 2 (mm)
2	20	M	III	104, 19	Floating	Recurrent	Occipital	50	No	No	0	+17.0
3	39	F	IVA	144, 50	Rotational	Continuous	Occipital	50	No	No	+5.0	+16.0
4	35	F	IA	166, 50	No	No	Occipital	2	No	No	−3.5	+10.5
7	34	F	III	92, 25	No	Recurrent	Occipital	34	Conductive	Right	+10.0	+14.5
9	69	F	IA	150, 47	Floating/rotational	Continuous	Frontal	10	Mixed	No	+1.0	+14.0
23	26	F	IVB	142, 58	Staggering	Recurrent	Occipital	20	No	No	+1.0	+6.0
30	49	F	IVB	112, 35	Rotational	Recurrent	No	20	Mixed	Right	+1.0	+8.5
36	50	F	IA	140, 50	Floating	Recurrent	Temporal/occipital	30	Mixed	No	−6.0	+10.0
40	27	F	IVB	85, 36	No	No	Temporal	300	No	No	+0.5	+3.5

In BI, dens is projected above a line drawn from the anterior to the posterior margin of the foramen magnum (level 1). In basilar invagination, dens projecting 10 mm or more above a line drawn from the most posterior point of the hard palate to the posterior margin of the foramen magnum is recorded as abnormal (level 2).

Neurological Findings

Of all the patients, 25 reported lightheadedness (59.5%). In the neurological examination, all patients who were able to stand upright had normal Romberg's test, diadochokinesis, and finger–nose test. All but one patients presented with normal cranial nerve function; one patient had missing pharyngeal reflex. Thirteen patients had sensory defects of the skin, and five of these patients also presented with BI. None of the 42 patients had head-shaking nystagmus. The Dix-Hallpike's test was normal in all patients but two: it was positive bilaterally in a 51-year-old woman with OI type IA, and unilaterally in a 42-year-old woman. The first patient had moderate, mixed hearing loss (PTA 0.5–2 kHz 78/77 dB HL), temporal headache but no vertigo or BI. ENG was normal. The second patient with slight mental retardation due to congenital asphyxia also had moderate mixed hearing loss (PTA 0.5–2 kHz 73/85 dB HL). She had rapid attacks of slightly rotational vertigo and slipsfalls, temporo-occipital headache but no BI, while her ENG was slightly abnormal with directional preponderance value of 29.

Two patients had spontaneous nystagmus with Frenzel's glasses, both of them presenting with normal skull base, vertigo, hearing loss, and tinnitus. A 32-year-old woman with OI type III/IV and peculiar, medial-directed slow nystagmus of the left eye in all positions had previously been operated on with unsuccessful stapedotomy leading to deafness of the left ear (Table IV, patient 41). She had floating vertigo and nausea provoked by head movements. No spontaneous nystagmus was seen at ENG, the directional preponderance was 100%, and the caloric asymmetry 40%. The other patient was a 42-year-old woman with OI type IB and congenital asphyxia mentioned earlier, presenting with a right directional spontaneous nystagmus, which became left directional when the head was rotated to the left. Pressure changes subjectively worsened the rotating type vertigo. At ENG, no spontaneous nystagmus was seen, the directional preponderance was 29% and the caloric asymmetry 3%. OFI was abnormal, as was the calibration and pendel test, indicating a central lesion (Table IV, patient 39).

Subjective symptoms deriving from the central nervous system were reported by 11 patients: two patients had attacks of syncope, one of them also presenting with BI. The second patient was also examined with MRI, and BI was excluded. Seven patients presented with visual blurring or diplopia, three of them also suffering from BI. Three patients reported attacks of dysartria, and three patients suffered from sensory defects in the skin of the face; with one patient in each group also presenting with BI.

DISCUSSION

In this article, we have described the subjective symptoms and clinical signs of vestibular dysfunction in a group of 42 adult patients suffering from OI types I, III, and IV. No clinical studies on vertigo in OI patients have previously been performed. Progressive hearing loss, however, is one of the major symptoms in OI, and

TABLE IV. Abnormalities at Electronystagmography (ENG) in 14 Patients

Pt no.	Sex (male/female)	Class of OI	Age (years)	Hearing loss (right/left)	BI	Vertigo	Ear surgery (right/left)	Ang veloc (°/sec)	Dir prep (%)	Cal asymm (%)	OFI (%)	Abn eye mov
7	F	III	34	C/C	Yes	No	Right	0	−20.2	8.9	Normal	No
9	F	IA	69	M/no	Yes	Yes	—	0	16.8	−3.6	Normal	Yes[a]
14	F	IA	41	No/S	No	Yes	—	0	−21.5	−18.7	Normal	No
20	F	IVB	30	M/S	No	No	Left	0	−26.5	−57	Normal	No
21	F	IVB	33	M/M	No	No	—	0	−9.8	33	Normal	No
24	M	IVA	19	No/no	No	No	—	7.3	43.5	27.9	Normal	No
31	F	IA	53	S/M	No	No	Left	0	23.1	38.5	Normal	No
34	F	IA	51	M/S	No	No	Left	0	−12.5	−20.1	Normal	No
35	F	IA	25	S/S	No	Yes	Left	0	25	−50	Normal	No
37	M	IA	46	M/M	No	Yes	Right, left	0	−2.7	29.7	Normal	No
39	F	IB	52	M/M	No	Yes	—	0	29	3.2	Abnormal	Yes[b]
40	F	IVB	27	No/no	Yes	No	—	0	−18.4	−6.1	Normal	Yes[b]
41	F	III/IV	32	M/S	No	Yes	Left	0	100	40	Normal	No
42	M	IA	47	No/no	No	No	—	0	28.2	−7.7	Normal	No

Directional preponderance greater than 20%, angular velocity of the spontaneous nystagmus equal to or more than 7°/sec and a difference in response of the two labyrinths of 20% or more in the bithermal caloric testing points on the peripheral lesion, while abnormal findings in saccadic eye movements, in pursuit and optokinetic tests, and OFI less than 50% indicate central lesions. Type of hearing loss: conductive (C), mixed (M), or sensorineural (S). Ang veloc, angular velocity of the spontaneous nystagmus (°/sec); Dir prep, directional preponderance (%); Cal asymm, caloric asymmetry (%); Abn eye mov, abnormal eye movements.

[a]Abnormal saccades (dysmetric, slow, or irregular saccades).

[b]Abnormal pendel test and optokinetic nystagmus (gain abnormalities, saccadic, or ataxic eye movements).

vertigo is frequently associated with otosclerosis in which the hearing loss clinically resembles that in OI. Vertigo is also common in BI, which has been found in up to 25% of adult OI patients.

In this study, 52.4% of the 42 adult OI patients reported vertigo. It mostly presented as short episodes of floating or rotational sensation, often related to rapid head movements or altered position. Vestibular disturbances are common in otosclerosis [Morales-Garcia, 1972; Virolainen, 1972; Cody and Baker, 1978]. They may present as spontaneous attacks of rotational vertigo or imbalance, positional vertigo, or a sense of almost continuous imbalance [Cody and Baker, 1978; Thomas and Cody, 1981]. In BI of unspecified cause, vertigo has been reported in 68.7% of cases [Elies and Plester, 1980]. It has been described as oscillatory and vertical, rarely as rotational sensation, and also as a sensation of lightheadedness and unsteadiness, worse at dusk [Elies and Plester, 1980]. Vertigo especially precipitated by head movements has previously been reported as one of the principal neurological symptoms of BI [Tucker, 1979; Elies and Plester, 1980; Sawin and Menezes, 1997]. Vertigo related to head movements has also been reported in otosclerosis [Thomas and Cody, 1981; Sawin and Menezes, 1997]. In our study, head movements or altered position were found to be a common trigger of vertigo, provoking balance problems in 72.7% of the altogether 22 patients with vertigo.

In population studies, hearing loss has been reported in 22.7–57.9% of adult OI patients [Pedersen, 1984; Stewart and O'Reilly, 1989; Paterson et al., 2001; Kuurila et al., 2002]. In this study among 42 adult Finnish OI patients, progressive hearing loss was found in 25 (58.5%). In otosclerosis, the prevalence of vertigo has been reported to increase with increasing sensorineural component in the hearing pattern [Cody and Baker, 1978; Thomas and Cody, 1981]. No correlation between sensorineural hearing loss and vertigo was found in this study, although vertigo seemed to be associated with hearing loss. This correlation, however, was not statistically significant. Nausea has been reported to accompany the balance problems both in otosclerosis and BI, but only three patients out of the 22 in our study reported nausea during the attacks of vertigo [Tucker, 1979; Kayan, 1987; Sawin and Menezes, 1997].

Pathological ABR was found in 52% of the patients with hearing loss, but also in two patients with normal hearing. Long latency of the fifth wave in these two patients (6.9 and 6.6 msec) may be explained by the large size of the head (61.7 and 60.9 cm), which has been thought to influence the latency of the fifth wave in ABR [Yamaguchi et al., 1991; Nikiforidis et al., 1993]. Head size in patients with OI has been reported to be increased [Lund et al., 1999].

Abnormal OAE was found in 10 ears without hearing loss, whereas it was normal in five ears with mild sensorineural-type hearing loss (PTA 0.5–2 kHz 16.7–25 dB HL) and in one ear with conductive type hearing loss (PTA 0.5–2 kHz 30 dB HL). While normal OAE is known to be found in patients with degeneration in the acoustic nerve and preserved hair cells in the inner ear, pathological OAE in patients with normal hearing at

audiometry may be caused by partly loss of hair cells in the inner ear and, therefore, predict future hearing loss [Brown and Dort, 2001; Ohwatari et al., 2001].

ENG was abnormal in 14 patients (33.3%). No correlation between vertigo and deviant ENG results was found. In line with earlier reports concerning otosclerotic ears with cochlear involvement as compared with ears with pure conductive deafness, we also found significantly more ENG pathology in the OI patients with sensorineural or mixed-type hearing loss than in those with conductive hearing loss or normal hearing ($P = 0.0768$) [Morales-Garcia, 1972; Cody and Baker, 1978]. This implies extensive damage to the inner ear. In otosclerosis, ENG pathology has been reported in up to 59% of patients, generally consisting of positional nystagmus or unilateral depression of labyrinthine activity manifested as directional preponderance at ENG [Morales-Garcia, 1972; Virolainen, 1972]. Abnormality in caloric tests, however, has been reported in higher proportions than the occurrence of the symptom of vertigo [Cody and Baker, 1978; Thomas and Cody, 1981]. The ENG pathology in our patients was generally slight with caloric asymmetry or directional preponderance, and only six patients with deviant ENG had vertigo (27.3%).

Nine patients in this study presented with BI. Four of them also had hearing loss. Sensorineural hearing loss has previously been reported in BI, but no association between BI and presence or type of hearing loss could be found in our study [Sawin and Menezes, 1997]. In fact, sensorineural hearing loss was more common in patients with normal skull base (42%) than in patients with BI (11%). Vertigo, including vertigo related to rapid head movements tended to be more common in patients with BI than in patients with normal skull base, but the difference was not statistically significant. Patients with BI had significantly more symptoms deriving from the central nervous system, as described in earlier publications on BI [Elies and Plester, 1980; Charnas and Marini, 1993; Sillence, 1994; Sawin and Menezes, 1997; Hayes et al., 1999]. Although BI has been reported to be most prevalent in OI type IVB [Sillence, 1994], no correlation between the type of OI and presence of BI was found in our study. Moreover, BI has also been suggested to occur in more severely affected individuals with OI [Charnas and Marini, 1993]. In our study, three of the nine patients with BI had a severe form of OI, while two patients had a mild and four patients a moderate form of OI.

ENG-pathology and presence of BI did not statistically correlate in our patients, although those with BI seemed to have more central-type of ENG pathology ($P = 0.118$). In the literature, frequent pathology with horizontal or rotational nystagmus, diminished unilateral caloric response, and absence of caloric responses have been reported in BI patients [Sawin and Menezes, 1997]. Notably, only one patient with BI in our material had directional preponderance of more than 20%, while no patients with caloric asymmetry or spontaneous nystagmus were found.

This study confirms that vertigo, in addition to hearing loss, is common in patients with OI. As in otosclerosis, the true cause for sensorineural hearing loss, as well as for vertigo in OI still remains unknown. The fact that the patients with hearing loss in this study were found to have more vertigo, and the co-existence of sensorineural hearing loss and deviant vestibular findings at ENG imply damage to the inner ear as the main reason for vertigo in these OI patients. A pathology similar to otosclerotic foci in contact with the pars superior of the vestibule or the superior vestibule nerve, as well as otosclerotic vascular changes due to the disease or to biochemical changes in the inner ear fluids are possible, as implied in the literature [Virolainen, 1972; Ghorayeb, 1978]. Vertigo in OI patients might also be explained by BI. Still, some OI patients without hearing loss or BI also suffer from a vertigo clinically indistinguishable from the type of vertigo seen in patients with hearing loss or BI, making it even more challenging to elucidate the etiopathology of vertigo associated with OI. In clinical work, patients with OI should be informed about the frequency of vertigo in OI, not only as a symptom of BI, a feared complication of OI.

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Stapes surgery in Osteogenesis imperfecta in Finland

Kaija Kuurila[1]; Seppo Pynnönen[2]; Reidar Grénman[3]

[1]Department of Otorhinolaryngology-Head and Neck Surgery, Vaasa Central Hospital, [2]Department of Mathematics and Statistics, University of Vaasa; [3]Department of Otorhinolaryngology-Head and Neck Surgery, Turku University Hospital, Finland

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Corresponding author: Kaija Kuurila, MD, Department of Otorhinolaryngology-Head and Neck Surgery, Vasa Central Hospital, Sandviksg. 2-4, 65100 Vasa, Finland tel +358-6-3231111 email kaija.kuurila@vshp.fi fax +358-6-3231378

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Reprint requests: Ms Helina Kosonen, Department of Otorhinolaryngology-Head and Neck Surgery, Turku University Central Hospital, PL 52, 20521 Turku, Finland. Tel. +358-2-3130000 fax. +358-2-3133550, email helina.kosonen@tyks.fi

IV

ABSTRACT

In a nationwide study on ear surgery in Finnish Osteogenesis imperfecta patients between years 1961-2002, surgical findings and audiometric results of 33 patients (43 operations) are presented. The mean age at the time of the first operation was 30.1 years. Typical surgical findings described in OI with thick, fixated or obliterated footplate, thick and vascular mucosa with excessive bleeding tendency and elastic, fractured or atrophic stapes crurae were found. Compared with previous studies, the hearing gain was poorer and the remaining postoperative gap was greater in the 43 operations analyzed. The results of this nationwide study, however, may not be directly comparable with operative results of non-population studies. On the other hand, the hearing gain in our study was better in university hospitals than in central hospitals, and, furthermore, comparable with previous studies after surgery performed by a single surgeon in a university hospital.

OI-related conductive hearing loss may be successfully treated with surgery in most patients. The rarity of the disease leading to small annual numbers of surgery, the variable surgical findings and profuse bleeding tendency in the middle ear, as well as the audiometric results in this study support centralization of ear surgery in OI patients.

Abbreviations: OI: Osteogenesis imperfecta, DI: Dentinogenesis imperfecta, $PTA_{0.5\text{-}2kHz}$: pure tone average at frequencies of 0.5, 1 and 2 kHz, HL: hearing level

Key words: Osteogenesis imperfecta, hearing loss, ear surgery, stapedotomy

INTRODUCTION

Hearing loss is one of the main symptoms in Osteogenesis imperfecta (OI), a genetic disease of the skeleton. Patients with OI have remarkable bone fragility due to mild traumas, varying from a few fractures during the lifetime to hundreds in childhood. The sclerae may be blue, and dentin in the teeth may be fragile (Dentinogenesis imperfecta).[1]

In population studies, hearing loss has been found in 22.6%-58% of patients with OI.[2-8] The hearing loss most often begins in early adulthood, it is progressive, and proceeds from conductive hearing loss to a mixed and sensorineural type with increasing age.[2, 8-12] However, it may also begin in childhood, and some patients may present with a predominantly sensorineural pattern at the onset of the hearing loss.[2, 8, 12, 13]

In the sixties, otosclerosis was suggested to be a local form of Osteogenesis imperfecta.[14] Further studies, however, have revealed that hearing impairment in OI and otosclerosis are two histologically, enzymatically and etiologically distinct entities with clinical similarities.[15, 16] Compared with otosclerosis, hearing loss in OI has a tendency to earlier onset, more severe middle ear involvement and a higher incidence of sensorineural hearing loss.[6, 8, 12, 17, 18]

According to previous studies, the typical surgical findings in the middle ear of an OI patient with hearing loss are thick and fixated or obliterated footplate, thick and vascular mucosa

Table 1. The Sillence classification of Osteogenesis imperfecta, discussed and modified at the 8th International Conference on Osteogenesis imperfecta, Annecy, 2002.[29-32] AD, Autosomal Dominant; AR, Autosomal Recessive

OI Type	Clinical features	Inheritance
IA	Mild to severe bone fragility; normal or slightly short stature, intensely blue sclerae, presenile hearing loss, normal teeth	AD
IB	Mild to severe bone fragility; normal or slightly short stature, intensely blue sclerae, presenile hearing loss, dentinogenesis imperfecta	AD
II	Extremely severe bone fragility, lethal	AR/AD
III	Variable, often severe bone fragility in infancy with progressive skeletal deformity; short stature, bluish sclerae, variable dentin abnormality	AR/AD
IVA	Bone fragility with mild to moderate deformity, often short stature, normal sclerae, normal teeth	AD
IVB	Bone fragility with mild to moderate deformity, often short stature normal sclerae, dentinogenesis imperfecta	AD
V	At least one episode of hyperplastic callus formation, moderate to severe fragility of long bones and vertebral bodies, limitations of pronation/supination in forearms, ligamentous laxity, normal teeth	AD
VI	Vertebral compression fractures, moderate to severe bone fragility, white or faintly blue sclerae, normal teeth	?
Other types	Cole-Carpenter – OI type	AD
	Bruck - OI type	AR
	North American OI	AR
	Osteoporosis-pseudoglioma syndrome	AR
	Additional undefined types	

with excessive bleeding tendency, and brittle atrophic crura. Crural fractures, obliterated round window, fragile incus, and deficient, short ossicles are also reported.[19-21] Compared with the dense, hard bone seen in otosclerosis, the footplate area in OI often presents with soft, granular or iceberg type bone.[20, 22-24] Despite the challenges in surgery caused by the anatomic peculiarities in the middle ear, the postoperative hearing results have been encouraging, albeit not as satisfactory as in otosclerosis. [19-21, 25-28] Furthermore, it has also been suggested that conductive hearing loss in OI patients can be surgically relieved with about the same level of predictability as in those with otosclerosis.[21]

The minimum prevalence of OI is 1/10 000.[1] It is mostly inherited as an autosomal dominant trait, but there are also autosomal recessive forms of OI.[3, 29] OI is caused by mutations in one of the two genes, COL1A1 and COL1A2, which encode type 1 collagen.[1] David Sillence introduced the classification of OI in the late 1970's. It has later been modified on several occasions, and the earlier classification into congenita- and tarda –forms has been replaced. Currently, OI is divided into six major types on the basis of clinical, radiographic and genetic criteria (Table 1).[29-32]

The aim of this nationwide study was to analyse the results of middle ear surgery in Finnish OI patients and to study the eventual benefit of centralization of the treatment. Anatomical findings in the middle ear of these patients were also recorded.

MATERIALS AND METHODS

Altogether 254 Finnish patients with OI were ascertained in a nationwide search in 1998 through the patient register of the Department of Clinical Genetics, Helsinki University Central Hospital,

Table 2. The nineteen operations not included in this study.

Pt number	Ear	Op year	Age at surgery	Type of OI	Type of operation	The reason for exclusion from the study
1	dx	1999	30	I	Stapedotomy	No postoperative audiometry available
2	sin	1990	26	I	Crurotomy, partial stapedectomy	No audiometry available
13	sin	1996	30	I	Exploratory tympanotomy	Stapes superstructure removed, non-identifiable stapes footplate
17	dx	1986	21	I	Stapedectomy	No hearing gain was achieved, revision 8 months after primary surgery
17	dx	1991	26	I	Prosthesis from malleus to the oval window	Revision surgery after 5 years
23	dx	1987	29	I	Stapedectomy	No hearing gain was achieved, revision 3 months after primary surgery
27	dx	1997	43	I	Stapedotomy	Revision surgery after 10 years
28	dx	1993	40	I	Stapedotomy	No hearing gain was achieved, revision 1 month after primary surgery
31	dx	1973	15	I	Stapedectomy	First postoperative audiometry after 4 years
31	dx	1992	35	I	Exploratory tympanotomy	Fistula suspicion 19 years after primary surgery
32	dx	1983	29	IV	Stapedectomy	Revision surgery after 14 years
33	dx	1993	47	IV	Prosthesis from malleus to the oval window	Revision surgery after 9 years
34	sin	1963	21	I	Mobilization	No audiometry available
35	sin	1977	37	I	Stapedotomy	First postoperative audiometry after 16 years
36	dx	1982	1	I	Stapedectomy	Postoperative audiometries after 1 month and 12 years
37	dx	1995	36	I	Stapedotomy	Only postoperative audiometry after 1 month
38	sin	1987	36	IV	Stapedectomy	First postoperative audiometry after 3 years
39	dx	1968	24	IV	Exploratory tympanotomy	Missing stapedial plate, oval window replaced with hard promontorial bone
40	sin	1969	36	I	Stapedectomy	Postoperative audiometries after 3 months and 25 years

through the membership register of the Finnish OI association, and the HILMO care register from Finnish university and central hospitals (prevalence 4.9/100 000). The diagnosis was based on fracture history, clinical and radiographic findings, and family history. The patients were approached through a questionnaire. They were asked about subjective and objective onset of the hearing loss, the use of hearing devices and surgical treatment.

Of the patients ascertained, 204 consented to participate in the study (80.3%) by returning a questionnaire. By 2002 ear surgery was performed in 40 of these patients, and a total of 62 operations were performed.

Seven patients and totally 19 operations were excluded from the study. As may bee seen from table 2, most exclusions were made because of insufficient or lacking audiometric data. Four primary operations with no hearing gain resulted in revision surgery shortly after the primary operation, and in these cases, so only the results of the revision surgery were included in the study (patients 13, 17, 23 and 28). In addition, four late revisions (4-14 years) were not included (patients 17, 27, 32 and 33).

This study focuses on surgical and audiometric data of the 33 patients (43 operations), in whom the preoperative pure tone air ($PTA_{500-2000}$) thresholds on speech frequencies could be compared with audiometry performed under standard conditions in a soundproof room at a local or university hospital 6 to 12 months after surgery.

The mean age of the 9 men and 24 women was 30.1 years by the time of the surgery (SD 9.4, range 15-53 years). The type of OI according to the criteria introduced by Sillence is presented in Figure 1. Subclassification of OI type I and IV into A and B on the basis of presence or absence of Dentinogenesis imperfecta (DI) was not used in our audiological study, since dentinal manifestations in OI apparently form a continuum from normal dentin to severe DI, and visual evaluation does not reveal the changes seen in X-ray or light and transmission electron microscopy.[33]

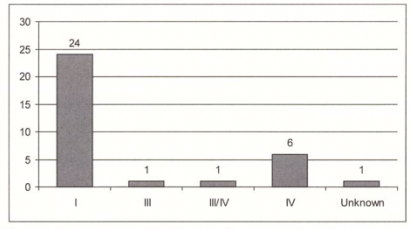

Figure 1. The type of OI in the 33 operated patients

The major statistical analyses were performed by utilizing paired t-tests for comparisons of the mean postoperative changes, the usual two-sample t-test and the chi-square test to analyze differences between patient groups. In addition, the *F*-test was used to analyze the homogeneity (equality of variances) of the postoperative results.

RESULTS

Of the 33 operated patients included in this study, all except three had blue sclerae. Nineteen patients had familial type of OI, while 14 patients were sporadic cases. The mean age at the onset of the subjective hearing loss was 20.8 years (SD 6.1, range 10-35 years). Most patients had bilateral hearing loss, with only three patients presenting with unilateral hearing loss. Thirteen patients used a hearing aid.

On all, 43 surgical procedures performed on the 33 patients from 1961 to 2001 were analysed (Figure 2, A-C). Three of the operations were revisions due to an unsatisfactory hearing result in the primary operation, performed by the same surgeon one, three and eight months after the primary surgery, respectively (Figure 2 B, case 23 dx and figure 2 C, cases 17 dx and 28 dx). Additionally, one patient with previous explorative tympanotomy and removal of the stapes superstructure in a local central hospital was one year later operated in a university hospital (Figure 2 A, case number 13 sin). The stapes surgery was bilateral in 10 patients. The mean follow-up time after the surgery was 5.6 years (SD 5.2, range 6 months-19 years). In 13 out of 43 operations the diagnosis OI was unnoticed, although 12 of these patients had blue sclerae, DI was present in five patients and all but one patient had had fractures.

TABLE 3. The type of middle ear operation in the 43 surgical procedures performed in Finland 1961-2002.

Type of operation	Number	%
Stapedotomy	19	44,2
Stapedectomy	13	30.2
Crurotomy, partial stapedectomy	6	14
Mobilization	2	4,7
Columellization	2	4,7
Prosthesis in a narrow oval window	1	2,3
Total	43	100

Table 3 presents the type of operation performed, and the most frequent surgical findings in the middle ear on the 43 operations are shown in Table 4. Figure 2 presents the pre- and postoperative hearing level in the 43 ears operated, with stapedotomies (A), stapedectomies (B) and other methods (C) presented separately. In all the patients, the hearing gain was statistically significant in both type I OI and in patients with more severe types of OI. The improvement was slightly, but not statistically significantly better on patients with type I OI.

TABLE 4. The most frequent form of anatomical findings in the 43 operated middle ears.

		Number of ears
Mucosa	Vascular	16
	Thick	8
Fibrosis		8
Footplate	Thick, sclerotic	19
	Obliterative	8
	Thin	4
Stapes crurae	Elastic	7
	Fractured	4
	Atrophic	4

Twenty different surgeons performed the 43 operations included in this study. One surgeon operated three ears, five surgeons operated two ears each, while thirteen surgeons operated one ear each. In addition, one surgeon performed sixteen operations. Thirty-six ears were operated in five university hospitals and seven ears in five central hospitals. The seven operations performed in central hospitals are presented in Figure 2 B and C.

The preoperative $PTA_{500\text{-}2000}$ in university and central hospitals was similar, with difference neither in variability nor on the average. Compared with central hospitals, the $PTA_{500\text{-}2000}$ was improved considerably in university hospitals, being also statistically significant with t-value of 2.6 and p-value 0.005, while the change in $PTA_{500\text{-}2000}$ in central hospitals was marginal (3.8dB). Standard deviations of $PTA_{500\text{-}2000}$, however, seemed to increase noticeably, close to double, after ear surgery in both hospital groups suggesting some obvious failures in both groups. Furthermore, in the university group, the 16 operations performed by a single surgeon differed from the others particularly in terms of statistically significantly smaller variability in postoperative $PTA_{500\text{-}2000}$, i.e. the results were more homogenous. Also the hearing gain was better in the 16 operations, although the difference was not statistically significant.

The pre- and postoperative gaps are illustrated in Figure 3.

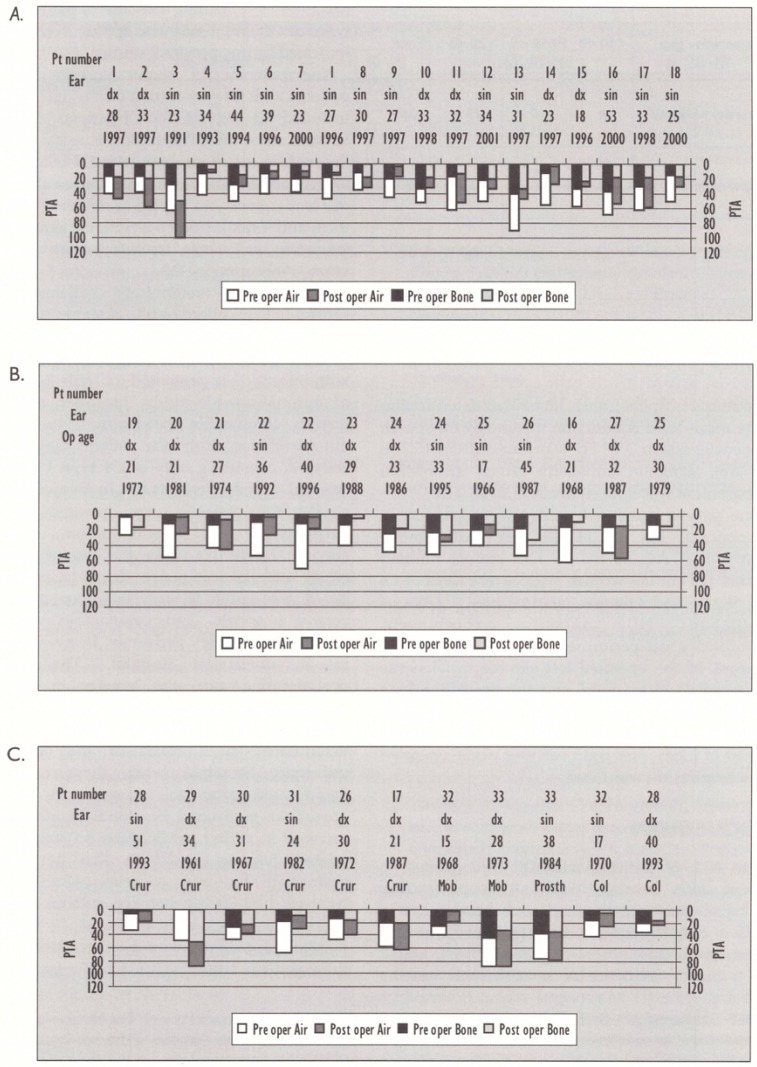

Figure 2. Individual mean pre- and postoperative air- and bone conduction thresholds on speech frequencies on the 43 operated ears, presented according to the operation method used. Patients are numbered from 1 to 33, the ear operated is presented, as well as the age of the patient at the time of the operation, and the year of operation.
A. Stapedotomy. All these cases were performed in university hospitals, and cases 4 sin to 18 sin were operated by a single surgeon (16 operations).
B. Stapedectomy. The first three operations (19 dx to 21 dx) were performed in central hospitals.
C. Other methods. Cases 28 sin, 29 dx, 33 sin, and 28 dx were performed in central hospitals. Methods used: Crur; Crurotomy, partial stapedectomy, Mob; Mobilization, Prosth; Prosthesis in a narrow oval window, Col; Columellization

Pre oper Air; preoperative air conduction thresholds on frequencies 500, 1000 and 2000 Hz
Pre oper Bone; preoperative bone conduction thresholds on frequencies 500, 1000 and 2000 Hz
Post oper Air; postoperative air conduction thresholds on frequencies 500, 1000 and 2000 Hz
Post oper Bone; postoperative bone conduction thresholds on frequencies 500, 1000 and 2000 Hz

Postoperative gap	<10 dB	10-20 dB	>20 dB	Total
University hospitals	8	13	15	36
Central hospitals	2	2	3	7
Total	10	15	18	43

Figure 3. Mean pre- and postoperative gap in the operated 43 ears. The results are presented separately for university and central hospitals.

In central hospital group, an overall deterioration in the mean bone conduction thresholds on speech frequencies 500 to 2000 Hz was found (from 15.7 to 18.6 dB), while in university group, an overall improvement was noticed (from 21.0/16.2 dB).

One patient presented with severe deafness in the operated left ear at the first postoperative follow-up ($PTA_{500-2000}$ 100 dB HL; patient number 3). This 110 cm tall woman with severe form of OI type III/IV had bilateral hearing loss ($PTA_{500-2000}$ 23.3/63.3dB HL dx/sin) preoperatively. Stapedotomy was performed year 1991 at the age of 23 years. In the operated left ear the facial nerve was reported to be nude, and the threadlike long process of the incus was in supine position making it necessary to place the prosthesis in the malleus instead of incus. Her right ear was never operated and a hearing aid was fitted.

DISCUSSION

Up to 60% of patients with OI are reported to present with hearing loss.[2-8] Although having clinical similarities with otosclerosis, hearing loss in OI is a distinct entity with a tendency toward earlier onset, more severe middle ear involvement and a higher incidence of sensorineural hearing loss.[6, 8, 12, 15-18] Hearing loss affects patients with all types of OI, and it may also appear in childhood, as indicated by our previous studies.[8, 13]

The outcome of stapes surgery in Finnish patients with OI was carried out in 2002. This study included 43 ears of 33 OI-patients operated in Finland since 1961.The typical surgical findings in the middle ear of patients with OI-related hearing loss reported in previous studies were also found in our study: thick and fixated or obliterated footplate, thick and vascular mucosa with excessive bleeding tendency, and elastic, fractured or atrophic stapes crura were common. [19-21]

Despite the anatomical challenges, mostly satisfying to excellent results of stapes surgery in OI have been presented in earlier studies.[19-21, 24, 28] The hearing gain found in earlier studies compared with our study is presented in Table 5. The results of our nationwide study, however, may not be directly comparable with former studies. Unlike our population study, the patients may have been selected, consisting only of OI type I patients, or selected by other criteria.[19-21] In two of the earlier studies the patients were operated by several surgeons[20, 28] and in two studies, by a single surgeon.[19, 21] In our study, the hearing gain in the whole material was poorer than in former studies, but it was better in university hospitals than in central hospitals, and comparable with earlier studies after surgery performed by a single surgeon in a university hospital. The remaining postoperative gap was greater in our study compared with earlier studies (Table 5).

The mean bone conduction thresholds were not deteriorated as a result of the operation in university hospitals, while they were slightly deteriorated after the surgery in the seven operations performed in central hospitals, as also reported in earlier publications on surgery on OI-related hearing loss.[20, 28] The 16 operations performed by one surgeon were performed between 1993-2002, when the authors studied OI-related hearing loss in Finland.[8, 13, 34] Also this number of patients may be considered low. All these patients were known to suffer from OI, while

TABLE 5. Hearing gain 6-12 months after stapes surgery in different studies on patients with OI. Notable is, that in our present study, air conduction thresholds improved in all ears operated by a single surgeon, and a hearing gain equal or more than 10 dB was achieved in 14 out of the 16 operations (87.5%). No ears deteriorated as the result of the operation in this group. Of the previous studies, only Pedersens Danish study was a population study including all types of OI, while Garretsens Dutch study only included OI type I patients. [20 28]

Author	Number of ears	Hearing gain ≥10dB	Hearing gain <10dB	Deterioration
Shea&Postma 1982[19]	51	43 (84%)	6 (12%)	2 (4%)
Pedersen 1983[20] *	42	37 (88%)	0	0
Garretsen&Cremers 1990[28]	46	37 (81%)	5 (11%)	4 (9%)
This study 2002	43	30 (70%)	4 (9%)	9 (21%)

* after 3 months

TABLE 6. The air-bone gap in different studies after stapes surgery. Notable again is, that except for the present study, Pedersens Danish study is the only population study including all types of OI.

Study	No of ears	< 10 dB	10-20 dB	> 20 dB
Shea and Postma 1982[19]	51	38 (75%)	?	?
Armstrong 1984[21]	30	23 (77%)	6 (20%)	1 (3%)
Pedersen 1983[20] *	42	26 (62%)	(9 21%)	7 (17%)
Garretsen 1990[37]	40	31 (78%)	5 (13%)	4 (10%)
This study	43	18 (42%)	13 (30%)	12 (28%)

* after 3 months

in thirteen out of the 27 operations in central hospitals and other university hospitals the surgeon was unaware of the diagnosis OI by the time of the surgery, and the patients were treated as patients with a far more common otosclerosis.

One patient presented with severe deafness in the operated left ear at the first postoperative follow-up. Ear surgery leading to severe deafness ($PTA_{500\text{-}2000a}$ more than 90 dB) has been reported in earlier studies in OI [22, 24, 28] as well as in otosclerosis surgery.[25-27]

Although the rarity of OI, modest centralization of the treatment of OI-related entities has been established in Finland so far. OI may still be unrecognised; especially the mild forms, and the patients may be treated as otosclerosis. The results of this study indicate, that the surgical outcome may be better at university hospitals than in local central hospitals with smaller annual number of otosclerosis surgery, and, like this study indicates, even fewer patients with OI-related hearing loss. In addition to more experienced surgeons, the difference in operative results may be explained by the anatomic peculiarities in the middle ear in OI. Particularly the profuse bleeding tendency and obliterative disease of the oval window may cause technical problems in an operation unit without laser, which is known to make stapes surgery easier.[35, 36] Therefore, a prerequisite for successful surgery is correct preoperative differential diagnosis of OI-related hearing loss and otosclerosis, which may be difficult in patients with mild type of OI often presenting with almost normal phenotype, perhaps with exception of slightly short stature or blue sclerae.

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Osteogenesis Imperfecta: Correlation of Molecular Genetic Findings with Hearing Loss and Other Clinical Features

H. Hartikka[1], K. Kuurila[2], Jarmo Körkkö[3], I. Kaitila[4], R. Grénman[5], S. Pynnönen[6], JC. Hyland[7,8], L. Ala-Kokko[1,7,9]

[1]*Collagen Research Unit, Biocenter and Department of Medical Biochemistry and Molecular Biology, University of Oulu, Oulu;* [2]*Department of Otorhinolaryngology-Head and neck surgery, Vaasa Central Hospital, Vaasa;* [3]*Department of Clinical Genetics, University of Oulu, Oulu;* [4]*Department of Clinical Genetics, Helsinki University Central Hospital, Helsinki;* [5]*Department of Otorhinolaryngology-Head and Neck Surgery, Turku University Central Hospital, Turku;* [6]*Department of Mathematics and Statistics, University of Vaasa, Vaasa, Finland;* [7]*Center for Gene Therapy and* [8]*Departments of Pathology and Laboratory Medicine, and* [9]*Medicine, Tulane University Health Sciences Center, New Orleans, Louisiana, U.S.A.*

The first two authors made equal contributions.

Corresponding author: Kaija Kuurila, MD
Department of Otorhinolaryngology, Vaasa Central Hospital
Hietalahdenkatu 2-4, 65100 Vaasa, Finland
Tel +358-6-3231111 fax, +358-6-3231378, email kaija.kuurila@vshp.fi

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Abstract

Osteogenesis imperfecta (OI) is caused by mutations in *COL1A1* and *COL1A2* that code for the α1 and α2 chains of type I collagen. Phenotypes correlate with the mutation types in that *COL1A1* null mutations lead to OI type I, and structural mutations in α1(I) or α2(I) lead to more severe OI types (II to IV). However, correlative analysis between mutation types and OI associated hearing loss has not been previously performed. Fifty-four Finnish OI patients with previously diagnosed hearing loss or age 35 and above were analyzed here for mutations in *COL1A1* or *COL1A2*. Altogether 49 mutations were identified. They represented the molecular genetic background of 41.1 % of the Finnish OI population. Thirty-eight mutations were in *COL1A1* and 11 in *COL1A2*. Sixteen of them were glycine substitutions and 16 were splicing mutations in α1(I) or α2(I). In addition, 17 null allele mutations were detected in *COL1A1*. Thirty-two patients (65.3 %) with a mutation had hearing loss. That is slightly more than in our previous population study on Finnish adults with OI (57.9 %). The association between the mutation types and OI type was statistically evident. Patients with *COL1A1* mutations more frequently had blue sclerae than those with *COL1A2* mutations. In addition, patients with *COL1A2* mutations tended to be shorter than those with *COL1A1* mutations. However, no correlation was found between the mutated gene or mutation type and hearing pattern. These results suggest that the basis of hearing loss in OI is complex, and it is a result of multifactorial, still unknown genetic effects.

Introduction

Osteogenesis imperfecta (OI) is an inherited disorder of connective tissue. It is characterized by a variable propensity to skeletal fractures following mild trauma, and to secondary deformities in the extremities, spine and skull, and short stature. [1] Progressive hearing loss, dentinogenesis imperfecta (DI), blue sclerae, joint hypermobility and easy bruising are also common. [1-3] The minimum prevalence of OI is 1/10 000-1/30 000. [1,4] Variable clinical expression, under-reporting, and a lack of patient registers may explain variation in the reported prevalence figures. [1,4]

Classification of OI into types I to IV based on the clinical and radiographic criteria and mode of inheritance was introduced by Sillence in the late 1970's. [5] This classification has been modified on several occasions. [2,6,7] Currently, two new OI types, OI types V and VI have been added to the pre-existing types (Table 1). [8,9] Most patients with OI types I to IV have mutations in the *COL1A1* and *COL1A2* genes encoding the proα1 and proα2 chains of type I collagen. [1,8,9]

Hundreds of mutations in the *COL1A*1 and *COL1A2* genes have been detected in OI patients. [1,8-13] The variety of mutations partially explains the clinical variability of OI that ranges from mild to lethal. [14] It has been proposed, in general, that mutations manifested by a reduction of type I collagen lead to the mild variant, OI type I. These consist of nonsense and frameshift mutations occur, throughout *COL1A1*, and lead to premature translation termination resulting in null alleles.[15] Mutations that alter the structure of the proα chains and result in the formation of abnormal molecules usually cause more severe phenotypes. The majority

of mutations are single base substitutions converting the codons for obligatory glycine residues to those coding for amino acids with bulkier side chains. [10,16]

Table I Classification of osteogenesis imperfecta

OI type[a]	Clinical features	Inheritance
IA	Mild to severe bone fragility normal or slightly short stature, blue sclerae, presenile hearing loss, normal teeth	AD[b]
IB	Mild to severe bone fragility normal or slightly short stature, blue sclerae, presenile hearing loss, DI	AD
II	Extremely severe bone fragility, lethal	AD/AR[c]
III	Variable, often severe bone fragility in infancy with progressive skeletal deformity; short stature,bluish sclerae, variable dentin abnormality	AD/AR
IVA	Bone fragility with mild to moderate deformity, often short stature, normal sclerae, normal teeth	AD
IVB	Bone fragility with mild to moderate deformity, often short stature normal sclerae, dentinogenesis imperfecta	AD
V	At least one episode of hyperplastic callus formation, moderate to severe fragility of long bones and vertebral bodies, limitations of pronation/supination in forearms, ligamentous laxity, normal sclerae, normal teeth	AD
VI	Vertebral compression fractures, moderate to severe bone fragility, white or faintly blue sclerae, normal teeth	?

[a]Sillence *et al*[1,5]; Hall *et al*[7]
[b]AD=Autosomal Dominant
[c]AR=Autosomal Recessive

Additionally, mutation location also correlates somewhat with the severity of the phenotype. C-terminal glycine substitutions and other C-terminal mutations that alter the structure of the procollagen α chains often result in more severe phenotypes than similar N-terminal mutations. [1,17-19] This correlation is more evident with the mutations in *COL1A1* than in *COL1A2*.

Population studies have revealed progressive hearing loss in up to 58% of adult OI patients. [4,20-24] Hearing loss in OI clinically resembles otosclerosis, but differs by earlier onset, more severe middle ear involvement and a higher incidence of a sensorineural component. [20,23,25-27] Hearing loss in OI typically begins in the early adulthood. It proceeds from a conductive hearing loss to a mixed or sensorineural type. [4,20,25,28-30] However, some patients present at onset with a predominantly sensorineural pattern. [4,20,25,31] Hearing loss has been suggested to be more frequent in OI type I than in OI type IV. [2,6,24] In our previous study, however, no correlation was found between the OI types and the presence, type or severity of hearing loss. [20]

Previously, genotype-phenotype correlations in OI have mainly been focused on the severity of the disease. The correlation between mutations and hearing loss has not been extensively studied. [13] Therefore, the goal of this study was to perform genotype-phenotype correlation emphasizing hearing loss in OI.

Materials and Methods

Patient population

Since 1998 a nationwide search has been performed through the patient register of the Department of Clinical Genetics, Helsinki University Central Hospital, through the membership register of the Finnish Osteogenesis Imperfecta Association, and the patient care register from the Finnish University and Central Hospitals (HILMO). 299 Finnish OI patients were identified (prevalence 5.7/100 000) and 205 patients consented to participate in the nationwide study by returning a questionnaire. Audiometry was performed on 183 of the patients between 4 to 81 years of age. Results of these studies have been reported earlier. [20,31,32]

The inclusion criteria of the study subjects were: Diagnosis of OI based on multiple skeletal fractures due to inappropriately mild traumas, particularly occurring in childhood, a previous audiometric study and either a previously diagnosed hearing loss or an age of greater than 35 years. This age limit was used since the hearing loss in OI is known to manifest during the second to third decades of the life. [4,25,28-30] A clinical geneticist established the diagnosis and classification of OI, based on the fracture history, clinical and radiographic findings, and family history. Since dental manifestations in OI form a continuum from normal dentin to severe DI, and visual evaluation does not reveal the changes that can be detected by X-ray or light and transmission electron microscopy, subclassification of OI types I and IV into A and B on the basis of the presence or absence of DI was not used in this study. [3]

Blood samples were available from 54 unrelated patients fulfilling the inclusion criteria. The study was approved by the Joint Ethical Committee of Helsinki University Central Hospital. Permission to acquire the patient data was granted by the Ministry of Health and Social Affairs.

Table 2 Clinical data and mutations

N (sex)	F/S[a]	Type of OI	Adult height (cm)	Blue sclerae	Fracture rate[c]	Age[d]	HL[e]	Age at HL[f]	PTA dx/sin (dB)[g]	Hearing pattern dx/sin[h]	esHL[i]	MT[j]	Mutation
1(F)	S	III	92	-	+++	32	+	20	61.7/40	m/c	-	1	G589S
2(F)	S	IV	110	?	+++	28	+	15	81.7/61.7	m/m	+	1	G121R
3(M)	F	I	150	+	+	39	-		0/1.7	n/n	-	1	G349A
4(M)	S	I	150	+	+++	30	+	21	25/66.7	s/m	+	1	G127R
5(F)	S	IV	75[b]	+	+++	12	+	12	28/33.3	c/c	-	1	G610C
6(F)	F	I	152	+	+	34	-	28	13.3/0	n/n	-	1	G901S
7(F)	S	IV	101	+	++	33	+	30	30/50	m/m	-	2	G370S[k]
8(M)	S	IV	110[b]	?	+++	18	+	15	40/28	c/c	-	2	G922S[k]
9(F)	F	I	150	+	+	23	+	17	51.7/28.3	m/s	+	2	G814R[k]
10(M)	F	I	174	-	++	43	-		10/13.3	n/n	-	2	G1012S[k]
11(F)	F	I	158	-	+	33	+	14	51.7/6.7	c/n	-	2	G232S[k]
12(F)	F	I	163[b]	+	0	16	+	15	36.7/36.7	c/c	-	2	G769S[k]
13(F)	F	IV	91	?	+++	30	-		3.3/0.0	n/n	-	2	G280A[k]
14(F)	S	III/IV	111	+	+++	23	+	20	23.3/63.3	s/m	+	2	G703R[k]
15(F)	F	IV	130	-	++	27	+	20	36.7/45	s/m	+	2	G316S[k]
16(F)	F	IV	147	?	+	31	+	30	46.7/33.3	m/s	+	2	G505S[k]
17(F)	S	I	151	+	++	49	+	15	117/117	a/a	+	3	IVS30+2T>G
18(F)	F	I	166	+	+	35	-		0/0	n/n	-	3	IVS36-1G>A
19(M)	F	IV	145	+	+++	33	-		11.7/5.0	n/n	-	3	IVS43+1G>A[k]
20(F)	F	I	153	+	+	44	+	25	41.7/50	m/m	+	3	IVS42-2A>G
21(M)	F	I	166	+	++	53	+	35	93.3/30	m/s	-	3	IVS17del+to+6
22(F)	F	I	150	+	+	54	-		15/15	n/n	-	3	IVS44+5G>T
23(M)	F	I	190	+	++	31	+	24	1.7/31.7	n/m	-	3	IVS31+2T>G
24(M)	F	I	170	+	++	37	-		1.7/3.3	n/n	-	3	IVS-1-3C>G
25(M)	S	III	120	+	+++	35	+	20	41.7/38.3	c/c	-	3	c.2268-2269delTC
26(M)	S	III	120	+	+++	49	-		11.7/11.7	n/n	-	3	IVS17+1G>C
27(F)	F	I	152	+	+	41	-		13.3/10.0	n/n	-	3	IVS1+1G>A
28(F)	S	III	100	+	+++	47	+	30	65/96.7	m/s	+	3	IVS44-2A>C
29(M)	S	I	172	+	++	33	+	20	50/31.7	m/c	-	3	IVS40+3A>C
30(M)	F	I	175	+	++	35	-		11.7/10	n/n	-	3	IVS9-2A>G
31(F)	F	I	160	+	+	46	-		0/0	n/n	-	3	IVS3+2T>C
32(M)	S	I	173	-	+++	37	+	35	6.7/41.7	n/c	-	3	IVS3-9A>G
33(M)	F	I	165	+	+	59	-		13.3/15.0	n/n	-	4	c.532delC
34(M)	F	I	178	+	+	42	-		1.7/5.0	n/n	-	4	W1147X
35(F)	F	I	172	+	+	34	+	30	43.3/41.7	m/c	-	4	c.3560delG
36(F)	S	I	147	+	+	39	+	36	55/46.7	m/s	+	4	R704X
37(F)	S	I	158	+	+++	36	+	24	45/53.3	m/c	-	4	c.2451delT
38(M)	F	I	167	+	+++	55	+	25	35/38.3	m/m	-	4	c.243delC
39(F)	F	I	150	+	+	48	+	?	15/18.3	n/s	-	4	c.610delC
40(M)	F	I	178	+	++	34	+	26	51.7/21.7	m/c	+	4	c.2646-2648delAG
41(F)	F	I	160	+	++	39	+	34	35/40	c/c	-	4	R848X
42(F)	F	I	158	+	+	56	-		3.3/5.0	n/n	-	4	c.299insC
43(F)	S	I	161	+	+++	24	+	13	45/35	m/m	+	4	c.2550insT
44(F)	F	I	155	+	++	41	+	?	13.3/18.3	n/s	-	4	c.3160delT
45(M)	F	I	167[b]	+	+	17	+	16	33.3/40	s/m	+	4	c.299insC
46(F)	F	I	150	+	+++	50	+	25	81.7/83.3	m/m	+	4	R848X
47(F)	F	I	154	+	+	21	+	19	51.7/61.7	c/m	+	4	c.389insA
48(M)	F	I	170	+	++	47	-		10.0/6.7	n/n	-	4	c.3064delG
49(F)	?	I	158	+	++	30	-		3.3/3.3	n/n	-	4	c.1994delG

[a]F=familial OI; S=sporadic OI.
[b]Height at the time of the audiometry
[c]+=<10; ++=10-30; +++=>30.
[d]Age at audiometry. Preoperative age is given for the surgical patients.
[e]HL=hearing loss.
[f]Age at the onset of hearing loss.
[g]PTA dx/sin: Pure tone thresholds on speech frequencies (500, 1000, 2000 Hz); right/left ear.
[h]n=normal; c=conductive; m=mixed; s=sensorineural; a=anacusis.
[i]esHL=early sensorineural hearing loss.
[j]MT=mutation type; 1=substitution of glycine residues in pro 1(I); 2=substitution of glycine residues in pro 2(I); 3=splicing mutation; 4=null allele mutation.
[k]mutation in *COL1A2*.

Analyses of the *COL1A1* and *COL1A2* genes

Genomic DNA was isolated from blood samples by standard procedures. Conformation sensitive gel electrophoresis (CSGE) was used to scan the *COL1A1* and *COL1A2* genes for mutations in the 54 unrelated patients with OI. All 51 exons of *COL1A1* and all 52 exons of *COL1A2* and the flanking sequences were amplified by PCR as described previously. [33] The PCR products were about 200-450 bp in size and contained at least 60 bp of exon flanking sequences. PCR amplifications were carried out in a reaction volume of 23 µl containing 40 ng of genomic DNA, 200 µM of each dNTP, 0.25 µM of each primer, and 1 unit of Taq polymerase (AmpliTaq Gold, Applied Biosystems). The PCR conditions were an initial denaturation at 95°C for 10 min, followed by 95°C for 40 sec, 54-64°C for 40 sec, and 72°C for 40 sec for 35 cycles. To generate heteroduplexes for CSGE analysis the PCR products were denatured at 98°C for 3 min and annealed at 68°C for 30 min. The PCR products were analyzed on an agarose gel to check the quantity and quality of the products.

Scanning of the PCR products was performed by CSGE as previously described with the exception that the gels were stained with SYBR Gold nucleic acid gel stain (Eugene) instead of ethidium bromide. [34] PCR products that contained heteroduplexes were sequenced with PCR primers by an automated instrument (ABI PRISM 377 or 3100 Sequencers and ABI PRISM Dye Terminator Cycle Sequencing Ready Kit, Applied Biosystems). Prior to sequencing, the samples were treated with exonuclease I and shrimp alkaline phosphatase. [35,36]

Audiometric evaluation

Pure tone audiometry was performed under standard conditions in soundproof rooms. Normal hearing was defined as pure tone average (PTA) 0.5-2 kHz equal to or better than 15 dB hearing level (HL) in patients under 60 years of age, and equal to or better than 20 dB HL in patients 60 years or older. The following definition for different types of hearing loss paralleling definitions by Shapiro et al. and Pedersen was used. [4,26]

1. Conductive hearing loss: average air-bone-gap for the frequencies 0.5, 1 and 2 kHz greater than 15 dB, with corresponding bone conduction threshold smaller than 15 dB.

2. Sensorineural hearing loss: air conduction thresholds for the frequencies 0.5, 1 and 2 kHz equal to or greater than 15 dB, with corresponding air-bone-gap smaller than 15 dB.

3. Mixed hearing loss: average air-bone-gap for the frequencies 0.5, 1 and 2 kHz greater than 15 dB, with corresponding bone conduction threshold equal to or greater than 15 dB.

The age of the patients at the time of audiometry was recorded. Audiometry was determined prior to surgery since surgery may affect pure tone thresholds greatly. Early sensorineural hearing loss was defined as mean bone conduction thresholds on speech frequencies more than 20 dB HL at the age of less than 30 years.

Genotype-phenotype correlation

To perform genotype-phenotype correlation, *COL1A1* and *COL1A2* mutations were classified in two ways. In the first classification, mutations were divided into those that were found in *COL1A1* and those localized in *COL1A2*. In the second classification, the mutations were divided into four subgroups. Subgroup one and two contained single base mutations resulting in glycine substitution in proα1(I) and proα2(I), respectively. The third subgroup consisted of mutations that altered consensus RNA splicing sequences. These mutations usually lead to exon skipping or intron inclusion or the use of a cryptic splice site. [37,38] Mutations affecting splice sites can be manifested in a qualitative or quantitative manner. Because cultured cells were not available, mRNA and protein studies could not be performed. The fourth subgroup included nonsense and frameshift mutations, which typically lead to haploinsufficiency through nonsense-mediated mRNA decay.

Statistical methods

Statistical dependencies were determined between the mutation types and the onset, presence, type, special characteristics and severity of hearing loss. In addition, correlations between the mutations, OI types and clinical features, such as blue sclerae, height and fracture rate were performed.

Statistical analyses of dependencies were carried out by utilising t-tests and cross-tables (contingency tables). Due to the small sample size, we utilized in the latter case the Fisher's exact test instead of the traditional chi-square test. The major advantage of the Fisher procedure is that it does not rely on a large sample number as does chi-square analysis, and hence should be more powerful in detection of possible dependencies.

Results

General characteristics

Forty-nine patients were found to have a mutation either in *COL1A1* or *COL1A2* (Table 2). The mean age of the patients, 19 men and 30 females, at the time of the audiometry was 36.4 years (SD 11.1 years, range 12-59 years). Most of the patients had OI type I (Figure 1). Forty had blue sclerae (81.6 %), and all but one suffered fractures. Thirty-two of the 49 patients had familial OI and 16 had sporadic disease. One patient could not be classified as

familial or sporadic, since the family members could not be examined. The 32 patients with familial OI had 74 relatives with OI, and therefore, these 49 patients represent the molecular genetic background of 123 of the known 299 Finnish OI patients (41.1 %) by the year 2003.

Molecular genetic findings

Mutation analysis by CSGE resulted in identification of *COL1A1* or *COL1A2* mutations in 49 patients. CSGE analysis and complete sequencing of the genes did not detect mutations in five patients. A total of 47 different mutations were identified. An identical mutation was present in two non-related patients. Thirty-eight of the mutations were in *COL1A1* and eleven in *COL1A2*. Sixteen patients had a mutation resulting in glycine substitutions in *COL1A1* or *COL1A2*. Six glycine mutations were in *COL1A1* and they were located between exons 13 and 45. These patients had OI type I, III or IV. Ten of the 16 glycine substitutions were in *COL1A2*. They were located between exons 19 and 49. Three patients had OI type I, five had OI type IV and one patient had OI type III. No correlation was observed between the locations of the glycine substitutions and the severity of OI (Table 2).

Mutations in the consensus RNA splicing sequences were found in twelve patients with OI type I, in three patients with OI type III and in one patient with OI type IV. The mutations were located between introns 1 and 44 of *COL1A1* and one was in intron 43 of *COL1A2*. One OI type III patient with a two nucleotide deletion in exon 33/34 of *COL1A1* (c.2268-2269delTC) was included in this group. Since this patient had severe OI, the mutation was predicted to result in non-sense mediated exon skipping instead of haploinsufficiency. [39] However, RNA was not available to confirm this prediction.

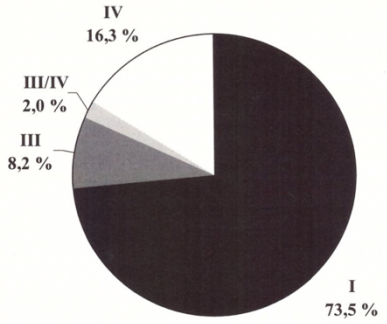

Figure 1 The OI type in the 49 patients. OI type I (I) was most common. One patient could not be classified as OI type III or IV (III/IV).
III=OI type III; IV=OI type IV.

Four nonsense mutations and thirteen frameshift mutations, predicted to result in nonsense-mediated mRNA decay, were found in *COL1A1*. Nine of the frameshift mutations were deletions and four were insertions. Two unrelated patients had the same

Table 3 Clinical findings and mutation types

Mutation type	N	Mean age (range)	Blue sclerae	Mean height (range)	Hearing loss				OI type		
					Present	es[a]	No variation[b]	Variation[c]	I	III	IV
Gly substitution α1(I)	6	29.2 (12-39)	4	121.5 cm (75-152)	4	2	0	0	3	1	2
Gly substitution α2(I)	10	27.7 (16-43)	4	133.5 cm (91-174)	8	4	2	0	4	1[d]	5
Splicing α1(I) or α2(I)	16	41.2 (31-54)	15	153.9 cm (100-190)	8	3	3	2	12	3	1
Null allele α1(I)	17	39.5 (17-59)	17	161.6 cm (147-178)	12	7	3	4	17	0	0
Total	49	36.4 (12-59)	40	148.5 (75-190)	32	16	8	6	36	5	8

[a]es=early sensorineural hearing loss
[b]No variation=no variation in expression of the hearing loss in family
[c]Variation=variation in expression of the hearing loss in family
[d]A patient classified as OI type III/IV

Table 4 Type of OI related to the mutated genes and mutations types.

OI type	*COLIA1*	*COLIA2*	Gly α1(I)	Gly α2(I)	Splicing α1(I) or α2(I)	Null allele α1(I)
I	32	4	3	4	12	17
III	4	1[a]	1	1	3[a]	0
IV	2	6	2	5	1	0
Total	38	11	6	10	16	17

[a]Includes one patient with OI type III/IV

c.299insC mutation. Also a nonsense mutation, R848X, occurred in two unrelated patients. All of these patients had OI type I.

Audiometric findings

Of the 49 patients with a mutation in either *COL1A1* or *COL1A2*, 32 had hearing loss (65.3 %) (Table 3). It was bilateral in 24 patients and unilateral in 8 patients. The mean age at the onset of hearing loss was 23.9 years (SD 8.0, range 12-45 years). The hearing pattern as related to the OI type in the 49 patients is presented in Figure 2. Hearing loss was found in all OI types.

According to the audiometric studies, 15 of the 32 patients with hearing loss had early sensorineural hearing loss, i.e., bone conduction thresholds on speech frequencies 500-2000 Hz were 20 dB HL or more before the age of thirty years in a previous audiometry. One patient had anacusis. This individual with OI type I had blue sclerae, normal teeth by visual evaluation and multiple fractures. Audiometry was first performed when she was 20 years of age. She was found to have a progressive hearing loss of mixed type. Rapid progression of the hearing loss resulted in total anacusis by the age of 22 years (Table 2, patient number 17).

The pattern of familial hearing loss could be studied in 14 cases. The remaining patients with familial OI either had no adult relatives with OI or the hearing pattern in the relatives was unknown. The hearing loss in the other affected family members was similar in five familial cases. It varied in three patients. On the other hand, three patients with normal hearing had relatives with normal hearing, while three other patients with normal hearing had relatives with hearing loss. No correlation was found between the OI type and expression of hearing loss in families.

Other clinical findings

The mutation and clinical data of the 49 patients is presented in Table 2. The mutated genes correlated with the OI types. *COL1A1* mutations were more frequent in OI types I and III, and *COL1A2* mutations were so in OI type IV (p=0.00086).

The association between the four different mutation subgroups and clinical OI types were statistically significant (p=0.0010), predominantly due to the association of null allele mutations with OI type I and partially due to the association of glycine substitutions in proα2(I) with OI type IV (Table 4). Patients with *COL1A1* mutations more often had blue sclerae than the patients with *COL1A2* mutations (p=0.0327). Furthermore, patients with *COL1A2* mutations tended to be shorter than those with *COL1A1* mutations (p=0.0443, t-test). Table 3 presents the OI type, height and hearing status of the patients with *COL1A1* and *COL1A2* mutations. No correlation was found between the mutation types and the fracture rates or between the mutation types and sporadic or familial cases.

Statistical analysis indicated that mutations in *COL1A1* were as likely to cause hearing loss as were *COL1A2* mutations (p=0.725). The presence of hearing loss did not correlate with the different mutation types (p=0.452), nor did the type or severity of the hearing loss correlate with the

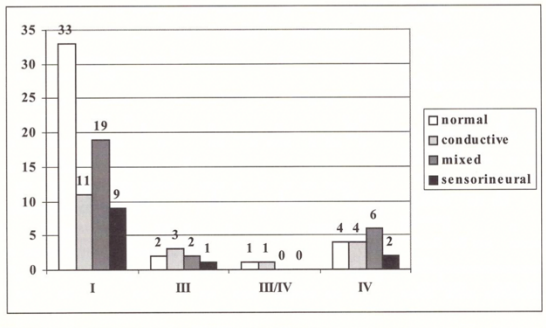

Figure 2 Hearing pattern in the different OI types in the 98 ears of the 49 patients. Hearing loss was found in all OI types. Ears with anacusis are included in sensorineural hearing loss.

mutated genes or different mutation types. Early sensorineural hearing loss, or expression of hearing loss in the families also did not correlate with the mutation types.

Patients without detectable mutations

COL1A1 or *COL1A2* mutations were not detected by CSGE or sequencing of all exons in five patients. A 55-year-old woman (height 163 cm) with familial OI type I and a moderate fracture rate had mixed hearing loss bilaterally. Two patients had sporadic OI type I and moderate bone fragility. A 69-year-old woman (height 153 cm) had normal hearing, and a 41-year-old woman (height 161 cm) had conductive/mixed hearing loss (right/left ear). A 41-year-old, 157 cm tall woman with sporadic OI type IV and moderate bone fragility had white sclerae and normal hearing. In addition, a 48-year old, 130 cm tall man with sporadic OI type V had moderate bone fragility and normal hearing.

Discussion

We present here the clinical and mutation data of 49 OI patients, representing 41.1 % of the known Finnish OI population. Forty-one of these mutations are presented here for the first time.

Earlier reports suggest that mutations resulting in a reduction of the amount of type I collagen produce the mildest phenotypes, while mutations altering the structure of type I collagen produce a wide range of phenotypes. [1,17] The association between the mutation type and the OI type was also evident in this study. Null allele mutations most often produced OI type I, while single base substitutions resulting in glycine substitutions in proα2(I) tended to produce OI types I and IV. Patients with *COL1A1* mutations more often had blue sclerae whereas patients with *COL1A2* mutations tended to be shorter. No statistical dependency between the mutation type and fracture rate or familial OI was found.

In the present study, hearing loss was found in 32 patients (65.3 %). In population studies, progressive hearing loss has been found in up to 58% of the patients. [4,20-24] In this study involving pre-selected patient material, hearing loss was slightly more common than that previously observed in Finnish OI adults.[20]

Although conductive hearing loss often proceeds to a mixed or exclusively sensorineural type with increasing age, some patients present with a predominantly sensorineural pattern at the onset of hearing loss. [4,20,25,28-31] Fifteen patients with hearing loss had a clear sensorineural component before the age of 30. Hearing loss has been suggested to be more common in patients with *COL1A1* mutations than in patients with mutations in *COL1A2*. [13] Thirty-eight mutations were found in *COL1A1* and eleven mutations in *COL1A2*. No correlation between the mutated gene and hearing pattern was found. Furthermore, hearing loss has been suggested to be more common in OI type I than in OI type IV. [24,40] However, in this study hearing loss in all OI types was found to be approximately equal.

A variety OI causing mutations have been identified in *COL1A1* and *COL1A2*. They include single base mutations resulting in the substitution of obligatory glycine residues, frameshift mutations, null allele mutations, splicing mutations, multiexon rearrangements and mutations in the C-terminal propeptides that interfere with molecular assembly. [10,15,17] We found 16 single base mutations resulting in glycine substitution, 16 RNA splicing mutations and 17 null allele mutations. The types of mutations did not correlate with the presence, type or severity of hearing loss, the age at the onset of the hearing loss or special features of the hearing loss such as early sensorineural hearing loss or the expression of hearing loss in the family. On the contrary, the different mutation types found in the 49 patients resulted in overlapping hearing phenotypes. Two patients (patients 42 and 45) with the same *COL1A1* null allele mutation, c.299insC, had different hearing patterns. Patient 42 had normal hearing at 56 years, while patient 45 already had a moderate mixed/sensorineural hearing loss (right/left ear) at the age of 17 years. On the other the R848X *COL1A1* mutation resulted in a conductive hearing loss in patient 41 at the age of 39 years, and mixed hearing loss in patient 46 at the age of 50 years.

In five patients, no mutation could be found in *COL1A1* or *COL1A2* by CSGE analysis or sequencing of all exons. While all these patients had clinically confirmed OI, they may have had large gene rearrangements that could not be detected by the mutation detection approach used here or these patients may demonstrate the heterogeneity of OI. [41,42] One of these patients had OI type V, a type in which *COL1A1* and *COL1A2* mutations have not been reported. [9]

This study suggests that the basis of hearing loss in OI is complex and the hearing pattern does not correlate with the mutation types in *COL1A1* and *COL1A2*. Apparently, hearing loss in OI is a result of multifactorial, still unknown genetic effects.

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