

## Osteogenesis Imperfecta at the Beginning of Bone and Joint Decade

Dragan Primorac, David W. Rowe<sup>1</sup>, Monica Mottes<sup>2</sup>, Ingeborg Barišić<sup>3</sup>, Darko Antičević<sup>4</sup>, Stefania Mirandola<sup>2</sup>, Macarena Gomez Lira<sup>2</sup>, Ivo Kalajzić<sup>1</sup>, Vesna Kušec<sup>3</sup>, Francis H. Glorieux<sup>5</sup>

*Laboratory for Clinical and Forensic Genetics, Split University Hospital and School of Medicine, Split, Croatia;*  
<sup>1</sup>*Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, Conn, USA;* <sup>2</sup>*Department of Mother and Child, Biology and Genetics, University of Verona, Verona, Italy;*  
<sup>3</sup>*Department of Medical Genetics, Children's Hospital Zagreb;* <sup>4</sup>*Department of Orthopedics, Zagreb University Hospital Center, Zagreb, Croatia;* and <sup>5</sup>*Genetics Unit, Shriners Hospital for Children and McGill University, Montreal, QC, Canada*

Osteogenesis imperfecta (OI), or brittle bone disease, is a heritable disorder characterized by increased bone fragility. Four different types of the disease are commonly distinguished, ranging from a mild condition (type I) to a lethal one (type II). Types III and IV are the severe forms surviving the neonatal period. In most cases, there is a reduction in the production of normal type I collagen or the synthesis of abnormal collagen as a result of mutations in the type I collagen genes. These "classic" forms of OI are described in this review. There are instances, however, where alterations in bone matrix components, other than type I collagen, are the basic abnormalities of the OI. Recently, three such discrete types have been identified by histomorphometric evaluation (types V and VI) and linkage analysis (Rhizomelic OI). They provide evidence for the as yet poorly understood complexity of the phenotype-genotype correlation in OI. We also discuss bisphosphonates treatment as well as fracture management and surgical correction of deformities observed in the patients with OI. However, ultimately, strengthening bone in OI will involve steps to correct the underlying genetic mutations that are responsible for this disorder. Thus, we also describe different genetic therapeutic approaches that have been tested either on OI cells or on available OI murine models.

**Key words:** collagen; dentinogenesis imperfecta; gene therapy; genetics; mutation; osteogenesis imperfecta; syndrome

Since the beginning of the century, many classifications of osteogenesis imperfecta (OI) have been proposed to evaluate clinical status, give life prognosis for the affected children, and provide genetic information to the parents. The criteria have included clinical presentation, radiographic findings, and the type of inheritance (1-6). The wide spectrum of the disease and considerable interfamilial and intrafamilial variability often made such classifications difficult. The biochemical, genetic, and clinical research efforts in recent years have contributed to a better understanding of the pathogenesis of the disorder, but have not been able to provide accurate clinical classification. In spite of the new knowledge, OI is still commonly classified by Sillence's classification into four major types according to the clinical features, radiographic findings, and the type of inheritance (5,6). Recently, a new group of patients has been identified at the clinical and molecular level and added to the present classification as OI type V, OI type VI, and Rhizomelic OI (7). For purposes of correlating clinical phenotype with the underlying genetic and pathophysiological basis of the disease, grouping the classes into deforming and nondeforming bone disease can be helpful (Table 1).

### Nondeforming Osteogenesis Imperfecta: Type I

Osteogenesis imperfecta type I is a dominantly inherited disease characterized mainly by blue sclerae and increased tendency to sustain fractures even from minimal trauma. Most of the OI patients (60%) belong to this group. The prevalence of OI type I has been estimated to be 1 in 15,000-20,000 infants, but mild cases are often overlooked. New mutations are frequent and in some studies account for almost half of the patients. Fractures are rare in the perinatal period and chiefly occur from childhood to puberty. The long bones of the arms and legs and small bones of the hands and feet are most frequently broken. There is significant interfamilial and intrafamilial heterogeneity suggesting that there are other factors than the type and the location of collagen gene mutations which can influence the clinical phenotype (8-10). In the mild cases only few fractures and/or mild osteopenia can be observed, whereas the patients with more severe forms can have more than 50 fractures. Fractures heal with good callus formation and only 15% of patients develop deformities of the long bones (11). The number of fractures decreases through adulthood and then often increases in

**Table 1.** Clinical and molecular classification of osteogenesis imperfecta (OI)

Molecular classification	Clinical classification	Clinical severity	Molecular mechanism
Dominant Negative	Type II	Perinatal, lethal	Glycine substitutions preferentially located in C terminal helical domain of either collagen chain
Dominant Negative	Type III	Progressive, deforming	Glycine substitutions preferentially located in mid helical domain of either collagen chain
Dominant Negative	Type IV	Moderately deforming	Glycine substitutions preferentially located in mid helical domain of the $\alpha_2$ collagen chain
?	Type V	Moderately deforming	Non type I collagen gene mutation
?	Type VI	Moderate to severe deforming	Non type I collagen gene mutation
?	Rhizomelic OI	Moderate to severe deforming	Non type I collagen gene mutation
Haploid Insufficiency	Type I	Classical mild OI	Complete non-functional COL1A1 allele usually due to premature stop codon

menopause in women and after the sixth decade in man. On radiographs, bone morphology is generally normal at birth, although femoral bowing and mild osteopenia are sometimes found. However, wormian bones are present in 60% of cases (11). Vertebral morphology is normal at an earlier age, but often a typical "cod fish" appearance is gradually formed. The size of the skull and jaws of OI type I patients is generally slightly reduced, but otherwise craniofacial morphology is normal (12). All OI type I patients have intensely blue sclerae that retain their color throughout life. Hearing loss occurs in about 50% of families, beginning usually during the second decade as a conductive loss and leading gradually to profound mixed or sensorineural deafness (13,14). Women are twice as often affected as males. Hearing loss in children is not frequent, but it is recommended that audiometry is performed in affected children every 3 years after the age of 10 (15). It has been proposed that OI type I patients should be subdivided into groups A and B on the basis of the absence or presence of dentinogenesis imperfecta (DI), respectively (16). This classification is to some extent controversial because dental manifestations form a continuous spectrum ranging from normal dentin structure to severe DI with no clear-cut limit. Difficulties in diagnosing mild DI also often make assignment to the specific group questionable (17,18). In different studies, therefore, the proportion of OI patients with DI ranges between 10% and 50% (11,19). Hypoplasia of dentin and pulp with translucency of teeth, which have a yellowish or grey coloration, susceptibility to caries, and irregular placement and eruption of teeth are the main dental problems. Patients with associated DI usually have a more severe form of the disease, with greater fracture rates and growth retardation (20). It is suspected that at least some OI type I patients with DI belong to OI type IVB in which the scleral hue is more pronounced (21).

Non-mineralized collagen-containing tissues, such as the sclerae, cornea, skin, heart valves, or tendons, show a reduction in amounts and thickness. Hence additional clinical findings are found, reflecting connective tissue malfunction, such as thin skin, hernias, and generalized joint hypermobility. Increased liability to bruising and other bleeding problems mainly due to vascular fragility are also present (22). Mitral valve prolapse, aortic valve insufficiency, and larger than normal aortic root diameter have been occasionally identified (23).

The height of type I patients is normal or postnatally slightly reduced (11). Disproportion in stature

in the OI type I is caused mainly by spinal involvement, mostly platyspondylia and scoliosis, which is present in about a quarter of patients (11,24). Muscle strength is normal except for the periarticular hip muscles (25). A delay exists in achieving motor milestones, comparable to the 95th percentile of the normal population (24). A recent study shows that community ambulation without the use of crutches is achieved in only 52% of cases of OI type I (26). Non-motor developmental skills are usually normal. Although children with OI have normal intelligence, they may underachieve in school because of the nature of the illness and physical disability (27). In adolescence and adulthood they have problems emerging from the discrepancy between their apparently normal appearance and their underlying illness. Competition with healthy children carries the possibility of new injuries. Normal life challenges, such as a demanding job, pregnancy, or military service for affected individuals are risky situations requiring additional psychological strength and family support (27). Patients with OI type I usually have a normal lifespan and die of unrelated illnesses (28). Perhaps the most important reason for identifying these individuals is to initiate anti-osteoporosis therapy as soon as menopausal symptoms have developed because these individuals are particularly susceptible to accelerated bone loss when sex steroids are lost (29).

#### **Deforming Forms of Osteogenesis Imperfecta (Type II, III, IV, V, VI, and Rhizomelic Osteogenesis Imperfecta)**

A unifying feature of all the deforming forms of OI is the strong dominant negative properties of the mutation. In those cases where affected individuals choose to have children, the disease is passed on in an autosomal dominant manner, except Rhizomelic OI, which is inherited as an autosomal recessive trait. However, in most cases the disease results from a random and isolated mutation in the germ cell of a parent. Usually, this event is a one-time occurrence and the likelihood of another affected individual is extremely small. However in 5-10% of cases one of the parents sustained a somatic mutation during his or her embryonic development, such that a proportion of their somatic and germ cells have an OI mutation (30,31). The mosaic parent does not have features of OI and most of their children have normal bones. However, if the child is conceived with the germ cell carrying the OI mutation, then full-blown OI results. As a result of this possibility for recurrence, families

with a child with OI who wish to have more children are advised to undergo prenatal testing (32) or sensitive ultrasound analysis with the possibility that a subsequent child might be affected (33,34).

#### *Osteogenesis Imperfecta Type II*

OI type II is the most severe form of the disease, affecting 1 in 20,000-60,000 infants. Prematurity and intrauterine growth retardation are often present. Newborns have soft calvarial bones, distinctive triangular face, bluish sclerae, and beaked nose. A narrow thorax, short and deformed extremities with multiple fractures, and a typical frog-like position are the main features. Death usually occurs in the neonatal period from infection and pulmonary and/or cardiac insufficiency. Survival beyond a year is extremely rare (35). OI type II has been associated with neuropathological alterations, perivenous microcalcifications, and impaired neuronal migration (36,37).

Bone changes can already be seen by prenatal ultrasound examination and are invariably present at birth. The hallmarks of OI type II include shortened and deformed limbs in the presence of normal birth weight and length. Ossa suturalia, poor calvarial ossification, shortness and angulation of the long bones, and multiple intrauterine fractures with callus formation are the main radiological features. By using radiological criteria, Sillence et al (38) have subdivided OI type II patients into three categories: groups A, B, and C. *Group A* is the most frequent and presents with short, broad femora with wave-like contours. Multiple fractures give the ribs a continuously beaded appearance. *Group B* is also characterized with short, broad, accordion-like femora, but with normal ribs or few fractures. *Group C* has long, slender bones and thin, continuously beaded ribs. Different empirical recurrence risks have been proposed for each subgroup, reflecting heterogeneity of type II that was also confirmed by biochemical studies. Most cases result from new dominant mutations in one of the genes for type I collagen. The recent study also showed that the activity of prolidase (an enzyme essential for collagen synthesis and cell growth) is also significantly reduced in the cells isolated from the patient with OI type II (39). Recurrence among sibs is estimated to be 6-7% and is usually due to parental mosaicism (35).

#### *Osteogenesis Imperfecta Type III*

Type III OI occurs in approximately 20% of all patients with OI (40). All infants born with fractures and deformity who survive the perinatal period belong to this group. OI type III is usually recognized at birth, because intrauterine fractures produce deformities of the long bones and severe skeletal changes. Most patients have intrauterine growth retardation, and further progressive growth failure continues during childhood as a result of long bone deformations and spinal involvement. A significant proportion of patients have large and asymmetric heads, while the face is usually triangular. In infancy, sclerae are often pale blue or grey but they regain normal color by puberty. The maxilla is frequently posteriorly inclined, and most craniofacial size measurements are reduced (12). Class III dental malocclusion occurs in 80% of

OI type III patients with a high incidence of anterior and posterior cross bites and open bites (30). DI is present in more than 80% of the primary dentition. Primary teeth are more frequently affected with discolorations and abrasions than permanent teeth. More severe attrition and enamel fracture have been observed on primary teeth with yellow/brown discoloration. A delay in dental development is observed in 21% of patients (41). Radiological abnormalities are found in both abraded and/or discolored teeth, as well as in clinically normal appearing teeth. In most cases cub-shaped extensions of the pulp chambers and obliterations of the root canals are found (20). Periapical cystic mandibular radiolucencies are also seen occasionally (42).

During the first 10 years of life, the number of fractures and the extent of skeletal changes is approximately the same in type III and type IV of OI. About 30% of patients experience recurrent abdominal pain due to chronic constipation and pelvic deformity with severe acetabular protrusion (43). Occasionally, congenital cardiac malformations, hemihypertrophy, papillary calcifications or kidney stones, as well as hypercalciuria, are seen (11,44).

A child who can sit by the age of nine to ten months is likely to achieve independent walking (26,45). The development of all motor milestones is significantly delayed compared with the type I and IV, with a discrepancy between static and dynamic milestones (46). In the study by Engelbert et al (26), 27% of children with OI type III achieved household ambulation with crutches, whereas 45% were restricted to the use of wheelchairs. Most children who are independent in ambulation have poor joint alignment, poor balance, and low endurance. Bending and angulations of the long bones, hip contractures, and pelvic deformities are present in the most severe cases, hindering independent walking. Joint laxity results in hyperextension and valgus position of the knees and feet. Muscle strength is usually severely decreased, with a muscular imbalance around the hip joint (25). Children who are not ambulatory usually have, in addition, joint contractures and malalignment of the upper extremities, leading to recurrent fractures (4). Osteopenia and joint hyperlaxity often lead to progressive kyphoscoliosis and chest wall abnormalities. Radiographic findings of six or more biconcave vertebrae before puberty is a sign that severe scoliosis is likely to develop (47). Thoracic scoliosis of more than 60° has a severe negative influence on pulmonary function (48). Multiple microfractures of the vertebral bodies lead to further deformities by damaging the vertebral growth plates. Low back pain resulting from vertebral compression fractures following minimal trauma is often experienced. Other complications, such as spondylolysis and spondylolisthesis are also seen. The most severe neurological complications result from the craniocervical instability caused by laxity at the C1-C2 vertebrae. This can lead to the progressive shifting of the tip of the dens of the C2 vertebrae into the foramen magnum resulting in basilar invagination and compression of the lower part of the medulla oblongata and the cervical part of the spinal medulla. Basilar invagination generally progresses

slowly causing many neurological signs and symptoms, which can be very subtle at first (49-51). Platybasia leads to the stretching of the lower cranial nerves and can cause hearing problems, swallowing difficulties, and ataxia. In most severe cases invaginated dens of C2 can compress the middle brain, resulting in hydrocephalus (51). At birth, undermineralized calvarium, and elongated long bones and ribs are seen radiologically. Angulation deformities resulting from poor healing often lead to further pathological fractures. Recurrent microfractures of the growth plate appear by the age of two years. They form cystic structures in the epiphyseal region of the long bones (popcorn epiphyses) and arrest growth (52). Growth rate is severely reduced from birth to about 6-7 years of age and then stops completely (11). Although severely short stature is a rule in type III of OI patients, the serum insulin-like growth factor (IGF) I is normal (11,53).

The extent to which the life style of the patient is affected correlates with the severity of the physical impairments. Concerns about physical appearance are intensified in puberty, and depression related to the feelings of inadequacy may appear in adulthood (27). Early mortality in OI type III is due to respiratory illness or complications resulting from basilar invagination or injury. Intracranial hemorrhage can result even from minor trauma and can cause rapid death (28,50). Patients surviving beyond 10 years have a better prognosis, because the mortality rate is the highest before that age (54). There are rare autosomal recessive forms. Most cases are presumably dominant new mutations in both type I collagen genes (9,55).

#### *Osteogenesis Imperfecta Type IV*

OI type IV is a dominantly inherited form, which differs from the type I by normal or slightly grayish sclerae, shorter stature, and common presence of DI. The severity of the bone disease is intermediate between OI type I and III. Although usually there is no intrauterine growth retardation, postnatal growth velocity is very reduced (11). There is division into group A and B, according to the presence or absence of DI. Craniofacial size-measurements are moderately affected, generally more compared to type I patients (12). Class III dental malocclusion is present in 70% and acceleration in the tooth development in 23% of OI type IV patients (41). Hearing loss affects only some families. There is marked intrafamilial and interfamilial heterogeneity. A smaller proportion of infants already have bone deformities and fractures at birth. The frequency of fractures is usually highest from childhood to puberty, and then again increases in older age. Hyperplastic callus formation has been reported as a serious complication, but probably refers to OI type V (see below). In infants with normal teeth and with no radiological findings, including wormian bones, child abuse must be ruled out (56).

In OI type IV, new deformities of both the lower and upper extremities develop during the first 10 years (11). Upper and lower extremities are equally malaligned (43). Muscle strength is mainly decreased in the proximal muscles of the upper and lower extremities (25). Retardation in motor development de-

velops after the milestone "sitting without support" is achieved (24). According to a recent study of 18 patients (26), community walking is possible in 26%, whereas 57% are fit for household walking without crutches. Even when ambulation is achieved, it can be lost because of progressive spinal deformation, increasing use of a wheelchair, or loss of interest for physical therapy (26). In one third of the patients, there is progressive kyphoscoliosis, often leading to severe cardiopulmonary and neurological complications. Basilar impression occurs in 71% of patients with OI type IV B, and as many as 50% of patients show signs of compression of structures in the posterior fossa (50). The earliest manifestations of basilar invagination are hypotonia or communicating hydrocephalus, which can develop within the first five years of life (49). Neurological symptoms, such as headaches, coughing, or trigeminal neuralgia, indicate prompt and detailed neurological investigation (57). Life expectancy in this group of OI patients is only minimally impaired (28,53).

#### *Osteogenesis Imperfecta Type V*

It is characterized by moderate to severe bone fragility and osteopenia, and frequent development of hypertrophic calluses following fracture healing or corrective surgery (58). In subjects with positive family history, there is an autosomal dominant pattern of inheritance. All subjects had limitations in the range of pronation and supination in one or both forearms, associated with radiologically apparent calcification of the interosseous membrane. Dislocation of the radial head was seen in three patients (58). A radiodense metaphyseal band immediately adjacent to the growth plate was a constant feature in young subjects. None had blue sclerae or dentinogenesis imperfecta. Histo-morphometric analysis of iliac crest biopsies shows irregular mesh-like organization of the matrix lamellae, and depressed bone formation. By both copy DNA (cDNA) and genomic DNA (gDNA) analyses, no alterations in the structure of the two genes encoding the type I collagen molecule could be found in this group of patients.

#### *Osteogenesis Imperfecta Type VI*

Glorieux and his group (59) recently described another group of 8 patients initially diagnosed with OI type IV, who shared both unique and common characteristics. Fractures were first documented between four and 18 months of age. OI type VI patients sustained more frequent fractures than those with OI type IV. Sclerae were white or faintly blue and dentinogenesis imperfecta was uniformly absent. All patients had vertebral compression fractures. None showed radiological signs of rickets. Lumbar spine bone mineral density (BMD) was low and similar to age-matched patients with OI type IV. Serum alkaline phosphatase levels were slightly elevated compared to age-matched OI type IV patients. Other biochemical parameters of bone and mineral metabolism were within the reference range. Mutation screening of the coding regions and exon/intron boundaries of both collagen type I genes did not reveal any mutations, and type I collagen protein analyses were normal. Qualitative histology of iliac crest bone biopsies

showed an absence of the birefringent pattern of normal lamellar bone under polarized light, often with a "fish-scale" pattern. Quantitative histomorphometry revealed thin cortices, hyperostoidosis, and a prolonged mineralization lag time in the presence of a decreased mineral apposition rate. Thus OI type VI is a moderate to severe form of brittle bone disease, with accumulation of osteoid due to a mineralization defect, in the absence of a disturbance of mineral metabolism. The underlying genetic defect remains to be elucidated.

#### *Rhizomelic Osteogenesis Imperfecta*

This novel form of autosomal recessive OI in 8 individuals living in an isolated Native American community from Northern Quebec have been recently reported (60). The moderate to severe phenotype is characterized by fractures at birth, bluish sclerae, early lower limb deformities, coxa vara, and osteopenia. Rhizomelia (shorter humeri and femora) is a striking feature. Histomorphometric analyses of iliac crest biopsies were not different from those done in OI type I, with decreased cortical width and trabecular number, increased bone turnover and preservation of the lamellar organization of the bone matrix. By linkage analysis, the disease locus is localized on the short arm of chromosome 3, which is outside the loci for the type I collagen genes. No candidate gene has as yet been identified in the chromosomal segment of interest.

#### **Molecular Basis of Osteogenesis Imperfecta**

A comprehensive listing of the mutations within type I collagen genes resulting in OI (61) is now maintained in the OI mutation database ([www.le.ac.uk/genetics/collagen](http://www.le.ac.uk/genetics/collagen)). They can be broadly correlated with clinical severity of the deforming forms of OI being associated with mutations that interrupt the helical stability of the collagen molecule, whereas most forms of OI type I are being associated with underproduction of an otherwise normal type I collagen.

#### *Deforming Osteogenesis Imperfecta*

Essentially, all of these mutations act in a dominant negative manner, ie, it is the presence of the abnormal gene product that causes the disease. The abnormal gene product is a collagen chain that alters the three-dimensional structure of the collagen fibril. Foremost is a glycine substitution in the collagen (gly-x-y) triplet followed by an inframe deletion, an inframe insertion, or by exon skipping. Depending on the helical location of a mutation, these produce a variety of clinical pictures, which range from lethal (OI type II) to severely deforming (OI type III) to mildly deforming (OI type IV). Since the helix assembles from the C-terminal propeptide, a mutation in the C-terminal helical and propeptide region results in greater instability and more severe disease, whereas mutations located in the mid-helical domain tend to be less severe. However, mutations within the mid helical domain can have a severe phenotype suggesting that subdomains within the helix are critical for function beyond just contributing to an intact helical structure. Mutations located at the N-terminal domain

of either chain can be extremely mild and fall into the category of OI type I.

Because the exons that encode the helical domain maintain the reading frame, mutations in the consensus donor or acceptor site can lead to exon skipping, producing a shortened helix that has the same effect on helical stability as a glycine substitution (62). Much less common are mutations that delete a portion of the gene and along with it a number of inframe exons (63) or mutations that insert a segment of intron that remains inframe with the entire transcript. In the latter case, a non-helical segment is inserted within the helical domain disrupting the structure of the collagen helix (64).

The one exception to the statement that severe disease results from a dominant negative mutation in either type I collagen gene is a null mutation of the COL1A2 gene. Formation of the heterotrimeric collagen molecule requires that the  $\alpha 2(I)$  chain accounts for 50% of the available chains at the time the procollagen molecule is assembled. When this requirement is not met, either because of under production of the  $\alpha 2(I)$  chain or over production of the  $\alpha 1(I)$  chain, then homotrimeric molecules are formed. Severity of disease depends on the balance between homotrimeric and heterotrimeric molecules within the bone matrix.

#### *Nondeforming Osteogenesis Imperfecta*

The most common mutation causing OI type I reduces the output of otherwise normal type I collagen. Because of the 2:1 requirement for formation of heterotrimeric collagen, the level of COL1A1 production directly influences the accumulation of normal type I collagen molecules. Reduced output from a single COL1A1 allele reduces the production of heterotrimeric collagen and the unincorporated  $\alpha 2(I)$  chains are degraded intracellularly. Mutations introducing a premature stop codon are the most frequent cause for a null COL1A1 allele (65-69). Premature stop codons arising in all but the terminal exon of a gene usually lead to rapid destruction of the transcript by a cellular mechanism termed nonsense mediated RNA decay (70). This appears to be an important mechanism for preventing a truncated protein from expression, thus saving the cell from proteins with unintended function.

A second mechanism for producing a null COL1A1 allele is retention of an intron within the mature transcript. Intron retention instead of exon skipping can result when a mutation of a splice donor site is located in a small intron such that the combination of the intron and the flanking upstream and downstream exon is regarded as an acceptable exon (71,72). However, the presence of the mutant donor site retains the transcript within the splicing apparatus of the nucleus (S35 domain) and it is eventually destroyed (73).

#### **Murine Models of Osteogenesis Imperfecta**

Murine models are increasingly being developed for understanding pathogenesis of OI and as a platform to investigate new therapeutic strategies. The MOV-13 mice were generated by exposing mouse

embryos to Moloney murine leukemia virus. This resulted in an integration of a proviral copy into the first intron of the  $\alpha 1(I)$  collagen gene, preventing the transcription initiation of the murine COL1A1 gene. When observed as a heterozygous mutation, it results in a reduced amount of collagen in nonmineralized tissues, hearing loss, and fragile bones when tested by biomechanical studies, but with no evidence of spontaneous fractures. Therefore, heterozygous MOV-13 mice do not exhibit any significant phenotypic difference from wild type mice and are clinically similar to OI type I (74). Table 2 is showing available murine models for OI and their clinical phenotype (75,76, 78-81).

**Table 2.** Available murine models for OI (from ref. 75)

Clinical phenotype	Terminology (ref. No.)
Type I	MOV 13 (heterozygous) (74)
Type II	MOV 13 (homozygous) (76)
Type II	Transgenic - COL1A1 mutation (78)
Variable	Transgenic COL1A1 minigene mouse (79)
Type II	Knock-in BrtIII (80)
Type III	OIM mice (81)
Type III/IV	Knock-in BrtIV (80)

The MOV-13 mice homozygous for the null mutation do not produce type I collagen and exhibit an embryonic lethal phenotype at mid-gestation due to defects in development of the vascular organs (76). This MOV-13 homozygous model was used to study the role of type I collagen during embryonic development. These studies showed that the absence of type I collagen had the most pronounced effect on development, starting after 12-14 days. Homozygous MOV-13 mice exhibited two major alterations: necrosis of erythropoietic and mesenchymal cells and rupture of major blood vessels, often causing a collapse of the vascular system (77). Mice with a COL1A1 mutation, resulting in a perinatal lethal phenotype, were generated by Stacey et al (78) by introducing a transgene with a mutation resulting in the substitution of a glycine residue at position 859 of the  $\alpha 1(I)$  chain. It has been described that less than 10% of the expression of mutated COL1A1 was sufficient to decrease type I collagen content by 50%. Transgenic mice expressing a human COL1A1 minigene under the control of a 2.5 kb collagen promoter were generated by Khillan et al (79). The minigene contained the 5' and 3' end of the COL1A1 gene but lacked a central region excluding 41 exons. Transgenic mice harboring this construct exhibited a variable phenotype. Collagen molecules incorporating the shortened pro $\alpha 1(I)$  chain were rapidly eliminated by intracellular degradation. Mice with the lethal phenotype had extensive fractures of the ribs and long bone and expressed much higher levels of minigene compared to transgenic mice without the lethal phenotype (79). Knock-in murine models of OI have been generated using a Cre/lox recombination system (80). Cre recombinase recognizes the loxP sequence and excises the containing sequence that is located between two directly repeating loxP sites. A targeting construct contained a G to T transversion that resulted in a change of the amino acid Gly349 to

Cys in the  $\alpha 1(I)$  chain. Chimeric mice were generated and F1 heterozygous mutant mice exhibited a lethal phenotype. This unpredicted result was due to an alternative splicing into the floxed stop cassette. These mice were named Brittle (BrtIII). Another line of mice was generated in which lox cassette was removed. This step generated heterozygous mutant mice carrying a change of Gly-Cys at the 349 position and avoided the untoward splicing events. So far a large number of animals have been examined. Significant variability of the phenotype ranged from smaller size, deformity of the rib cage, and undermineralization of the skeleton, to disorganized vertebral bodies and osteoporotic bones, to a phenotype that was lethal. Further examination of these mice revealed that levels of mutant collagen mRNA and protein expression were very similar in mice with highly variable clinical phenotypes. Some of this variability can be explained by the genetic background of the mouse and suggests that other pathophysiological modifiers can determine clinical severity of bone disease. Osteogenesis imperfecta murine (OIM) mice is a phenocopy of the naturally occurring mutation causing human type III OI (81). These mice are deficient in pro $\alpha 2(I)$  collagen due to a G deletion at nt 3983 of the COL1A2 gene that causes a frameshift in the last 48 amino acid sequence of the C-terminal propeptide preventing the incorporation of pro $\alpha 2(I)$  chain into the collagen heterotrimer. As a consequence, accumulation of pro $\alpha 1(I)$  homotrimeric molecules in the extracellular matrix occurs. The mice homozygous for the mutation (oim/oim) can be identified at birth by a smaller size, presence of hemorrhages in the joints, visible breakage of the bones, and subluxation of the forepaws. Radiologically, evidence of fresh or healed fractures and generalized osteopenia can be observed. It has been described that collagen in oim/oim mice showed reduced resistance against tensile stress (82). X-ray diffraction studies revealed that the absence of  $\alpha 2(I)$  chain decreased the order of axial packing and caused a loss of crystalline lateral packing of fibrillar collagen molecules (83). The bones from oim/oim mice show reduced mechanical properties due to both lower collagen content and changes in the mineral composition and organization and alignment of mineral crystals (84).

### Pathophysiology of Osteogenesis Imperfecta

Intact bone is able to sense its mechanical environment and initiate a new round of bone formation when damaged or weakened bone is encountered. This fundamental principle of bone biology is continuously called upon in OI because the matrix that is produced is never able to support the load placed on the skeleton. This situation is illustrated in the histology of OI bone, which shows a state of high turnover characterized by increased numbers of osteoblasts and osteocytes (85) and an increased number of osteoclasts. Dynamic labeling shows that increased number of double-labeled surfaces with reduced mineral apposition rate (86). Analysis of murine models is particularly instructive in appreciating the pathophysiology of the OI mutation. Because net total bone

formation in OI bone is low and its intrinsic properties for matrix production in culture are impaired, the OI osteoblast or its lineage is viewed as underproductive. However, the OI osteoblast lineage is under constant stimulation to proliferate to build up sufficient numbers of precursor cells to progress to full osteoblast differentiation and produce the matrix that was resorbed by the activated osteoclasts. The activated osteoblastic lineage can be demonstrated by measuring the content of COL1A1 messenger RNA (mRNA) in OI bone or the activity of a type I collagen promoter transgene, which is sensitive to osteoblastic activity. In both cases, a high level of transcriptional activity for type I collagen can be demonstrated relative to normal bone. The OIM mouse model illustrates that it is the balance between matrix formation and resorption that determines bone strength in OI. In effect, the pathophysiology of OI can be viewed as a consequence of the activated osteoclast lineage, and this probably explains the success of bisphosphonate treatment in OI.

### Molecular Diagnosis of Osteogenesis Imperfecta

It has been estimated that >90% of patients with OI have a mutation in either gene coding for type I procollagen (COL1A1 and COL1A2). Almost 300 different mutations have been reported in the Human Type I Collagen Mutation Database: ([www.le.ac.uk/genetics/collagen/index.html](http://www.le.ac.uk/genetics/collagen/index.html)), testifying to the high allelic heterogeneity of the disease. The high rate of new, private mutations, with virtually no recurrent hot spot, is common also to other collagen disorders (due to defects in types II, III, and V collagens, respectively) and represents a major obstacle in the development of efficient molecular diagnostic protocols.

Whereas the diagnosis is still based on clinical and radiological grounds, there is a growing demand for molecular studies, ie, identification of the causal mutation. In the mildest forms (OI type I according to Sillence's classification) clinical signs may not be so obvious and, as it has happened in our experience, a molecular reason for inexplicable fractures can be conclusive in distinguishing OI from child abuse. In moderately severe forms (type IV/III), the identification of the molecular defect may be useful for genotype-to-phenotype correlations and also for prenatal diagnosis, since affected individuals, who have a 50% risk of transmitting the disease to their offspring, tend to condition their reproductive options to the availability of a prenatal test. Prenatal diagnosis, however, is mostly sought by healthy parents who have had a severely affected child (OI type II or III). A prenatal screening on DNA from chorionic villus sample is reasonably feasible only when the causal mutation in the affected sibling has been characterized previously. Although the vast majority of these cases is due to fresh mutations, recurrence of affected siblings due to germline mosaicism in either parent may not be excluded *a priori*: the risk of recurrence in type II has been empirically estimated between 2% and 7% (87).

### Methodological Approaches

#### *Direct Search for the Causal Mutation*

Traditionally and ideally, the search for type I collagen mutations is supported by biochemical studies (35). The starting material is a skin biopsy from which a fibroblastic line is obtained. Anomalous electrophoretic migration of  $\alpha 1(I)$  and  $\alpha 2(I)$  chains in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels due to posttranslational overmodifications, and, more importantly, cyanogen bromide peptide mapping of abnormal chains, give fundamental hints about the nature (e.g., glycine substitution) and the position of the mutation within the triple helical domain.

Unfortunately, collagen biochemistry is not available in many laboratories and is also rather expensive. As a more approachable alternative, some researchers have developed protocols to amplify all 103 exons and exon boundaries of both COL1A1 and COL1A2 genes by polymerase chain reaction (PCR); in this case the starting material is a peripheral blood sample from which the patient's genomic DNA is extracted. Since a "brute force" sequencing approach would be inconceivable, the PCR products are first scanned for heteroduplexes by conformation-sensitive gel electrophoresis (CSGE) and only positive samples are then sequenced (88). Alternatively to CSGE, denaturing high performance liquid chromatography (DHPLC) might be employed for screening, as it has been done for fibrillin-1 (FBN1) gene mutations causing Marfan syndrome (89). This approach, although very efficient (80% mutations detected), is still very laborious and expensive and it does not seem applicable as a routine method in any laboratory. Most of the molecular studies on OI patients have been done by limiting the screening to cDNA sequences of both COL1A1 and COL1A2 genes. Total RNA is isolated routinely from cultured skin fibroblasts by RNawiz (Ambion Inc., Austin, TX, USA) and treated with RNase-free DNase. Total DNA is extracted as well, by standard procedures.

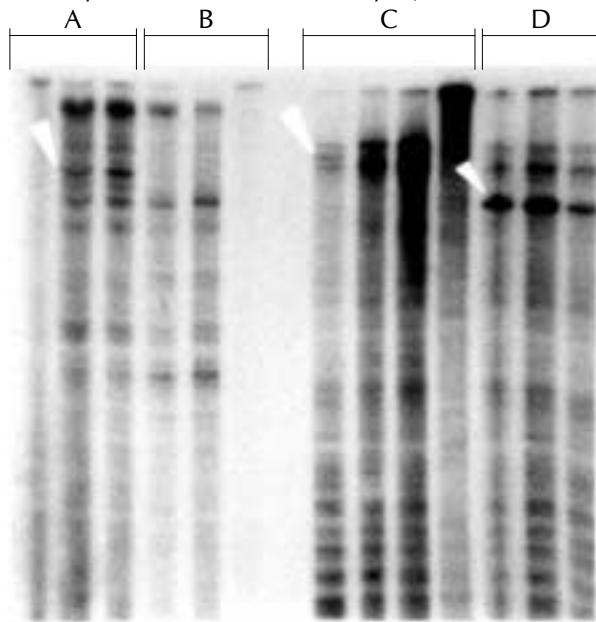
In the course of the years, M. Mottes and her group applied alternatively three different screening methods, each of which presents pros and cons, to localize the mutations which, in all cases, have been sequenced afterwards (below).

*Chemical cleavage of mismatch (CCM)*. This method has been employed successfully in OI by W. G. Cole and collaborators (90) and by M. Mottes and her group (91). Mutant RNA/wild type cDNA hybrids are treated with hydroxylamine and piperidine. We have designed five and four overlapping cDNA segments covering the entire COL1A1 and COL1A2 coding regions, respectively. The method is very efficient in pinpointing nucleotide changes leading to glycine substitutions (therefore involving a G-N base change), which are the most common mutations in OI type II and III. Some disadvantages of the technique, which is not very popular, are the following: the need for hazardous chemicals, the need for radioactive labeling of the cDNA probes, and the cautious and the expert handling of the wt probe, which must be flawless (no nicks) to sustain the chemical cleavage. Three ex-

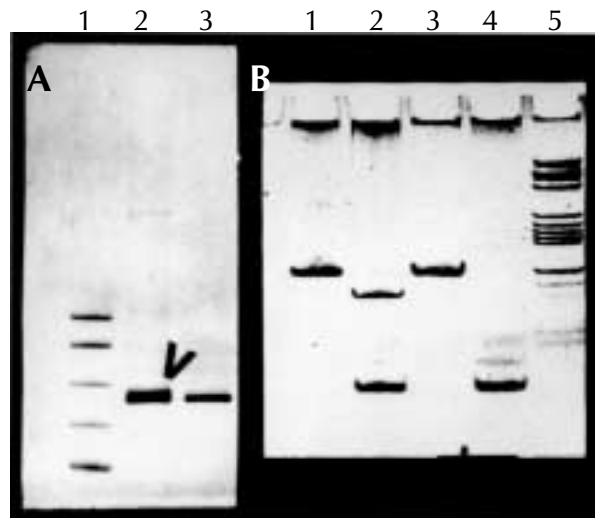
amples of mutation detection by CCM in OI patients are shown in Figure 1.

*Heteroduplex analysis and/or single-strand conformational polymorphism (HA/SSCP).* These two techniques are very popular among laboratories involved in mutation screening. They are simple and rapid to perform and they do not necessarily involve the use of radioactively labeled material. Their efficiency varies greatly. The two techniques appear almost equally efficient when applied to COL1A1 and COL1A2 coding sequences. In our experience, several polymorphisms and unique mutations were characterized by this approach (92,93), but certainly other causal mutations were missed (4 unsuccessful screenings out of 10). In this approach, COL1A1 and COL1A2 coding sequences are amplified by reverse transcriptase (RT)-PCR in a series of overlapping fragments of 300-600 bp each and screened by electrophoresis on appropriate gels (MDE Hydrolink for HA, 6% polyacrylamide plus 5% glycerol for SSCP).

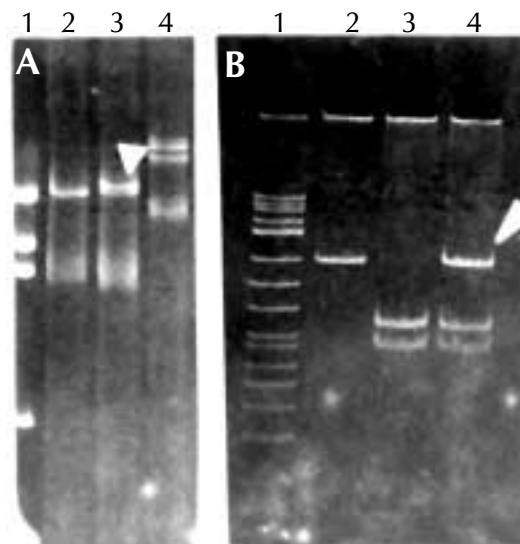
An example of mutation screening by HA is shown in Figure 2. After sequence determination of the positive cDNA fragment, confirmation of the mutation at the genomic level was achieved in the proband by PCR and restriction analysis, since the nucle-



**Figure 1.** Mismatch analysis on mRNA:cDNA heteroduplexes. Total RNA from three probands (lanes A, C, D) and one control (B) was hybridized to either a XhoI-NcoI 1586 bp  $\alpha$ 1(I) cDNA probe (lanes A,B) or a NcoI-NcoI 1382 bp  $\alpha$ 2(I) cDNA probe. Both probes had been 3'-end-labeled with [ $\alpha$ <sup>32</sup>P] dCTP. Heteroduplexes were reacted with hydroxylamine for 0, 30, and 60 min, respectively, and subsequently treated with piperidine. Cleavage products were subjected to electrophoresis on a 5% polyacrylamide 7 mol/L urea gel and autoradiography. Specific bands generated by cell culture model (CCM) are indicated by arrows, other many common bands visible in all lanes were generated by nonspecific degradation of the probes. Subsequent sequencing of the candidate regions revealed the following mutations: A) type II OI, G2084A (COL1A1), Gly478Ser; B) control, no mutation; C) type II OI, G2498T (COL1A2), Gly697Cys; and D) type II OI, G2327T (Col1a2), GLY640Cys.



**Figure 2. Panel A.** Heteroduplex analysis on MDE gel of a 214 bp  $\alpha$ 1(I)cDNA fragment obtained by reverse transcription-polymerase chain reaction (RT-PCR) from total fibroblastic RNA of a type II OI newborn (lane 2, arrow) and of a control (lane 3). Sequencing revealed a G1815A transition in one COL1A1 allele (G566Arg). **Panel B.** Genomic DNA analysis of the G1815A mutation, which abolishes a MspI restriction site. Lane 1, 124 bp PCR product from the proband DNA. Lane 2, MspI digestion pattern. Lane 3, 124 bp PCR product from a chorionic villus sampling (CVS) DNA from the mother's second pregnancy. Lane 4, MspI digestion pattern. Lane 5, MW marker. The higher MW band visible in lane 2, which corresponds to the mutant allele, is not present in lane 4, therefore indicating an healthy foetus.



**Figure 3. Panel A.** Single-strand conformational polymorphism (SSCP) analysis of amplified genomic COL1A1 DNA fragments from OI type I probands. The arrow points to an anomalously migrating band in lane 3. Sequence analysis of the region revealed an heterozygous C4856T transition causing a premature termination codon (TGA) at Arg75. **Panel B.** Genomic restriction assay diagnostic for the C4856T mutation. Lane 1, MW marker. Lane 2, 320 bp polymerase chain reaction (PCR) product from a control DNA. Lane 3, Aval digestion pattern of control. Lane 4, Aval digestion pattern of the proband's DNA. The uncut band (arrow) corresponds to the mutant allele, since the C-T transition disrupts the Aval restriction site.

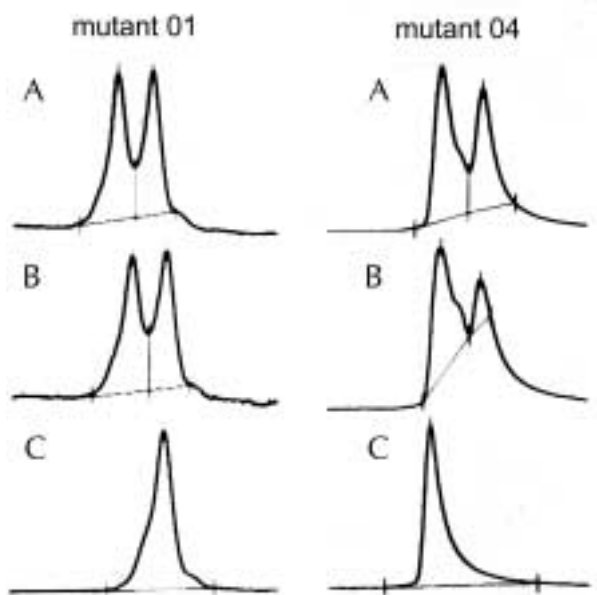


otide substitution affected a MspI recognition site. This simple specific restriction assay could also be applied for parental screening at the genomic level to exclude gonadal mosaicism, and also for prenatal testing on DNA obtained by chorionic villus sampling (CVS) in a subsequent pregnancy in that family (Fig. 2).

An example of mutation detection by SSCP is shown in Figure 3. In this particular case, the analysis was performed on PCR products obtained from genomic DNA, instead of RT-PCR products. The choice of screening the patient at the genomic level was forced by the finding of a "null allele phenotype", ie, there was evidence of the mutant transcript not being present in the cytoplasmic mRNA pool (see below).

*Null Allele Test*

It is well known that the majority of OI type I patients have a quantitative defect in type I collagen, due to various mutations in the COL1A1 gene, which result in a functionally null allele (67,94). The generation of a premature termination codon or of an aberrant splicing site typically leads to unstable nuclear transcripts, which are not found in the cytoplasm. The RT-PCR approach for direct mutation detection described earlier is therefore useless, and mutation screening at the genomic level is the only direct approach possible.



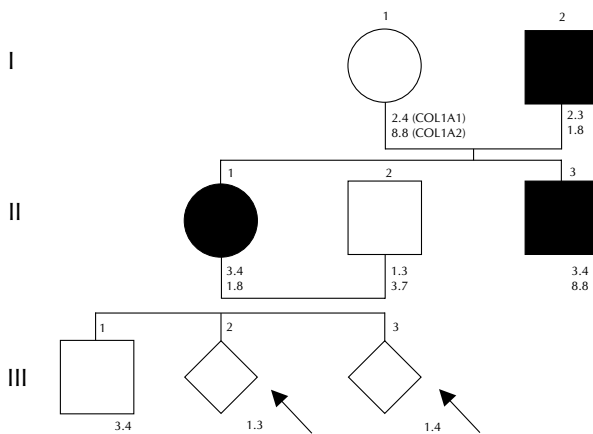
**Figure 4.** Analysis of the 4bp insertion polymorphism in the 3'UTR of COL1A1 in two OI type I patients (null allele phenotypes). Amplification of the region by polymerase chain reaction (PCR) was achieved with 5'fluorescein-labelled oligonucleotides, peaks corresponding to the two expected alleles were separated on a LKB ALF-DNA Sequencer (Pharmacia Biotech, Uppsala, Sweden). A, analysis at the genomic DNA level. B, analysis of reverse transcriptase (RT)-PCR products obtained from purified nuclear RNA extracts. C, analysis of RT-PCR products obtained from cytoplasmic RNA. The presence of only one peak in panel C) suggests loss of heterozygosity, ie, loss of the mutant transcript in both cases. Mutant 01 was further characterized at the molecular level, as shown in Figure 3.

In patients who show the mildest clinical phenotype of OI, a molecular confirmation of the diagnosis nevertheless can be achieved even if the causal mutation remains unknown; cultured fibroblasts are needed as a source of both RNA and DNA. Subjects are first genotyped for two polymorphic markers within the COL1A1 coding sequence: MnlI restriction fragment length polymorphism (RFLP) (95) and the 4 bp insertion polymorphism (96). Once heterozygosity for at least one marker has been proven, a loss of heterozygosity (a proof of null allele phenotype) can be monitored by testing the same polymorphism at the cDNA level (obtained by RT-PCR). The test is simple and rapid (Fig. 4); the only limitation being genotypic informativity (ie, heterozygosity). Where the null allele phenotype was proven in this way, further characterization of the causal mutation was performed (an R75X case is shown in Figure 3).

*Linkage Analysis*

The phenotypically less severe forms of OI (type I and type IV) do not impair fitness considerably, therefore they can be transmitted through generations. Prenatal and/or presymptomatic diagnosis in newborns from an affected parent are frequently requested; linkage analysis with various polymorphic markers at both loci generally allows identification of the allele segregating with the disease in one given family (Fig. 5).

Due to the high allele heterogeneity, locus heterogeneity, and large gene sizes (18 kb for COL1A1, 38 kb for COL2A2), the search for causal mutations in OI is somehow difficult, time-consuming, and expensive. For the above reasons, and also considering that OI is a relatively rare disease (1:10,000 estimated incidence), molecular diagnosis of that illness has been developed in only a few dedicated laboratories in the world.



**Figure 5.** Linkage analysis in a OI type I family. Genotypes for two informative polymorphic markers (MnlI COL1A1, ref. 11, and COL1A1 VNTR, ref. 13) are reported. Discordance of segregation of the disease with COL1A2 is demonstrated by the fact that two affected sibs (II-1, closed circle, and II-3, closed square) received different alleles from their affected father (I-2, closed square). The disease is segregating with allele 3 at COL1A1 in this family; on this assumption two prenatal diagnoses were performed: pregnancy III-2 (1.3, open rhomb) was terminated since the fetus was predicted to be affected having inherited allele 3 from his/her affected mother, pregnancy III-3 (1.4, open rhomb) yielded a healthy newborn male, as predicted by the prenatal molecular testing.

### Usefulness of Bone Markers in Osteogenesis Imperfecta

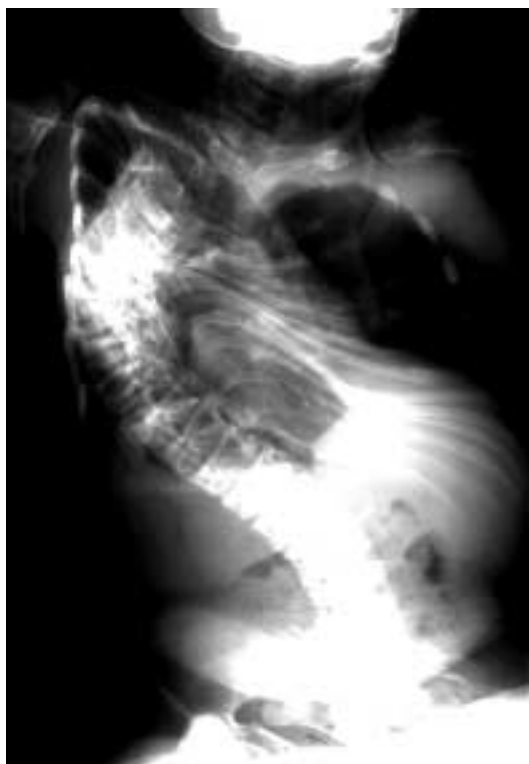
The human skeleton is in the process of constant renewal and minute architectural changes. Interaction of bone cells, ie, osteoblasts and osteoclasts, with other cells of the skeletal tissues result in basic actions: growth, modelling, repair, remodeling, and homeostasis, characteristic of bone tissue. Bone remodeling, which occurs mostly in adults, comprises coupled actions of osteoclasts and osteoblasts. Osteoclasts are derived from the monocyte-macrophage lineage and are capable of resorbing and degrading mineralized bone. Bone-forming cells, osteoblasts, are of mesenchymal origin and synthesize proteins, which make up bone matrix and allow its mineralization. Products of osteoclast or osteoblast cell actions are released into extracellular fluid and can consequently be measured in blood or urine. Measurement of these analytes or bone markers allows indirect insight and assessment of intensity of bone remodeling. Availability of methods for determination of bone markers has provided data on bone turnover in OI patients and their application in evaluation of bisphosphonate treatment. Reduced levels of procollagen were found in OI children (all types), in comparison to controls, confirming the defect in collagen type I biosynthesis. Changes of other bone formation or collagen degradation markers were not statistically significant (97). Similar results were reported by another group of authors (98), with decreased procollagen, both the carboxy- and amino-propeptides. The serum levels were lower in patients with quantitative collagen defects. Bone resorption markers were increased in severely affected adults, with the same underlying OI defect. However, both normal and reduced values were also found. These findings correlated with the *in vitro* results of collagen I SDS-PAGE (98). The two studies are mostly concordant with regard to procollagen levels, and would probably also agree in relationship to collagen degradation products but for a relatively small number of OI subjects in the former study, which thus lacked statistical strength. In OI children, classified as type III, IV, V, or unclassified, both formation and resorption markers (alkaline phosphatase and urinary N-telopeptide, respectively) were increased (99). In one report abnormally low concentrations of procollagen type I (both carboxy- and amino-propeptides) were measured in amniotic fluid during pregnancy and afterwards in serum of the same fetus/infant with mild osteogenesis imperfecta (100). Bone turnover as assessed by bone markers was found reduced in OI children and mildly affected OI adults, whereas bone resorption was elevated in severely affected adults. Treatment with bisphosphonates produced reduction of bone markers and bone turnover, among other beneficial effects (99,101,102). Levels of procollagen were below or within the reference range for premenopausal women in the majority of patients who were younger than 15 years. Children of this age normally undergo several phases of intensive skeletal growth and can expect high levels of procollagen. Decreased procollagen in OI patients probably reflects less collagen

type I formation and perhaps retarded skeletal growth. Effect of bisphosphonate therapy was observed as decrease in procollagen after commencement (OI type I) or as continuous decrease under therapy during one year (OI type III). Bone resorption in some children younger than 10 years was below upper limit of reference range for premenopausal women and above in older OI patients. Similar to procollagen results, much higher levels characteristic of intensive growth and turnover would be expected. It can be assumed that growth impairment is reflected in decreased cross-laps in serum. Optimal choice of a marker for patient follow-up or treatment efficacy assessment, with properties of least biological variability and the greatest clinically significant change could not be recommended so far for partly obvious reasons of limited patient sample and experience in the field of bone markers. A simple transfer of data from other metabolic bone disease may not prove adequate. Usefulness of bone markers diagnosis and decision-making regarding therapy in OI maybe helpful, but still needs further evaluation.

Unfortunately, so far no study has been conducted that could directly relate bone histology to the levels of bone markers. Static and dynamic histomorphometric parameters have rendered evidence of defects in mechanisms of bone growth and modelling, which normally ensure adaptation of the skeleton to the increasing mechanical needs during growth (86). Low bone turnover and reduced bone volumes were found in 8 patients with OI type IA (103). Histological evidence of low bone turnover and bone formation impairment would thus not entirely agree with the diversified results on bone markers, which could be normal, reduced, or increased. This is not further elucidated by positive correlations of procollagen and densitometry of the lumbar spine and os calcis (97). It can be expected that clinical usefulness of bone markers in OI patients will be validated after more experience is gathered in bisphosphonate treatment and biochemical assessment. In analogy with other metabolic bone disease, some benefit can be expected of a choice of specific bone marker and its degree of change in monitoring can be expected.

### Orthopedic Management of Osteogenesis Imperfecta

The most important goal in the orthopedic therapy of OI should be directed toward reducing deformity and promoting normal function. The typical clinical feature seen in OI patients include involvement of bones and joints, ie, fragility of the bone, osteopenia, ligamentous laxity, short stature, and difficulties in patient's ambulation. All features can present themselves in variable patterns depending on age of the patient and the severity of the disease, which is the result of genetic heterogeneity and variable expressivity. Multiple clinical problems of the locomotor system could be encountered in individual patients with OI. These problems are accordingly divided to specific anatomical regions: 1) the spine, 2) the long bones of upper extremities, 3) the long bones of lower extremities, and 4) growth plate. The pur-



**Figure 6.** Antero-posterior X ray of the spine of the 14-year-old girl with osteogenesis imperfecta type III. Serious scoliotic deformation compromises pulmonary function.

pose of this section is to review the numerous skeletal problems in patients with OI and to outline current principles of orthopedic management.

### Spine

Spinal problems in patients with OI, e.g., scoliosis and kyphosis, basilar impression, spinal fractures, and spondylolisthesis are described in orthopedic literature (104-110).

The high prevalence of scoliosis in OI population was described as early as 1975. Further, it is known that the incidence of scoliosis is increasing with the age of the patient. Those patients with chest deformities and those who were non-ambulatory had a predilection for development of scoliosis (111) (Fig. 6). Children with dentinogenesis imperfecta (DI) had also predilection for scoliosis. Overall, in children with OI incidence and severity of scoliosis was increasing with severity and the type of the disease and age (112,113). A review of the literature has revealed various percentages of scoliosis (20-80%) in children with OI (47,49,106,111,114). The most recent cross-section study on the prevalence of scoliosis in the OI population was published by Karbowski et al (115). They found scoliosis in 75% of patients out of 102 patients aged from 3 to 71 years. In 20 patients the severity of scoliosis was higher than 40°, and 56 patients had scoliosis of less than 40°. Non-scoliotic were 26 patients with OI. Pathogenesis of scoliosis in patients with OI is not precisely known. Most likely, primary triggering factors are vertebral microfractures caused by bone fragility, as well as injury to vertebral

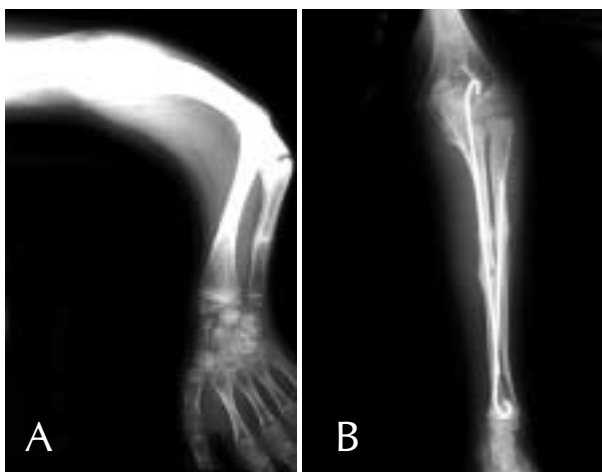
growth plates. Secondary factors, such as ligamentous laxity, limb length inequality, pelvic obliquity, and inter-vertebral disc abnormalities, may contribute to continuous progression of the scoliotic curve. This thought is supported by several investigators (47,106,116). However, other authors did not consider generalized ligamentous laxity as contributing factor in pathogenesis of spine deformities in patients with OI (49). Vertebral body shape was identified by Ishikawa et al (47) as a predictor of spinal deformity in OI. On lateral roentgenograms of the spine, four types of vertebral body deformities were identified: biconcave, flattened, wedged, and unclassifiable. On the basis of the study of 44 patients with OI, authors concluded that, in the presence of six biconcave vertebrae or more before puberty, severe scoliosis (>50°) was likely to develop (47). Thoracic and lumbar vertebrae usually have "codfish" appearance. Repeated compression micro-fractures produced "codfish vertebrae", which have osteoporosis, reduced height, biconcave shape, and adjacent biconvex discs (117). As a rule, the natural history of scoliosis in patients with OI is a curve progression. Scoliosis progression was studied in detail by Hanscom et al (3) and Hanscom and Bloom (118), who were using radiographic criteria to identify six grades (A-F) of the disease. Patients with type A disease have a mild form of OI and their scoliosis could be prevented from progression by arthrodesis of the spine. On the other side of the OI spectrum is the patient with grade F disease, who has a severe form of OI that is incompatible with survival. Patients with type B, C, D, and E grade of disease almost always had progression of scoliosis, and results of the spine arthrodesis were variable. Therapy of scoliosis is either nonoperative or operative. The therapy of scoliosis in patients with OI is particularly difficult because patient's short stature and deformity of thoracic cage, ribs, and vertebrae. Bony anatomical structures are fragile and soft to adequately transmit corrective forces of conventional orthosis to the spine or to accept forces of currently available instrumentation. Nonoperative treatment of scoliosis in OI has included observation and brace treatment. Monitoring of the spine status in all children with OI is mandatory because progression of spine deformity is very likely to occur. Orthotic treatment is almost always ineffective (111,114). The soft cast brace could be indicated in relatively limited group of patients with OI and scoliosis who were suffering of spinal pain or sitting discomfort; had Cobb angle less than 30° and are below 9 years of age (113,119). In patients with thoracic scoliosis of over 60°, significantly decreased vital capacity is regularly present, as well as restrictive pulmonary disease (120). Surgery is indicated when spinal deformity is progressive and scoliosis is causing pain. The principal goals of surgery are to maximize function and to minimize deformity and disability. Surgical options available for patients with OI are far from ideal. A conventional method of scoliosis correction with a Harrington rod is not very helpful due to fragile vertebrae where hooks should be placed. Therefore, methylmethacrylate was used as adjunct for better and safer hook purchase (121). For some surgeons, Luque type of segmental instrumentation is

an option to obtain sufficient stability but special caution should be made while tightening wires to pedicles (122). Preliminary results are given in five patients using method of fusion *in situ* with Kiel bone graft (123). Regardless of the technique of spine fusion, no significant amount of correction seems to be possible. The percentage of correction is in the range of 17% on average to 36% of scoliosis curve correction (104,122). If progression of the scoliotic curve has reached 35-40° of Cobb angle, there is an indication for early spine fusion regardless of the age of the patient, provided that no medical contraindication exists (106,124). Maybe surgeons should aim at stabilizing the scoliotic curve rather than correcting it (123,125,126). Complications of scoliosis surgery in patients with OI are common and should be anticipated especially in patients with associated kyphotic deformity, where instability of the metal implanted is very likely to occur (106,127). In a series of 60 patients surgically treated for scoliosis, Yong-Hing and MacEwan (104) found 33 complications in 20 patients. The most common complications were the following: Harrington hooks cut out during or after operation (10 patients), excessive blood loss (>2.5 L) (9 patients), and pseudarthrosis (5 patients). Those children with OI and progressive scoliosis who were treated with biphosphonates could have better bone stock and less fragile vertebrae and would have a chance for less complications after spine surgery and instrumentation failures.

#### *Basilar Impression*

Basilar impression is slowly progressive and has potentially serious complication in patients with OI, although many patients may be asymptomatic (108, 127). Basilar impression denotes elevation of the floor of the posterior cranial fossa, including medial migration of occipital condyles and infolding of the margins of the foramen magnum (106,128). Basilar invagination denotes cephalad migration of upper cervical vertebrae into this cranial depression. Before 1993, the frequency of basilar impression with neurologic complication has traditionally been thought to be rare in patients with OI. In English medical literature no more than 20 cases had been published until 1993. In a large clinic screening study of 87 patients with OI, Sillence (128,129) reported over-all frequency of basilar impression in 25% of patients. He found that basilar impression occurred in the highest frequency, 71% (10 out of 14 patients), in OI type IV B, and that 50% of this group of patients had neurologic signs of compression of posterior fossa structures (128). In a recent study, basilar impression was found in 17% of 47 screened patients, or in eight patients but seven of them were type III (49). Sawin and Menezes (108) reported basilar impression in 18 OI patients with following distribution: 10 patients with type III; 6 patients with type IV, and 2 patients with type I of the disease. In conclusion, basilar impression is more frequent than it was thought; all patients with OI should be screened for basilar impression with emphasis on patients with type III and IV and those with the dentinogenesis imperfecta subtype (128).

Pozo et al (130) suggested that repeated microfractures in the region of the foramen magnum caused gradual infolding of its rim. Sillence (129) indicated three possible pathogenetic mechanisms of basilar impression in certain types of OI: 1) difference in the nature of molecular defects; 2) greater osseous plasticity in OI type IV; and 3) earlier upright posture/sitting of patients with OI type IV as against OI type III. In series of Sawin and Menezes (108), patient's mean age at presentation was 12 years (range, 1-20 years), with 56% of patients (14 out of 25) presenting between the ages of 11 and 15 years. This finding suggests that adolescence is a critical period in the worsening of basilar impression. However, Sawin and Menezes (108) consider true pathogenesis of basilar impression in OI patients to be obscure. Clinical picture in patients with basilar impression is usually developing slowly but progressively. Radiological features may be present for several years before progression of neurologic signs. Sillence et al (129) found that basilar impression was radiologically present long before puberty with the youngest subject being two years of age. Furthermore, neurologic signs may be present before symptoms and those signs are the following: 1) nystagmus, 2) facial spasms, 3) nerve palsy, 4) pyramidal tract signs, 5) proprioceptive deficits, and 6) papilledema in case of hydrocephalus. On the contrary, neurologic symptoms may develop later, including 1) headache (neck and occiput), which is worse on movement, cough, sneezing, or straining; 2) trigeminal neuralgia; 3) imbalance; 4) weakness in arms and legs; and 5) bladder disorders (128). The catastrophic consequences of basilar impression include brain stem compression, tetraplegia, respiratory arrest, and sudden death (131). The diagnosis of basilar impression is a radiographic one (106). Following neurological evaluation, the next step is a plain lateral cervical spine and cranial radiograph, which will show translocation of the upper cervical vertebral column into the posterior fossa. In doubtful cases lateral craniometry is helpful, such as detection of McRea's, Chamberlain's, and McGregor's lines (132). The most useful is the McGregor's line, which is drawn from the upper surface of the posterior edge of the hard palate to the lowest point of the occipital curve of the skull. The measurement is considered pathological when the tip of the dens projects more than 7 millimeters above McGregor's line (128). In patients with basilar impression and OI who have characteristic shape of the skull, two patterns can be seen. One is a "Tam O'Shanter" appearance of the skull with overhang of temporal and occipital bones resembling the floppy beret worn in Scotland; and the other is a "Darth Vader" appearance of the skull, with flattening in interparietal area resembling shape of helmet worn by Darth Vader, a *Star Wars* movie character (133,106). In patients with short neck and severely deformed skull computed tomographic (CT) reconstruction and magnetic resonance imaging (MRI) could show more valuable details when surgical options are considered. MRI is not recommended for basilar impression screening purposes in patients with OI (128). Patients with neurologic deficit and symptoms and basilar impression, docu-



**Figure 7.** **A.** Pre-surgical X-ray of the left forearm of the 9-year-old girl with osteogenesis imperfecta type III. **B.** Postsurgical antero-posterior X-ray of the left forearm of the 11-year-old girl with osteogenesis imperfecta type III, after intramedullary fixation with 2 non-elongating rods.

mented with appropriate imaging studies, should be surgically treated by transoral occipital neural decompression, followed by dorsal occipitocervical fusion with contoured loop instrumentation (133,108). According to Sawin and Menezes (108), in 80% of patients, despite solid bone fusion, basilar impression progressed and prolonged cervical bracing was needed to prevent further progression, particularly in the period of adolescence. The primary goal of surgery for basilar impression in patients with OI is to preserve neurological function as long as possible (108). The natural history of basilar impression in each type of OI, as far as neurologic complications are concerned, has to be established. Until then, neurologic and radiographic screening for all patients with OI is recommended and follow-up for all positive cases as well as type IB, type III, and type IV patients. Follow-up should be repeated every 2 to 3 years until skeletal maturity (128,134).

#### *Upper Limb Surgery in Patients with Osteogenesis Imperfecta*

Upper limb surgery is rarely performed but when the hand function is severely impaired due to forearm deformity, consideration to multiple osteotomies, alignment, and intramedullary rodding should be given (Fig. 7) (135). Upper limb deformities are more often seen in the patients with severe forms of OI. Bilateral isolated olecranon fractures before age of one year is very rare and when such problem arises, one should consider other pathologies than simple trauma, e.g., osteogenesis imperfecta (136). Displaced fractures of the olecranon after trivial trauma is highly suspicious for OI. Seven cases of such olecranon fracture were reported and surgical fixation with absorbable pins and sutures was recommended. Eight tension band wiring should be used (137). Aneurysmal bone cysts of the radius in a patient with OI three years after fracture have been described (138). The functional outcome after Sofield proce-

dures in the upper limb was investigated in five patients, with 2-18 years of follow-up (139). There were 14 upper limb Sofield procedures and all five patients showed improved upper limb function.

#### *Lower Limb Surgery in Patients with Osteogenesis Imperfecta*

The goal of orthopedic treatment in lower extremities is to prevent fractures and deformities, correct existing deformities, and enhance the patient's ambulation and overall function (Fig. 8). Use of intramedullary rods with multiple osteotomies was first described more than 40 years ago and has gained wide acceptance in treating children who have OI. Problems with non-elongating rods are that when a child outgrows the rod, fracture or angulation is likely to occur in the region of non-protected bone. Elongating rods (Bailey-Dubow, ie, B-D rod and Sofield type rod) have established their role in improving patient's ability to walk (140). Despite general acceptance of intramedullary elongating nails in the treatment of children with OI, questions will arise on the matter of timing of surgery and the choice of an appropriate type of nail for a certain bone. Porat and co-workers (141) evaluated functional results of surgery with 32 elongating B-D rods and 24 non-elongating rods. They found that the rate of complication was 72% for B-D rods and 50% for non-elongating rods. Authors concluded that the type of nail used remains the surgeon's personal choice. Another study evaluating complications after 108 procedures of intramedullary rodding (42 B-D rods and 66 non-elongating rods) has shown that the overall complication rate was 60-69% for B-D rods and 55% for non-elongating rods. Forty-seven percent of bones were reoperated on with a higher rate for non-elongating rods, ie, 29% vs 19% for B-D rods (142).

No growth disturbance resulted from the use of the elongating rod system (143). Wide experience has



**Figure 8.** **A.** Pre-surgical lateral X-ray of the lower leg of the 8-year-old girl with osteogenesis imperfecta type III. **B.** Postsurgical antero-posterior X-ray of the lower leg after intramedullary fixation with a non-elongating rod.

been gained with B-D elongating nails and recently several groups of authors reported on complications of its use (144-147). Zions and co-workers (144) studied 40 B-D intramedullary nailing procedures in 15 children. There were 23 procedures on the femur and 17 on the tibia. They concluded that risk factors for complications in using B-D rods are children younger than five years and performing surgery on the tibia. Jerosch and colleagues (145) studied procedures with B-D rods on 107 long bones in 29 patients. The authors concluded that this procedure is the most successful way to stabilize the growing long bones. However, there is the high rate of complications especially in the tibia. Janus and co-workers (146) found that there was no influence of location of the nail (femur or tibia) on the complication rate. The rate of the reoperation was 29% in 110 B-D nails inserted into the femur or tibia of 34 children. Karbowski and co-workers (147) studied 63 patients with OI who had 186 primary intramedullary fixation with B-D rods. Complication rate for tibia rodding was 52% and for femur 21%. Thus, the authors concluded that the use of B-D rods in childhood could be recommended for the femur.

There is constant effort to improve surgical technique of intramedullary surgery in patients with OI. Li and co-workers (148) emphasize principles of minimal surgical trauma to avoid devascularization of the bone with rod placing under the control of an image intensifier. Mulpuri and Joseph (149) analyzed application of the Sofield intramedullary rod in 16 children with 66 lower limb segments (42 elongating and 24 non-elongating rods). They found that Sofield intramedullary rod was significantly better with regard to frequency of complications requiring reoperations and longevity of the rods. In conclusion, this review of various rodding systems for treating long bones deformities and fractures in children with OI has shown that, during the past 40 years, there were steady efforts to optimize hardware design and surgical technique for improving patients' chances of functional ability and integration into the society.

Other problems associated with orthopedic care of OI are the following: 1) differential diagnosis, 2) specific OI problems in very young and adult patients, 3) patient's walking prognosis, and 4) orthotic management and physiotherapy.

#### *Differential Diagnosis*

From the orthopedic standpoint, differential diagnosis should include non-accidental injury, such as child abuse; temporary brittle bone disease; idiopathic juvenile osteoporosis; hypophosphatasia; and rare leukemia (113,150-154). Occasionally, one could have difficulties differentiating OI from child abuse. Although OI is much less common than child abuse, one should keep in mind that children with OI are not immune to non-accidental injury. Some skeletal fractures in children younger than three years of age are highly associated with non-accidental injury, such as metaphyseal corner fractures, multiple fractures at different stages of healing, posterior rib fractures, fractures of scapulae, vertebrae, outer ends of the clavicles, spiral fractures of femur and humerus,

and bilateral fractures (113,154). Diagnosis of OI should not be ruled out on the basis of skeletal radiographs alone. It is well known that OI can occur in a patient who presented him/herself with radiological features of a metaphyseal corner fracture and who has not have typical OI findings, e.g., positive family history, blue sclerae, osteopenia, and Wormian bones (152). When diagnosis of OI or non-accidental injury is not clear, medico-legal implications could be serious (151). The family and medical history, careful physical examination, radiographic features of fractures, and biochemical analysis of skin collagen are four cornerstones of correct diagnosis (131,154). Expert laboratories using modern techniques could detect abnormalities in dermal type I procollagen in up to 87% of patients with OI (150). The existence of a temporary brittle bone disease (TBBD) is suggested by Paterson et al (152). According to Paterson, TBBD is a distinct disorder because multiple fractures, especially in the ribs, occur without evidence of trauma in first 12 months of life (usually in first six months); infants are usually born pre-term and in twins. Child abuse can be excluded with confidence in those patients because when children are returned to their parents, no subsequent evidence of fractures has been found (155). Joint laxity was frequent in the families of patients with TBBD and there are no fractures later in patient's life. Although Miller and Hangartner (153) could demonstrate association of decreased fetal movement and osteopenia in patients with TBBD, its existence is more a matter of clinical opinion than high science.

#### *Specific Musculoskeletal Problems in Adolescent and Adult Patients with Osteogenesis Imperfecta*

If existing orthopedic literature is reviewed regarding OI problems where skeletally immature adolescents are compared to adults, one will be surprised to realize that adults with OI are on the fringe of orthopedic surgeons' interest. A multidisciplinary approach to the management of adults is lacking, and relevant literature is scant. There are several specific musculoskeletal problems in adolescent and adult patients with OI that should be described briefly: transient osteoporosis, protrusion of acetabulum, lower limb deformities and osteoarthritis of hip and knee joints, hyperplastic callus, and differential diagnosis to osteosarcoma.

#### *Transient Osteoporosis*

The occurrence of transient osteoporosis in patients with OI is rare. There were approximately 20 cases published since its first description in 1968 (156). However, in adult patients with OI who presents with hip or groin pain and limp, a diagnosis of transient osteoporosis should be considered. Radiographs and bone scan should be used as first steps towards correct diagnosis. Further investigations include MRI, dual energy X-ray absorptiometry (DEXA), and CT of proximal femur (156,157). Avascular necrosis and malignancy should be considered in differential diagnosis but MRI could help to differentiate those conditions. Stress fracture was considered a triggering factor in etiology of transient osteoporosis

(157). Other authors considered etiology of transient osteoporosis uncertain and believed that microfractures might play a role in the early pathophysiologic process (156). Regional osteoporosis and occasional stress fracture of the femoral neck could be revealed on radiographs or on CT images. Transient osteoporosis could have migratory pattern and could happen in both hip joints sequentially (157) or in hip joint followed by ankle joint (156). A treatment regimen is non-weightbearing with or without physiotherapy or analgesics, and after 6-8 months, transient osteoporosis resolves spontaneously (156,157).

#### *Protrusion of the Acetabulum*

Prevalence of protrusion of the acetabulum in patients with OI is approximately 30%. In a series of 32 patients with OI type III and type IV, protrusion of the acetabulum was found in 29% and 27% of patients, respectively. Out of 28 patients with OI type I, only 7% of protrusions of the acetabuli was found (117). Pelvic deformity, such as severe bilateral protrusion of acetabulum in adolescents with OI, could cause distal obstruction of the colon due to the narrowed pelvis impinging on the sacrum. Successful treatment by bilateral triple pelvic osteotomies in a 14-year-old patient was reported (158). In a series of 43 patients who had OI type III, 12 patients (aged 2-44 years) with recurrent episodes of abdominal pain were found. Chronic constipation and abdominal pain were more frequent in patients with OI who had protrusion of acetabulum. Such patients should be referred to a gastrointestinal specialist for an early bowel program to prevent potential problems (43).

#### *Lower Limb Deformities*

Moorefield and Miller (159) studied the effects of OI and its treatment on adult life in 31 patients at an average of 19 years after last surgical procedure. Ninety-one percent of a total of 951 fractures had occurred before skeletal maturity, with 83% of fractures in lower extremity, mainly in the femur. Twenty-eight patients had a total of 174 operations. Slightly more than two thirds of the operations were multiple osteotomies with intramedullary rodding. In 17 patients, improvement gained at operation was preserved at follow-up and those patients were ambulatory. Approximately 50% of the patients (fourteen) had scoliosis averaging 31°. Additionally, it was found that these patients were generally very productive and socially adaptable individuals. Residual deformity of long bones of the lower extremity, ligamentous laxity, and disease by itself are factors leading to osteoarthritis of hip and knee joints in adults with OI. Five total hip and three total knee replacements were performed in six adult OI patients. Successful mid-term results with follow-up of 7 years for hip arthroplasty and 10 years for knee joint arthroplasty were published (160). Twelve non-unions of fractures in 10 out of 52 patients who had OI were identified. Nine patients were with type III of the disease. There were 6 non-unions in upper extremity, 5 in femur, and one in pubis (161). Deformity and functional disability of involved upper or lower extremity were the principal indication for surgical intervention. Solid union was obtained in 8 of 9 ununited frac-

tures treated by the excision of non-union, intramedullary nailing, and bone grafting. Non-unions were more frequent than one may have thought in patients with severe type of OI (161). The Ilizarov method of correction and lengthening of lower limb deformity was preformed in 6 adult patients who had OI. In 4 patients, functional status was improved, but there were 18 complications in 6 patients. Thus, the Ilizarov method should be contemplated in those patients who have OI for whom there is no other alternative, who have severe angular and shortening deformity, and who are free from frequent fractures (162).

#### *Hyperplastic Callus Formation and Osteosarcoma*

One can encounter very serious diagnostic difficulties in an effort to differentiate osteosarcoma, which is rarely associated with OI, and hyperplastic callus formation, which is also unusual but benign complication of OI (163,164). It seems that the debate on hyperplastic callus and on pro-biopsy and no-biopsy issues are still ongoing (165-167). Hyperplastic callus formation is one of the main features in the newly proposed type V of OI and, being so, will rise many new questions. The other side of the problem is hyperplastic callus formation in patients with OI that resembles osteosarcoma, and orthopedic surgeon may be forced to biopsy the lesion (166). Some authors recommend MRI and CT studies of hyperplastic callus formation for better imaging of multiphasic nature of lesion and identification of fracture line (167,168). Careful synthesis and interpretation of clinical, laboratory, and imaging data are essential for correct diagnosis (168).

#### *Calcium Intake and Follow-up*

It has been suggested that patients with OI during puberty had increased demand for calcium that is needed for the growth spurt (169). Dietary calcium supply may not be sufficient, and this demand could be met by "borrowing" calcium from the cortical shell. Thus, disuse osteoporosis in pubertal OI patient during fracture treatment accompanied with "borrowing" of calcium will contribute to aggravation of skeletal deformities (169). In general, osteoporosis, joint hypermobility, and increase of fracture frequency in post-menopausal women or in men in the fifth to sixth decade should be addressed adequately (170). Some authors recommend regular monitoring with bone densitometry at 3-5 year interval between the age of 20 and 35-40 years. Under circumstances when life events are contributing to osteoporosis, in women after 35 and in men after 40 years of age, bone densitometry may be performed annually (170). An increased fracture rate after menopause in women with OI is the result of double effect, ie, age-related bone loss coupled with defective collagen structure of disease (171). Hormone replacement therapy is indicated in this group of patients from the time of the menopause (171). The treatment of osteoporosis in adults with OI using biphosphonate is discussed elsewhere in this article.

### Specific Musculoskeletal Problems in a Very Young Child with Osteogenesis Imperfecta

The fractures and bowing of long bones in the first years of life in children who have OI are difficult problems to solve. The usual procedure of fragmentation and intramedullary rodding with extensible nail in very young child is not easy to perform. Middleton (172) first reported closed intramedullary rodding combined with osteoclasia of long bones in three patients in 1984. The minimal exposure of the bone was used with osteoclasia instead of osteotomy in 14 children with severe types of the disease. Twelve patients had fractures either before or at birth. There were 55 primary operative procedures, or 38 procedures of the lower extremity and 17 of the upper extremity. Reoperations for nail-changing were necessary on 19 occasions. At most recent follow-up, of 3 years and one month at average, five patients were walking independently and four were walking with aid (173).

Percutaneous nailing after manual osteoclasia of deformed long bone was done in a 7-year-old girl with severe form of OI by Sijbrandij (174). Cole (175) reported the early surgical treatment of three patients with severe form of OI, aged between 18 months and 5 years. The replacing of non-extensible rods was needed within 2 to 3 years. McHale et al (176) published the study of percutaneous intramedullary fixation of long bones in seven patients with severe form of OI including a 2-11 year follow-up. The goals of treatment of these young children were to improve parental handling, prevent fractures, provide mobility, and increase function. This procedure appears to be useful in the tibiae in children younger than 2 years of age (176). Intramedullary rodding in ten patients with OI type III and the impact of surgery on neuromotor development was studied by Engelbert et al (177). They recommend that intramedullary rodding should be performed between two and three and half years of age. Rodding does not always improve neuromotor development.

### Biphosphonate Therapy

Until recently, treatment has focused on fracture management and surgical correction of deformities and supportive rehabilitation programs. All medical therapies other than those directed at symptomatic pain relief have been ineffective in altering the course of the disease. They include fluoride, magnesium oxide, calcitonin, and anabolic steroids (178).

Lately, cyclical intravenous treatment with pamidronate (3-amino-1-hydroxypropylidene-bisphosphonate) has proven to be of benefit to children with severe forms of OI (179). Bone mineral density (BMD) and physical activity increased markedly in these patients and fracture rate decreased. Pamidronate is a second-generation bisphosphonate with a chemical structure analogous to pyrophosphate, the one naturally occurring inhibitor of bone resorption (180). Bisphosphonates are potent inhibitors of bone resorption (181). These compounds are widely used for the treatment of adults suffering from bone loss and increased bone fragility, and there is increasing experi-

ence on the effect of these drugs in children. To our knowledge, there are no published studies of adult patients with OI treated with bisphosphonates.

The Glorieux's group currently treats with pamidronate more than 180 children with severe OI, and some of them have been receiving treatment for seven years. Analysis of the results of the first 30 children who have been receiving the treatment (1.5-3 mg/kg intravenously at 4-6 month intervals) for 1-4 years (105) showed that the bone mineral density increased by  $41.9 \pm 2.9\%$  annually, and the deviation from normal, as indicated by Z-score, improved from  $-5.3 \pm 1.2$  to  $-3.4 \pm 1.5$ . Metacarpal cortical width increased during treatment, and lateral spine radiographs suggested new bone formation in 25 children. The incidence of radiologically confirmed fractures fell by 1.7 fractures per year. Treatment did not alter fracture healing, growth rate, or growth plate appearances. Dependence on mobility aids was reduced in 16 children, and remained unchanged in the other 14. All subjects reported substantial relief of chronic pain and fatigue.

A particular group of patients is that of children under three years of age. Pamidronate treatment was given for 12 months to 9 patients severely affected with OI (type III and IV, age: 2.6 to 20.7 months at entry) (182). The drug was administered intravenously in cycles of three consecutive days. The patients received an average dose of 12.4 mg/kg/year. This group was compared to a historical control group consisting of six age-matched severely affected OI patients who had not received any medical treatment, but had followed the same multidisciplinary supportive program. Under cyclical pamidronate treatment, bone mineral density (BMD) increased between 65% and 227% in one year. The Z-score increased significantly, whereas in the control group no change in the BMD Z-score was observed. The vertebral coronal area increased in all treated patients and remained unchanged in the untreated group. In the treated patients, the fracture rate was also significantly lower than in the control group (2.6 vs 6.3 fractures/year). No adverse side effects were noted apart from the well-known acute phase reaction during the first infusion cycle. In the group of patients of less than two years of age, the response to treatment appeared to be faster and more pronounced than what had been observed in older children. Signs of bone pain (e.g., crying while being handled) disappeared within days and an increase in BMD was evident as early as six weeks after the start of treatment. Without exception, the gain in BMD was greater than the increase expected in healthy children. In contrast, no changes in BMD occurred in the untreated controls. This strongly suggests that the observed changes reflect a drug effect rather than the passing of time or the natural evolution of the disease. The vertebral size increased in all treated children, as should be expected in growing individuals. This rebuilding of previously crushed vertebrae was evident as early as four months after initiation of therapy. In contrast, a decrease in vertebral size was noted in half of the untreated children, indicating that vertebral collapse had occurred



in those patients. Thus, it appears that pamidronate infusions not only increased lumbar spine BMD, but also protected bone integrity. Concomitant with these radiological changes, fracture rate decreased significantly. Fracture incidence is a weak efficacy parameter in open therapeutic studies of OI patients, as it can be influenced by external factors (e.g., mode of handling, and mobility) and may also spontaneously decrease with age. Yet, despite higher risk of injury due to increased mobility, a marked decrease in fracture rate was noted, suggesting a direct effect of the therapy. The disappearance of bone pain and decreased fracture incidence may have contributed to greater mobility. Physical activity is an essential factor for the development of the skeletal system. Thus, increased mobility may synergize with the direct inhibitory effect of pamidronate on bone resorption to increase bone mass. The effect of bisphosphonate therapy on growth has been a matter of concern before the treatment was used in children. In animal studies, long-term treatment with bisphosphonates did not affect linear growth, unless very high doses were administered. In the group of OI patients, pamidronate did not have a detrimental effect on growth. Instead, the height Z-score increased in all the patients that have started treatment under three years of age. Although the magnitude of response and duration of treatment are still under evaluation, it already appears that a similar therapeutic approach may be considered for other osteopenic disorders in children.

### Gene Therapy of Osteogenesis Imperfecta

Since bisphosphonates cannot correct the primary cause of OI, and their long-term use and effectiveness are still uncertain, the possibilities to correct the underlying genetic mutation are being evaluated in both humans and mice. The possibility that gene therapy is a feasible strategy in OI treatment came from the analysis of individuals who are somatic mosaics for an OI mutation but do not have evidence of bone disease. Those studies suggest that the deleterious effect of OI cells can be neutralized by the presence of normal cells. Thus, if it were possible to introduce normal cells into an individual with OI, the severity of bone disease would be reduced. Furthermore, bone turnover in OI is high and endogenous osteoblast lineage is activated. The introduction of normal cells into such environment would rapidly populate the bone with cells having a normal proliferative rate and making a normal matrix, which would outproduce the resident OI cells. To achieve this goal, we need to develop a strategy by which the endogenous OI bone cells can be engineered *in vitro* to correct the primary defect in type I collagen production and then reintroduced into the affected host. This process involves two steps: the inhibition of the output from the mutant collagen allele and an insertion of replacement collagen gene for the inactivated mutant gene. Once corrected, the engineered cells should be able to engraft bone, proliferate, and participate in new bone formation. So far, none of these strategies have been developed completely.

Allele-specific suppression of a mutant collagen gene can be possible at either the genetic or RNA level. Targeting the endogenous gene with a triplex forming oligonucleotide (183) or with a chimeric RNA-DNA oligonucleotide (184) can silence a specified sequence. This recently developed genetic approach is likely to undergo further improvement, which could make it the method of choice. More experience has been gained from the use of vectors designed to reduce the output of the mutant RNA. Strategies such as hammerhead (185) and hairpin (186) ribozymes, U1snRNA (187), RNA transplicing (188,189), and RNase P (190) have the potential for allele specific targeting of a mutant transcript. It is unlikely that any of these approaches will be powerful and specific enough to inhibit the mutation-containing transcript, reducing thus the severity of the disease (191). However, this goal may be reached by combining two or more of these anti-RNA strategies that act on different cell compartments and through different molecular mechanisms.

The anti-RNA approach will require the introduction of a procollagen cDNA expression construct to replace the lost activity of the suppressed transcript. Probably a type I collagen promoter fragment is the most appropriate promoter to achieve a high level of regulated expression. Another possibility is the use of vector to deliver the correcting construct. Retrovectors have the size, capacity, and ability of permanent integration needed to contain and express a collagen promoter-collagen cDNA construct. However, the expression of transgene can be suppressed after the transduced cells are reintroduced into the host (192). Modification of the retrovector that removes sequences responsible for suppression appears to overcome this problem, allowing the osteoblast specific expression of the transgene throughout the life of the mouse (71). It still remains to be demonstrated that this vector approach can achieve the level of collagen cDNA expression equivalent to that of an endogenous collagen allele.

Despite the genetic engineering problems, the most severe hurdle for somatic gene therapy of OI is the reintroduction of cells capable of homing to bone and participating in new bone formation into a host. Although the ability of marrow stromal cells to differentiate into mature osteoblasts *in vitro* or in subcutaneous implant is not questionable (193,194), demonstration that this is possible when marrow stromal cells are administered systemically is still unconvincing. Most studies in humans and mice can demonstrate a low degree (1-5%) of engraftment of bone or bone marrow stroma, as assessed by a transgenic or unique endogenous genetic marker (195-197). In many cases, the marker gene does not discriminate whether this cell arises from a mesenchymal or macrophage lineage. Only one study has demonstrated the expression of a transgene that is a marker of a differentiated osteoblast (198), although the level of engraftment and its contribution to bone formation is difficult to assess.

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### References

- 1 Looser E. Zur Kenntnis der Osteogenesis imperfecta congenita und tarda, Mitteilungen Grenzgebiete. *Med Chir* 1906;15:161-207.
- 2 Shapiro F. Consequences of an osteogenesis imperfecta diagnosis for survival and ambulation. *J Pediatr Orthop* 1985;5:456-62.
- 3 Hanscom DA, Winter RB, Lutter L, Lonstein JE, Bloom BA, Bradford DS. Osteogenesis imperfecta. Radiographic classification, natural history, and treatment of spinal deformities. *J Bone Joint Surg Am* 1992;74:598-616.
- 4 Binder H, Conway A, Gerber LH. Rehabilitation approaches to children with osteogenesis imperfecta: a ten-year experience. *Arch Phys Med Rehabil* 1993;74:386-90.
- 5 Silience DO, Senn A, Danks DM. Genetic heterogeneity in osteogenesis imperfecta. *J Med Genet* 1979;16:101-16.
- 6 Silience DO. Osteogenesis imperfecta nosology and genetics. *Ann NY Acad Sci* 1988;543:1-15.
- 7 Glorieux FH, Rauch F, Plotkin H, Ward L, Travers R, Roughley P, et al. Type V osteogenesis imperfecta: a new form of brittle bone disease. *J Bone Miner Res* 2000;15:1650-8.
- 8 Zergollern Lj, Barišić I, Zergollern S. Heterogeneity in osteogenesis imperfecta [in Croatian]. *Acta Med Jugosl* 1988;41:115-31.
- 9 Byers PH. Osteogenesis imperfecta. In: Royce PM, Steinmann B, editors. *Connective tissue and its heritable disorders: molecular, genetic and medical aspects*. New York (NY): Wiley-Liss; 1993. p. 317-50.
- 10 Smith R. Osteogenesis imperfecta: from phenotype to genotype and back again. *Int J Exp Pathol* 1994;75:233-41.
- 11 Vetter U, Pontz B, Zauner E, Brenner RE, Spranger J. Osteogenesis imperfecta: a clinical study of the first ten years of life. *Calcif Tissue Int* 1992;50:36-41.
- 12 Jensen BL, Lund AM. Osteogenesis imperfecta: clinical, cephalometric, and biochemical investigations of OI types I, III, and IV. *J Craniofac Genet Dev Biol* 1997;17:121-32.
- 13 Verstreken M, Claes J, Van der Heyning PH. Osteogenesis imperfecta and hearing loss. *Acta Otorhinolaryngol Belg* 1996;50:91-8.
- 14 Garretsen AJ, Cremers CW, Huygen PL. Hearing loss (in nonoperated ears) in relation to age in osteogenesis imperfecta type I. *Ann Otol Rhinol Laryngol* 1997;106(7 Pt 1):575-82.
- 15 Kuurila K, Grenman R, Johansson R, Kaitila I. Hearing loss in children with osteogenesis imperfecta. *Eur J Pediatr* 2000;159:515-9.
- 16 Levin LS, Salinas CF, Jorgenson RJ. Classification of osteogenesis imperfecta by dental characteristics. *Lancet* 1978;1:332-3.
- 17 Waltimo J, Ojanotko-Harri A, Lukinmaa PL. Mild forms of dentinogenesis imperfecta in association with osteogenesis imperfecta as characterized by light and transmission electron microscopy. *J Oral Pathol Med* 1996;25:256-64.
- 18 Lygidakis NA, Smith R, Oulis CJ. Scanning electron microscopy of teeth in osteogenesis imperfecta type I. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1996;81:567-72.
- 19 Lukinmaa PL, Ranta H, Ranta K, Kaitila I, Hietanen J. Dental findings in osteogenesis imperfecta: II. Dysplastic and other developmental defects. *J Craniofac Genet Dev Biol* 1987;7:127-35.
- 20 Petersen K, Wetzel WE. Recent findings in classification of osteogenesis imperfecta by means of existing dental symptoms. *ASDC J Dent Child* 1998;65:305-9,354.
- 21 Wenstrup RJ, Willing MC, Starman BJ, Beyers PH. Distinct biochemical phenotypes predict clinical severity in nonlethal variants of osteogenesis imperfecta. *Am J Hum Genet* 1990;46:975-82.
- 22 Evensen SA, Myhre L, Stormorken H. Hemostatic studies in osteogenesis imperfecta. *Scand J Haematol* 1984;33:177-9.
- 23 White NJ, Winearls CG, Smith R. Cardiovascular abnormalities in osteogenesis imperfecta. *Am Heart J* 1983;106:1416-20.
- 24 Engelbert RH, Uiterwaal CS, Gulmans VA, Pruijs HE, Helden PJ. Osteogenesis imperfecta: profiles of motor development as assessed by a postal questionnaire. *Eur J Pediatr* 2000;159:615-20.
- 25 Engelbert RH, van der Graaf Y, van Empelen R, Beemer FA, Helden PJ. Osteogenesis imperfecta in childhood: impairment and disability. *Pediatrics* 1997;99:E3.
- 26 Engelbert RH, Uiterwaal CS, Gulmans VA, Pruijs H, Helden PJ. Osteogenesis imperfecta in childhood: prognosis for walking. *J Pediatr* 2000;137:397-402.
- 27 Cole DE. Psychosocial aspects of osteogenesis imperfecta: an update. *Am J Med Genet* 1993;45:207-11.
- 28 McAllion SJ, Paterson CR. Causes of death in osteogenesis imperfecta. *J Clin Pathol* 1996;49:627-30.
- 29 Bischoff H, Freitag P, Jundt G, Steinmann B, Tyndall A, Theiler R. Type I osteogenesis imperfecta: diagnostic difficulties. *Clin Rheumatol* 1999;18:48-51.
- 30 Cole WG, Dagleish R. Perinatal lethal osteogenesis imperfecta. *J Med Genet* 1995;32:284-9.
- 31 Lund AM, Nicholls AC, Schwartz M, Skovby F. Parental mosaicism and autosomal dominant mutations causing structural abnormalities of collagen I are frequent in families with osteogenesis imperfecta type III/IV. *Acta Paediatr* 1997;86:711-8.
- 32 Raghunath M, Steinmann B, Delozier-Blanchet C, Extermann P, Superti-Furga A. Prenatal diagnosis of collagen disorders by direct biochemical analysis of chorionic villus biopsies. *Pediatr Res* 1994;36:441-8.
- 33 Lachman RS. Fetal imaging in the skeletal dysplasias: overview and experience. *Pediatr Radiol* 1994;24:413-7.
- 34 Tretter AE, Saunders RC, Meyers CM, Dungan JS, Grumbach K, Sun CC, et al. Antenatal diagnosis of lethal skeletal dysplasias. *Am J Med Genet* 1998;75:518-22.
- 35 Byers PH, Tsiopoulos P, Bonadio JF, Starman BJ, Schwartz RC. Perinatal lethal osteogenesis imperfecta (OI type II): a biochemically heterogeneous disorder usually due to new mutations in the genes for the type I collagen. *Am J Hum Genet* 1988;42:237-48.
- 36 Verkh Z, Russel M, Miller CA. Osteogenesis imperfecta type II: microvascular changes in the CNS. *Clin Neuropathol* 1995;14:154-8.
- 37 Emery SC, Karpinski NC, Hansen L, Maslah E. Abnormalities in the central nervous system development in

- osteogenesis imperfecta type II. *Pediatr Dev Pathol* 1999;2:124-30.
- 38 Sillence DO, Barlow KK, Garber AP, Hall JG, Rimoin DL. Osteogenesis imperfecta type II. Delineation of phenotype with reference to genetic heterogeneity. *Am J Med Genet* 1984;17:407-23.
- 39 Galicka A, Wolczynski S, Anchim T, Surazynski A, Lesniewicz R, Palka J. Defects of type I procollagen metabolism correlated with decrease of prolydase activity in a case of lethal osteogenesis imperfecta. *Eur J Biochem* 2001;268:2172-8.
- 40 Rowe WD, Shapiro RY. Osteogenesis imperfecta. In: Avioli L, Krane S, editors. *Metabolic bone disease and clinical related disorders*. 2nd ed. Philadelphia: WB Saunders; 1990. p. 659-701
- 41 O'Connell AC, Marini JC. Evaluation of oral problems in an osteogenesis imperfecta population. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1999;87:189-96.
- 42 Levin LS, Wright JM, Byrd DL, Greenway G, Dorst JP, Irani RN, et al. Osteogenesis imperfecta with unusual skeletal lesions: report for three families. *Am J Med Genet* 1985;21:257-69.
- 43 Lee JH, Gamble JG, Moore RE, Rinsky LA. Gastrointestinal problems in patients who have type III osteogenesis imperfecta. *J Bone Joint Surg* 1995;77:1352-6.
- 44 Chines A, Bonicace A, McAlister W, Whyte M. Hypercalciuria in osteogenesis imperfecta: a follow-up stud to assess renal effects. *Bone* 1995;16:333-9.
- 45 Daly K, Wisbeach A, Sanpera I Jr, Fixsen JA. The prognosis for walking in osteogenesis imperfecta. *J Bone Joint Surg Br* 1996;78:477-80.
- 46 Engelbert RH, Pruijs HE, Beemer FA, Helders PJ. Osteogenesis imperfecta in childhood: treatment strategies. *Arch Phys Med Rehabil* 1998;79:1590-4.
- 47 Ishikawa S, Kumar SJ, Takahashi HE, Homma M. Vertebral body shape as a predictor of spinal deformity in osteogenesis imperfecta. *J Bone Joint Surg Am* 1996;78:212-9.
- 48 Widmann RF, Laplaza FJ, Bitan FD, DiMaio MF, Burke SW. Spine deformity and pulmonary compromise in osteogenesis imperfecta. In: *Organising Committee*, editor. Program and abstract book. Proceedings of the Seventh International Conference on Osteogenesis imperfecta; 1999 Aug 29 - Sep 1; Montreal, Canada. Poster P-5.
- 49 Engelbert RH, Gerver WJ, Breslau-Siderius LJ, van der Graaf Y, Pruijs HE, van Doorne JM, et al. Spinal complications in osteogenesis imperfecta: 47 patients 1-16 years of age. *Acta Orthop Scand* 1998;69:283-6.
- 50 Sillence DO. Craniocervical abnormalities in osteogenesis imperfecta: genetic and molecular correlation. *Pediatr Radiol* 1994;24:427-30.
- 51 Charnas LR, Marini JC. Communicating hydrocephalus, basilar invagination and other neurological features in osteogenesis imperfecta. *Neurology* 1993;43:2603-8.
- 52 Marini J, Gerber NL. Osteogenesis imperfecta: rehabilitation and prospects for gene therapy. *JAMA* 1997;277:746-50.
- 53 Lund AM, Muller J, Skovby F. Anthropometry of patients with osteogenesis imperfecta. *Arch Dis Child* 1999;80:524-8.
- 54 Paterson CR, Ogston SA, Henry RM. Life expectancy in osteogenesis imperfecta. *BMJ* 1996;312:51.
- 55 Lund AM, Astrom E, Soderhall S, Schwartz M, Skovby F. Osteogenesis imperfecta: mosaicism and refinement of the genotype-phenotype map in OI type III. Mutations in brief no. 242. Online. *Hum Mutat* 1999;13:503.
- 56 Chapman S, Hall CM. Non-accidental injury or brittle bones. *Pediatr Radiol* 1997;27:106-10.
- 57 Hayes M, Parker G, Ell J, Sillence D. Basilar impression complicating osteogenesis type IV: the clinical and neuroradiological findings in four cases. *J Neurol Neurosurg Psychiatry* 1999;66:357-4.
- 58 Glorieux FH, Ranch F, Poltroon H, Ward LM, Travers R, Roughley PJ, et al. Type V osteogenesis imperfecta: a new form of brittle bone disease. *J Bone Miner Res* 2000;15:1650-8.
- 59 Glorieux FH, Ward LM, Lalić L, Roughley PJ, Travers R. Osteogenesis imperfecta type VI: form of Brittle bone disease with a mineralization defect. *J Bone Miner Res*. In press 2001.
- 60 Ward LM, Rauch F, Travers R, Chabot G, Azouz EM, Lalić L, et al. Rhizomelic osteogenesis imperfecta: an autosomal recessive form of brittle bone disease in a Native American community. *Bone*. In press 2001.
- 61 Dalgleish R. The human type I collagen mutation database. *Nucleic Acids Res* 1997;25:181-7.
- 62 Byers PH, Shapiro JR, Rowe DW, David KE, Holbrook KA. Abnormal alpha 2-chain in type I collagen from a patient with a form of osteogenesis imperfecta. *J Clin Invest* 1983;71:689-97.
- 63 Mundlos S, Chan D, Weng YM, Sillence DO, Cole WG, Bateman JF. Multiexon deletions in the type I collagen COL1A2 gene in osteogenesis imperfecta type IB. Molecules containing the shortened alpha2(I) chains show differential incorporation into the bone and skin extracellular matrix. *J Biol Chem* 1996;271:21068-74.
- 64 Wang Q, Forlino A, Marini JC. Alternative splicing in COL1A1 mRNA leads to a partial null allele and two In-frame forms with structural defects in non-lethal osteogenesis imperfecta. *J Biol Chem* 1996;271:28617-23.
- 65 De Vos A, Sermon K, Van de Velde H, Joris H, Vandervorst M, Lissens W, et al. Two pregnancies after preimplantation genetic diagnosis for osteogenesis imperfecta type I and type IV. *Hum Genet* 2000;106:605-13.
- 66 Campbell BG, Wootton JA, Macleod JN, Minor RR. Canine COL1A2 mutation resulting in C-terminal truncation of pro-alpha2(I) and severe osteogenesis imperfecta. *J Bone Miner Res* 2001;16:1147-53.
- 67 Redford-Badwal DA, Stover ML, Valli M, McKinstry MB, Rowe DW. Nuclear retention of COL1A1 messenger RNA identifies null alleles causing mild osteogenesis imperfecta. *J Clin Invest* 1996;97:1035-40.
- 68 Slayton RL, Deschenes SP, Willing MC. Nonsense mutations in the COL1A1 gene preferentially reduce nuclear levels of mRNA but not hnRNA in osteogenesis imperfecta type I cell strains. *Matrix Biol* 2000;19:1-9.
- 69 Willing MC, Deschenes SP, Scott DA, Byers PH, Slayton RL, Pitts SH, et al. Osteogenesis imperfecta type I: molecular heterogeneity for COL1A1 null alleles of type I collagen. *Am J Hum Genet* 1994;55:638-47.
- 70 Serin G, Gersappe A, Black JD, Aronoff R, Maquat LE. Identification and characterization of human orthologues to *Saccharomyces cerevisiae* Upf2 protein and

- Upf3 protein (*Caenorhabditis elegans* SMG-4). *Mol Cell Biol* 2001;21:209-23.
- 71 Stover ML, Primorac D, Liu SC, McKinstry MB, Rowe DW. Defective splicing of mRNA from one COL1A1 allele of type I collagen in nondeforming (type I) osteogenesis imperfecta. *J Clin Invest* 1993;92:1994-2002.
- 72 Shapiro J, Primorac D, Rowe DW. Osteogenesis imperfecta: current concepts. In: Bilezikian J, Raisz L, Rodan G, editors. *Principles of bone biology*. New York (NY): Academic Press; 1996. p. 889-902.
- 73 Johnson C, Primorac D, McKinstry M, McNeil J, Rowe D, Lawrence JB. Tracking COL1A1 RNA in osteogenesis imperfecta. splice-defective transcripts initiate transport from the gene but are retained within the SC35 domain. *J Cell Biol* 2000;150:417-32.
- 74 Bonadio J, Saunders TL, Tsai E, Goldstein SA, Morris-Wiman J, Brinkley L, et al. Transgenic mouse model of the mild dominant form of osteogenesis imperfecta. *Proc Natl Acad Sci USA* 1990;87:7145-9.
- 75 Forlino A, Marini JC. Osteogenesis imperfecta: prospects for molecular therapeutics. *Mol Genet Metab* 2000;71:225-32.
- 76 Schnieke A, Harbers K, Jaenisch R. Embryonic lethal mutation in mice induced by retrovirus insertion into the alpha 1(I) collagen gene. *Nature* 1983;304:315-20.
- 77 Lohler J, Timpl R, Jaenisch R. Embryonic lethal mutation in mouse collagen I gene causes rupture of blood vessels and is associated with erythropoietic and mesenchymal cell death. *Cell* 1984;38:597-607.
- 78 Stacey A, Bateman J, Choi T, Mascara T, Cole W, Jaenisch R. Perinatal lethal osteogenesis imperfecta in transgenic mice bearing an engineered mutant pro-alpha 1(I) collagen gene. *Nature* 1988;332:131-6.
- 79 Khillan JS, Olsen AS, Kontusaari S, Sokolov B, Prockop DJ. Transgenic mice that express a mini-gene version of the human gene for type I procollagen (COL1A1) develop a phenotype resembling a lethal form of osteogenesis imperfecta. *J Biol Chem* 1991;266:23373-9.
- 80 Forlino A, Porter FD, Lee EJ, Westphal H, Marini JC. Use of the Cre/lox recombination system to develop a non-lethal knock-in murine model for osteogenesis imperfecta with an alpha1(I) G349C substitution. Variability in phenotype in BrlIV mice. *J Biol Chem* 1999;274:37923-31.
- 81 Chipman SD, Sweet HO, McBride DJ, Davisson MT, Marks SC, Shuldiner AR, et al. Defective pro alpha 2(I) collagen synthesis in a recessive mutation in mice: a model of human osteogenesis imperfecta. *Proc Natl Acad Sci USA* 1993;90:1701-5.
- 82 Misof K, Landis WJ, Klaushofer K, Fratzl P. Collagen from the osteogenesis imperfecta mouse model (oim) shows reduced resistance against tensile stress. *J Clin Invest* 1997;100:40-5.
- 83 McBride DJ, Choe V, Shapiro JR, Brodsky B. Altered collagen structure in mouse tail tendon lacking the alpha 2(I) chain. *J Mol Biol* 1997;270:275-84.
- 84 Fratzl P, Paris O, Klaushofer K, Landis WJ. Bone mineralization in an osteogenesis imperfecta mouse model studied by small-angle x-ray scattering. *J Clin Invest* 1996;97:396-402.
- 85 Jones SJ, Glorieux FH, Travers R, Boyde A. The microscopic structure of bone in normal children and patients with osteogenesis imperfecta: a survey using backscattered electron imaging. *Calcif Tissue Int* 1999;64:8-17.
- 86 Rauch F, Travers R, Parfitt AM, Glorieux FH. Static and dynamic bone histomorphometry in children with osteogenesis imperfecta. *Bone* 2000;26:581-9.
- 87 Pepin M, Atkinson M, Starman BJ, Byers PH. Strategies and outcomes of prenatal diagnosis for osteogenesis imperfecta: a review of biochemical and molecular studies completed in 129 pregnancies. *Prenat Diagn* 1997;17:559-70.
- 88 Körkkö J, Ala-Kokko L, De Paepe A, Nuytinck L, Earley J, Prockop DJ. Analysis of the COL1A1 and COL1A2 genes by PCR amplification and scanning by conformation sensitive gel electrophoresis identifies only COL1A1 mutations in 15 patients with osteogenesis imperfecta type I: identification of common sequences of null-allele mutations. *Am J Hum Genet* 1998;62:98-110.
- 89 Liu WO, Oefner PJ, Qian C, Odom RS, Francke U. Denaturing HPLC-identified novel FBN1 mutations, polymorphisms, and sequence variants in Marfan syndrome and related connective tissue disorders. *Genet Test* 1997-98;1:237-42.
- 90 LamandeSR, Dahl HHM, Cole WG, Bateman JF. Characterization of point mutations in the collagen COL1A1 and COL1A2 genes causing lethal perinatal osteogenesis imperfecta. *J Biol Chem* 1989;264:15809-12.
- 91 Mottes M, Sangalli A, Valli M, Gomez Lira M, Tenni R, Buttitta P, et al. Mild dominant osteogenesis imperfecta with intrafamilial variability: the cause is a serine for glycine  $\alpha 1(I)901$  substitution in a type I collagen gene. *Hum Genet* 1992;89:480-4.
- 92 Mottes M, Gomez Lira M, Zolezzi F, Valli M, Lisi V, Freisinger P. Four new cases of lethal osteogenesis imperfecta due to glycine substitutions in the COL1A1 and COL1A2 genes. *Hum Mutat* 1998;12:71-2.
- 93 Mirandola S, Pignatti PF, Mottes M. Three novel polymorphic sequence variants in the type I collagen gene COL1A1, the main disease locus for Osteogenesis Imperfecta. *Mol Cell Probes* 2000;14:329-32.
- 94 Willing MC, Deschenes SP, Slayton RL, Roberts EJ. Premature termination is a unifying Mechanism for COL1A1 null alleles in osteogenesis imperfecta type I cell strains. *Am J Hum Genet* 1996;59:799-809.
- 95 Cohn DH, Zhang XM. PCR-based linkage strategies for the type I collagen genes. In: Abstracts from the Third International Conference on Molecular Biology and Pathology of Matrix, 1990 June 13-16, Matrix 1990;10:236.
- 96 Nuytinck L, Coppin C, De Paepe A. A four base pair insertion polymorphism in the 3' untranslated region of the COL1A1 gene is highly informative for null allele testing in patients with osteogenesis imperfecta type I. *Matrix Biol* 1998;16:349-52.
- 97 Cepollaro C, Gonnelli S, Pondrelli C, Montagnani A, Martini S, Bruni D, et al. Osteogenesis imperfecta: bone turnover, bone density, and ultrasound parameters. *Calcif Tissue Int* 1999;65:129-32.
- 98 Lund AM, Hansen M, Kollerup G, Juul A, Teisner B, Skovby F. Collagen-derived markers of bone metabolism in osteogenesis imperfecta. *Acta Paediatr* 1998;87:1131-7.
- 99 Glorieux FH, Bishop NJ, Plotkin H, Chabot G, Lanoue G, Travers R. Cyclic administration of pamidronate in children with severe osteogenesis imperfecta *N Engl J Med* 1998;339:947-52.
- 100 Kauppila S, Tekay A, Risteli L, Koivisto M, Risteli J. Type I and type III procollagen propeptides in amnio-

- tic fluid of normal pregnancies and in a case of mild osteogenesis imperfecta. *Eur J Clin Invest* 1998; 28: 831-7.
- 101 Astrom E, Soderhall S. Beneficial effect of bisphosphonate during five years of treatment of severe osteogenesis imperfecta. *Acta Paediatr* 1998;87: 64-8.
- 102 Bishop N. Osteogenesis imperfecta - current perspectives and treatment. In: The European Calcified Tissue Society. Reviews on bone disease and bone biology. Available at: [http://www.ectsoc.org/reviews/002\\_bish.htm](http://www.ectsoc.org/reviews/002_bish.htm). Accessed June 27, 2001.
- 103 McCarthy EF, Earnest K, Rossiter K, Shapiro J. Bone histomorphometry in adults with type IA osteogenesis imperfecta. *Clin Orthop* 1997;336:254-62.
- 104 Yong-Hing K, MacEwen GD. Scoliosis associated with osteogenesis imperfecta. Results of treatment. *J Bone Joint Surg Br* 1982;64:36-43.
- 105 Bighton P, Spranger J, Versveld G. Skeletal complications in osteogenesis imperfecta. A review of 153 South African patients. *S Afr Med J* 1983;64:565-8.
- 106 Lubicky JP. The spine in osteogenesis imperfecta. In: Weinstein SL, editor. The pediatric spine: principles and practice. New York (NY): Raven Press; 1994. p. 943-58.
- 107 Meyer S, Villarreal M, Ziv I. A three-level fracture of the axis in a patient with osteogenesis imperfecta. *Spine* 1986;11:505-6.
- 108 Sawin PD, Menezes AH. Basilar invagination in osteogenesis imperfecta and related osteochondrodysplasias: medical and surgical management. *J Neurosurg* 1997;86:950-60.
- 109 Rao S, Patel A, Schildhauer T. Osteogenesis imperfecta as a differential diagnosis of pathologic burst fractures of the spine. A case report. *Clin Orthop* 1993;289:113-7.
- 110 Rask MR. Spondylolisthesis resulting from osteogenesis imperfecta. Report of a case. *Clin Orthop* 1979;139:164-6.
- 111 Benson DR, Donaldson DH, Millar EA. The spine in osteogenesis imperfecta. *J Bone Joint Surg Am* 1978;60:925-9.
- 112 Stoltz MR, Dietrich SL, Marshall GJ. Osteogenesis imperfecta. Perspectives. *Clin Orthop* 1989;(242): 120-36.
- 113 Kocher MS, Shapiro F. Osteogenesis imperfecta. *J Am Acad Orthop Surg* 1998;6:225-36.
- 114 Renshaw TS, Cook RS, Albright JA. Scoliosis in osteogenesis imperfecta. *Clin Orthop* 1979;145:163-7.
- 115 Karbowski A, Schwitalle M, Eckardt A. Scoliosis in patients with osteogenesis imperfecta: a federal nation-wide cross-sectional study [in German]. *Z Orthop Ihre Grenzgeb* 1999;137:219-22.
- 116 Norimatsu H, Mayuzumi T, Takahashi H. The development of the spinal deformities in osteogenesis imperfecta. *Clin Orthop* 1982;162:20-5.
- 117 King JD, Bobecko WP. Osteogenesis imperfecta. An orthopaedic description and surgical review. *J Bone Joint Surg Br* 1971;53:72-89.
- 118 Hanscom DA, Bloom BA. The spine in osteogenesis imperfecta. *Orthop Clin North Am* 1988;19:449-58.
- 119 Huystee PJ, Sakkers RJB, Pruijs HEH. Brace treatment for scoliosis in osteogenesis imperfecta, (im)possibilities. In: Program and abstract book. Proceedings of the Seventh International Conference on Osteogenesis imperfecta; 1999 Aug 29 - Sep 1; Montreal, Canada. Poster P-14.
- 120 Widmann RF, Bitan FD, Laplaza FJ, Burke SW, DiMaio MF, Schneider R. Spinal deformity, pulmonary compromise and quality of life in osteogenesis imperfecta. *Spine* 1999;24:1673-8.
- 121 Herron LD, Dawson E. Methylmethacrylate as an adjunct in spinal instrumentation. *J Bone Joint Surg Am* 1977;59:866-8.
- 122 Birch JG. Osteogenesis imperfecta: orthopedic aspects. In: Uthoff HK, editor. Current concepts in bone fragility. Proceedings of the 12th Applied Basic Science Course. 1985. Ottawa, Ontario, Canada. Berlin, Heidelberg: Springer Verlag; 1986. p. 221-5.
- 123 Livesley PJ, Webb PJ. Spinal fusion in situ in osteogenesis imperfecta. *Int Orthop* 1996;20:43-6.
- 124 Hensinger RN, Kanwaldeep SS. Complications of spine surgery in children. In: Epps CH Jr, Bowen JR, editors. Complications in pediatric orthopaedic surgery. Philadelphia (PA): Lippincott; 1995. p. 116.
- 125 Cristofaro RL, Hoek KJ, Bonnet CA, Brown JC. Operative treatment of spine deformity in osteogenesis imperfecta. *Clin Orthop* 1979;139:40-8.
- 126 Benson DR, Newman DC. The spine and surgical treatment in osteogenesis imperfecta. *Clin Orthop* 1981;159:147-53.
- 127 Lubicky JP. Spinal deformity in osteogenesis imperfecta. In: Program and abstract book. Proceedings of the Seventh International Conference on Osteogenesis imperfecta; 1999 Aug 29 - Sep 1; Montreal, Canada. Invited lecture I-13. Available at: <http://www.geocities.com/CapeCanaveral/Lab/3608/Lubicky.html>. Accessed: June 19, 2001.
- 128 Sillence DO. Craniocervical abnormalities in osteogenesis imperfecta: genetic and molecular correlation. *Pediatr Radiol* 1994;24:427-30.
- 129 Sillence DO, Wilson M, Morley K, Onikul E, Kozlowski K, Ell J. Basilar impression complicating osteogenesis imperfecta: natural history and correlation with OI type. In: Proceedings of the Fifth International Conference on Osteogenesis Imperfecta; 1993 Sep 27-30; Oxford, UK. p. 139-41.
- 130 Pozo JL, Crockard HA, Ransford AO. Basilar impression in osteogenesis imperfecta. A report of three cases in one family. *J Bone Joint Surg Br* 1984;66: 233-8.
- 131 Tosi LL. Osteogenesis imperfecta. *Curr Opin Pediatr* 1997;9:94-9.
- 132 Copley LA, Dormans JP. Cervical spine disorders in infants and children. *J Am Acad Orthop Surg* 1998;6: 204-14.
- 133 Harkey HL, Crockard AH, Stevens JM, Smith R, Ransford AO. The operative management of basilar impression in osteogenesis imperfecta. *Neurosurgery* 1990;27:782-6.
- 134 Hayes M, Parker G, Ell J, Sillence D. Basilar impression complicating osteogenesis imperfecta type IV: the clinical and neuroradiological findings in four cases. *J Neurol Neurosurg Psychiatry* 1999;66:357-6.
- 135 Mirbaha M. Multiple osteotomies and intramedullary fixation of the radius and the ulna to correct severe deformity and improve function in osteogenesis imperfecta. *J Bone Joint Surg Am* 1966;48:523-7.
- 136 DiCesare PE, Sew-Hoy A, Krom W. Bilateral isolated olecranon fractures in an infant as presentation of osteogenesis imperfecta. *Orthopedics* 1992;15: 741-3.
- 137 Stott NS, Zionts LE. Displaced fractures of the apophysis of the olecranon in children who have

- osteogenesis imperfecta. *J Bone Joint Surg Am* 1993; 75:1026-33.
- 138 Stig Jacobsen F. Aneurysmal bone cyst in a patient with osteogenesis imperfecta. *J Pediatr Orthop B* 1997;6:225-7.
  - 139 Khoshhal KI, Ellis RD. Functional outcome of Sofield procedure in the upper limb in osteogenesis imperfecta. *J Pediatr Orthop* 2001;21:236-7.
  - 140 Nicholas RW, James P. Telescoping intramedullary stabilisation of the lower extremities for severe osteogenesis imperfecta. *J Pediatr Orthop* 1990;10:219-23.
  - 141 Porat S, Heller E, Seidmann DS, Meyer S. Functional results of operating in osteogenesis imperfecta: Elongating and nonelongating rods. *J Pediatr Orthop* 1991;11:200-3.
  - 142 Gamble JG, Strudwick WJ, Rinsky LA, Bleck EE. Complications of intramedullary rods in osteogenesis imperfecta: Bailey-Dubow rods versus nonelongating rods. *J Pediatr Orthop* 1988;8:645-9.
  - 143 Luhmann SJ, Sheridan JJ, Capelli RN, Scoenecker PL. Management of lower-extremity deformities in osteogenesis imperfecta with extensible intramedullary rod technique: a 20-year experience. *J Pediatr Orthop* 1998;18:88-94.
  - 144 Zions LE, Ebramzadeh E, Stott NS. Complications in the use of the Bailey-Dubow extensible nail. *Clin Orthop* 1998;348:186-95.
  - 145 Jerosch J, Mazzotti I, Tomašević M. Complications after treatment of patients with osteogenesis imperfecta with a Bailey-Dubow rod. *Arch Orthop Trauma Surg* 1998;117:240-5.
  - 146 Janus GJ, Vanpaemel LA, Engelbert RH, Pruijs HE. Complications of the Bailey-Dubow elongating nail in osteogenesis imperfecta: 34 children with 110 nails. *J Pediatr Orthop B* 1999;8:203-7.
  - 147 Karbowski A, Schwitalle M, Brenner R, Lehmann H, Pontz B, Wörsdörfer O. Experience with Bailey-Dubow rodding in children with osteogenesis imperfecta. *Eur J Pediatr Surg* 2000;10:119-24.
  - 148 Li YH, Chow W, Leong JC. The Sofield-Millar operation in osteogenesis imperfecta. *J Bone Joint Surg Br* 2000;82:11-6.
  - 149 Mulpuri K, Joseph B. Intramedullary rodding in osteogenesis imperfecta. *J Pediatr Orthop* 2000;20:267-73.
  - 150 Gahagan S, Rimsza ME. Child abuse or osteogenesis imperfecta: how can we tell? *Pediatrics* 1991;88: 987-92.
  - 151 Smith R. Non-accidental injury. *Current Orthopaedics* 2000;14:193-6.
  - 152 Paterson CR, Burns J, McAllion SJ. Osteogenesis imperfecta: the distinction from child abuse and the recognition of a variant form. *Am J Med Genet* 1993;45:187-92.
  - 153 Miller ME, Hangartner TN. Temporary brittle bone disease: Association with decreased fetal movement and osteopenia. *Calcif Tissue Int* 1999;64:137-43.
  - 154 Zaleske DJ. Metabolic and endocrine abnormalities. Osteogenesis imperfecta. In: Morrissy RT, Weinstein SL, editors. *Lovell and Winter's Pediatric Orthopaedics*. 4th ed. Philadelphia (PA): Lippincott-Raven; 1996. p. 164-70.
  - 155 Paterson CR, Monk EA. Temporary brittle bone disease: follow up observations of children at home. In: Organising Committee, editor. *Program and abstract book. Proceedings of the Seventh International Conference on Osteogenesis imperfecta*; 1999 Aug 29 - Sep 1; Montreal, Canada. Poster P-32.
  - 156 Noorda RJ, van der Aa JP, Wuisman PI, David EF, Lips PT, van der Valk P. Transient osteoporosis and osteogenesis imperfecta. A case report. *Clin Orthop* 1997;337:249-55.
  - 157 Karagkevrekis CB, Ainscow DA. Transient osteoporosis of the hip associated with osteogenesis imperfecta. *J Bone Joint Surg Br* 1998;80:54-5.
  - 158 Wenger DR, Abrams RA, Yaru N, Leach J. Obstruction of the colon due to protrusio acetabuli in osteogenesis imperfecta: treatment by pelvic osteotomy. *J Bone Joint Surg Am* 1988;70:1103-7.
  - 159 Moorefield WG, Miller GR. Aftermath of osteogenesis imperfecta: the disease in adulthood. *J Bone Joint Surg Am* 1980;62:113-9.
  - 160 Papagelopoulos PJ, Morrey BF. Hip and knee replacement in osteogenesis imperfecta. *Bone Joint Surg Am* 1993;75:572-80.
  - 161 Gamble JG, Rinsky LA, Strudwick J, Bleck EE. Non-union of fractures in children who have osteogenesis imperfecta. *J Bone Joint Surg Am* 1988;70:439-43.
  - 162 Ring D, Jupiter JB, Labropoulos PK, Guggenheim JJ, Stanitsky DF, Spencer DM. Treatment of deformity of the lower limb in adults who have osteogenesis imperfecta. *J Bone Joint Surg Am* 1996;78:220-5.
  - 163 Rutkowski R, Resnick R, McMaster JH. Osteosarcoma occurring in osteogenesis imperfecta. *J Bone Joint Surg Am* 1979;61:606-8.
  - 164 Gagliardi JA, Evans EM, Chandnani VP, Myers JB, Pacheco CM. Osteogenesis imperfecta complicated by osteosarcoma. *Skeletal Radiol* 1995;24:308-10.
  - 165 Smith R. Hyperplastic callus and osteogenesis imperfecta. *Lancet* 2001;357:248-9.
  - 166 Kutsumi K, Nojima T, Yamashiro K, Hatae Y, Isu K, Ubayama Y, et al. Hyperplastic callus formation in both femurs in osteogenesis imperfecta. *Skeletal Radiol* 1996;25:384-7.
  - 167 Azouz EM, Fassier F. Hyperplastic callus formation in OI (letter). *Skeletal Radiol* 1997;26:744-5.
  - 168 Dobrocky I, Seidl G, Grill F. MRI and CT features of hyperplastic callus in osteogenesis imperfecta tarda. *Eur Radiol* 1999;9:665-8.
  - 169 Gorissen MD, Engelbert RH, Jansen M, Pruijs HE. Aggravation of skeletal deformities in osteogenesis imperfecta during puberty – cause or coincidence? In: Organising Committee, editor. *Program and abstract book. Proceedings of the Seventh International Conference on Osteogenesis imperfecta*; 1999 Aug 29 - Sep 1; Montreal, Canada. Poster, P-13.
  - 170 Sillence D. Management of osteogenesis imperfecta in adolescents and adults. In: Organising Committee, editor. *Program and abstract book. Proceedings of the Seventh International Conference on Osteogenesis imperfecta*; 1999 Aug 29 - Sep 1; Montreal, Canada. Invited lecture I-28. Available at: <http://www.geocities.com/CapeCanaveral/Lab/3608/Sillence.html>. Accessed: July 2, 2001.
  - 171 Paterson CR, McAllion S, Stellman JL. Osteogenesis imperfecta after the menopause. *N Engl J Med* 1984;310:1694-6.
  - 172 Middleton RW. Closed intramedullary rodding for osteogenesis imperfecta. *J Bone Joint Surg Br* 1984; 66:652-5.
  - 173 Ryöppy S, Alberty A, Kaitila I. Early semiclosed intramedullary stabilization in osteogenesis imperfecta. *J Pediatr Orthop* 1987;7:139-44.
  - 174 Sijbrandij ES, van Gils AP, van Hellemond FJ, Louwerens JW, de Lange EE. Assessing the subtalar

- joint: the Broden view revisited. *Foot Ankle Int* 2001; 22:329-34.
- 175 Cole WG. Early surgical management of severe forms of osteogenesis imperfecta. *Am J Med Genet* 1993;45: 270-4.
- 176 McHale KA, Tenuta JJ, Tosi LL, McKay DW. Percutaneous intramedullary fixation of long bone deformity in severe osteogenesis imperfecta. *Clin Orthop* 1994;305:242-8.
- 177 Engelbert RH, Helders PJ, Keessen W, Pruijs HE, Gooskens RH. Intramedullary rodding in type III osteogenesis imperfecta. *Acta Orthop Scand* 1995; 66:361-4.
- 178 Castells S. New approaches to treatment of osteogenesis imperfecta. *Clin Orthop* 1973;93:239-49.
- 179 Glorieux FH, Bishop NJ, Plotkin H, Chabot G, Lanoue G, Travers R. Cyclical pamidronate therapy in children with severe osteogenesis imperfecta. *N Engl J Med* 1998;339:947-52.
- 180 Fleisch H. Bisphosphonates in bone disease – from the laboratory to the patient. San Diego (CA): Academic Press; 2000.
- 181 Fleisch H. Bisphosphonates: mechanism of action. *Endocrine Rev* 1998;19:80-100.
- 182 Plotkin H, Rauch F, Bishop NJ, Montpetit K, Ruck-Gibis J, Travers R, et al. Pamidronate treatment of severe osteogenesis imperfecta in children under three years of age. *J Clin Endocrinol Metab* 2000;85: 1846-50.
- 183 Faruqi AF, Datta HJ, Carroll D, Seidman MM, Glazer PM. Triple-helix formation induces recombination in mammalian cells via a nucleotide excision repair-dependent pathway. *Mol Cell Biol* 2000;20:990-1000.
- 184 Kren BT, Metz R, Kumar R, Steer CJ. Gene repair using chimeric RNA/DNA oligonucleotides. *Semin Liver Dis* 1999;19:93-104.
- 185 Klebba C, Ottmann OG, Scherr M, Pape M, Engels JW, Grez M, et al. Retrovirally expressed anti-HIV ribozymes confer a selective survival advantage on CD4+ T cells in vitro. *Gene Ther* 2000;7:408-16.
- 186 Lian Y, De Young MB, Siwkowski A, Hampel A, Rappaport J. The sCYMV1 hairpin ribozyme: targeting rules and cleavage of heterologous RNA. *Gene Ther* 1999;6:1114-9.
- 187 Beckley SA, Liu P, Stover ML, Gunderson SI, Lichtler AC, Rowe DW. Reduction of target gene expression by a modified U1 snRNA. *Mol Cell Biol* 2001;21: 2815-25.
- 188 Caudevilla C, Serra D, Miliar A, Codony C, Asins G, Bach M, et al. Processing of carnitine octanoyltransferase pre-mRNAs by cis and trans-splicing. *Adv Exp Med Biol* 1999;466:95-102.
- 189 Mansfield SG, Kole J, Puttaraju M, Yang CC, Garcia-Blanco MA, Cohn JA, et al. Repair of CFTR mRNA by spliceosome-mediated RNA trans-splicing. *Gene Ther* 2000;7:1885-95.
- 190 Kawa D, Wang J, Yuan Y, Liu F. Inhibition of viral gene expression by human ribonuclease P. *RNA* 1998;4:1397-406.
- 191 Dawson PA, Marini JC. Hammerhead ribozymes selectively suppress mutant type I collagen mRNA in osteogenesis imperfecta fibroblasts. *Nucleic Acids Res* 2000;28:4013-20.
- 192 Cherry SR, Biniszkiwicz D, van Parijs L, Baltimore D, Jaenisch R. Retroviral expression in embryonic stem cells and hematopoietic stem cells. *Mol Cell Biol* 2000;20:7419-26.
- 193 Dennis JE, Merriam A, Awadallah A, Yoo JU, Johnstone B, Caplan AI. A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse. *J Bone Miner Res* 1999;14:700-9.
- 194 Krebsbach PH, Kuznetsov SA, Satomura K, Emmons RV, Rowe DW, Robey PG. Bone formation in vivo: comparison of osteogenesis by transplanted mouse and human marrow stromal fibroblasts. *Transplantation* 1997;63:1059-69.
- 195 Ding L, Lu S, Batchu R, III RS, Munshi N. Bone marrow stromal cells as a vehicle for gene transfer. *Gene Ther* 1999;6:1611-6.
- 196 Onyia JE, Clapp DW, Long H, Hock JM. Osteoprogenitor cells as targets for ex vivo gene transfer. *J Bone Miner Res* 1998;13:20-30.
- 197 Pereira RF, O'Hara MD, Laptev AV, Halford KW, Pollard MD, Class R, et al. Marrow stromal cells as a source of progenitor cells for nonhematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. *Proc Natl Acad Sci USA* 1998;95:1142-7.
- 198 Nilsson SK, Dooner MS, Weier HU, Frenkel B, Lian JB, Stein GS, et al. Cells capable of bone production engraft from whole bone marrow transplants in non-ablated mice. *J Exp Med* 1999;189:729-34.

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**Correspondence to:**

Dragan Primorac  
Laboratory for Clinical and Forensic Genetics  
Split University Hospital and School of Medicine  
Spinčićeva 1  
21 000 Split, Croatia  
dragan.primorac@st.tel.hr