Osteogenesis Imperfecta at the Beginning of Bone and Joint Decade

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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
menopause in women and after the sixth decade in men. 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 postnataally slightly reduced (11). Disproportion in stature in the OI type I is caused mainly by spinal involvement, mostly platyspondyly and scoliosis, which is present in about a quarter of patients (11,24). Muscle strength is normal except for the periaricular 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

<table>
<thead>
<tr>
<th>Molecular classification</th>
<th>Clinical classification</th>
<th>Clinical severity</th>
<th>Molecular mechanism</th>
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<tbody>
<tr>
<td>Dominant Negative</td>
<td>Type II</td>
<td>Perinatal, lethal</td>
<td>Glycine substitutions preferentially located in C terminal helical domain of either collagen chain</td>
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<td>Glycine substitutions preferentially located in mid helical domain of either collagen chain</td>
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<tr>
<td>Dominant Negative</td>
<td>Type IV</td>
<td>Moderately deforming</td>
<td>Glycine substitutions preferentially located in mid helical domain of the α2 collagen chain</td>
</tr>
<tr>
<td>Type V</td>
<td>Moderately deforming</td>
<td>Non type I collagen gene mutation</td>
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<tr>
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<td>Non type I collagen gene mutation</td>
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Table 1. Clinical and molecular classification of osteogenesis imperfecta (OI)

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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 neuropsychological alterations, periventricular 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 suturallia, 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 proidase (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 hypercalcuria, 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 wheelchair. 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 malignment 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 vertebral bodies 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 craniovascular 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). Platelet-basias 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 pathologic 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 complete absence 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 (23). Retardation in motor development develops 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, 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 interosseus membrane. Dislocation of the radial head was seen in three patients (58). A radio-dense metaphyseal band immediately adjacent to the growth plate was a constant feature in young subjects. None had blue sclerae or dentinogenesis imperfecta. Histomorphometric 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, hyperosteoiosis, 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/ge 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 intron deletion, an intrame 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 intron exons (63) or mutations that insert a segment of intron that remains intrame 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 COLA2 gene. Formation of the heterotrimeric collagen molecule requires that the α2(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 α2(I) chain or over production of the α1(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 COLA1 production directly influences the accumulation of normal type I collagen molecules. Reduced output from a single COLA1 allele reduces the production of heterotrimeric collagen and the unincorporated α2(I) chains are degraded intracellularly. Mutations introducing a premature stop codon are the most frequent cause for a null COLA1 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 COLA1 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 (53S 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 \(\text{COL1A1}\) 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 \(\text{OI}\) type I (74). Table 2 is showing available murine models for \(\text{OI}\) and their clinical phenotype (75,76, 78-81).

<table>
<thead>
<tr>
<th>Clinical phenotype</th>
<th>Terminology (ref. No.)</th>
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<tr>
<td>Type I</td>
<td>MOV 13 (heterozygous) (74)</td>
</tr>
<tr>
<td>Type II</td>
<td>MOV 13 (homozygous) (76)</td>
</tr>
<tr>
<td>Type II</td>
<td>Transgenic - (\text{COL1A1}) mutation (78)</td>
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<tr>
<td>Variable</td>
<td>Transgenic (\text{COL1A1}) minigene mouse (79)</td>
</tr>
<tr>
<td>Type II</td>
<td>Knock-in BrtlII (80)</td>
</tr>
<tr>
<td>Type III</td>
<td>OIM mice (81)</td>
</tr>
<tr>
<td>Type III/IV</td>
<td>Knock-in BrtlIV (80)</td>
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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 \(\text{COL1A1}\) 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. This step generated heterozygous mutant mice carrying a frameshift in the last 48 amino acid sequence of the C-terminal propeptide preventing the incorporation of \(\text{pro}2(I)\) chain into the collagen heterotrimer. As a consequence, accumulation of \(\text{pro}1(I)\) 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 \(\text{OI}\) 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 \(\text{OI}\) bone, which shows a state of high turnover characterized by increased numbers of osteoblasts and osteoclasts (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 \(\text{OI}\) 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/gentics/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 α1(I) and α2(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](image1.png)

**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 α1(I) cDNA probe (lanes A, B) or a NcoI-NcoI 1382 bp α2(I) cDNA probe. Both probes had been 3‘end-labeled with [α-32P] 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](image2.png)

**Figure 2. Panel A.** Heteroduplex analysis on MDE gel of a 214 bp α1(II) 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](image3.png)

**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.

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 4. Analysis of the 4pb 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.

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 an 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. Bone turnover in OI patients would probably depend on 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 clinical 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 modeling, 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-
pose of this section is to review the numerous skeletal problems in patients with OI and to outline current principles of orthopedic management.

**Spine**

Scoliosis 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 then 40°, and 56 patients had scoliosis of less then 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 arthrodasis 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 then 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 bisphosphonates 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 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 denticogenesis 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 36% 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 paresis, 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-
mented with appropriate imaging studies, should be surgically treated by transoral ventral 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 procedure 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 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.

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 136 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 osteoarthrosis 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 pathophysiological 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 nailing. 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 osteoarthrosis 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 fractures 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 multiplanar 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 children is not easy to perform. Middleton (172) first reported closed intramedullary rodding combined with osteolysis of long bones in three patients in 1984. The minimal exposure of the bone was used with osteolysis 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 osteolysis of deformed long bone was done in a 7-year-old girl with severe form of OI by Sibbrendj (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 experience 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±2.9% annually, and the deviation from normal, as indicated by Z-score, improved from -5.3±1.2 to -3.4±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 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 higher 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 transsplicing (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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