Fragile without fractures

Atul A Deodhar, Anthony D Woolf

Despite being the commonest inherited disease of bone, osteogenesis imperfecta (OI) is a rare cause of fractures in daily clinical practice. Its prevalence in the general population is 1 to 5 per 100,000. The clinical spectrum ranges from mild forms with relatively few fractures and normal mobility to the lethal form, with multiple intratraume fractures and death in perinatal period. The diagnosis of OI is based on fracture rates, family history, and clinical signs. However, the rarity of the condition coupled with the subtle signs of mild OI can lead to cases being missed altogether or misdiagnosed in some as “post-menopausal” osteoporosis. In asymptomatic members of a family with mild OI, it is even harder to decide whether they have the condition or not. It has recently been suggested that consultation by a physician familiar with the variability of OI (such as a medical geneticist) is essential for the early diagnosis. Clearly, this is possible only in selected centres of expertise, and in most clinical settings one is forced to tackle the problem with locally available resources.

Case history
A 30 year old lady with known OI presented to the “Bone Clinic” with her two children in February 1992. The diagnosis of OI in the family was established three years before this visit when her 3 year old daughter was brought to the local accident and emergency department for a skull fracture with minor trauma. This child had suffered two long bone fractures previously, also with minor trauma. This led to initial suspicion for “non-accidental injury” or child abuse, though the family history suggested otherwise. The child’s 65 year old grandmother had a history of four fractures, fracture of both “legs” (details unclear) at age 6 months, fracture of a bone in left foot age 16 and a fractured right forth finger after menopause. She was under treatment for “post-menopausal” osteoporosis after a bone densitometry examination had confirmed low bone mass in lumbar spine and femoral neck. The grandmother also remembered that her own deceased father had a “tendency to break bones easily” though the exact number and sites of fractures were not known. The child’s mother gave history of three fractures before menarche, but no fractures since the age of 14. Two of these three fractures involved fingers or toes and the other, a wrist fracture, was a result of moderate trauma (falling off a tree). Her 7 year old brother had never suffered any fractures despite usual childhood injuries. All family members were noted to have blue sclerae and the diagnosis of OI was established.

The woman reported that since that visit to the accident and emergency department in 1989, neither her daughter nor her son had suffered any more fractures. Her son, now 10 years old, wished to play rugby in school, but she was worried that he may also have OI despite no fractures to date, and came in for advice.

On examination, the woman and both her children had blue sclerae. At 5 foot, the lady was short and her daughter was 30th percentile in height. The boy however was of normal size, 65th and 60th percentile for height and weight respectively. There was evidence of joint hypermobility in the 6 year old girl but not in the boy. The young girl bore scars of orthopaedic surgeries on her right leg. The musculoskeletal examination of the boy was normal. There was no pre-senile deafness or dentinogenesis imperfecta in any family member.

The presence of blue sclerae and the family history suggested that the boy most probably had type I or “mild” OI (table 1). His mother was however hoping that his normal height and absence of fractures ruled out OI and he himself was very keen to take up rugby. They clearly needed confirmation to the diagnosis by genetic investigations, which were available only in few select centres in the country on “fee for service” basis. After discussing these factors with the family we became aware of their unwillingness to travel because of the general inconvenience and the expenses involved. There was also a possibility of not knowing the answer despite undergoing all the tests as the genetic and biochemical methods of diagnosis carried a 50% to 70% sensitivity and reliability.

This raised the question whether bone densitometry examination could be a surrogate for genetic testing to aid the diagnosis of OI. We therefore measured the bone mineral density (BMD) of the lumbar spine on the boy using dual energy x ray absorptiometry (DXA).

The measurement showed that his BMD at L1-L4 lumbar spine was 0.484 g/cm² with a Z score of −2.5. The femoral neck bone densitometry was not carried out, as the normal ranges in children were not available. Based on his lumbar spine measurement, we gave an estimate of severely increased fracture risk and recommended calcium and vitamin D.
Table 1  Sillence classification for clinical types of OI

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<thead>
<tr>
<th>OI Type</th>
<th>Clinical feature</th>
<th>Inheritance</th>
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<tbody>
<tr>
<td>I &quot;Mild&quot;</td>
<td>Normal stature, little or no deformity, blue sclera, hearing loss in 50%, dentinogenesis imperfecta is rare</td>
<td>AD</td>
</tr>
<tr>
<td>II &quot;Lethal&quot;</td>
<td>Lethal in the perinatal period, minimal calvarial mineralisation, beaded fractures at birth, marked long bone deformities, platyspondyly</td>
<td>AD (new) AR (rare)</td>
</tr>
<tr>
<td>III &quot;Severe&quot;</td>
<td>Short stature, progressive deforming bones, wheel chair bound by adult life, scleral color varied — blush gray or white — often lightens with age, dentinogenesis imperfecta and hearing loss common</td>
<td>AD AR (rare)</td>
</tr>
<tr>
<td>IV &quot;Moderate&quot;</td>
<td>Normal sclera, mild to moderate bony deformity, variable short stature, dentinogenesis imperfecta and hearing loss common</td>
<td>AD</td>
</tr>
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AD = autosomal dominant, AR = autosomal recessive.

supplementation, weight bearing exercise programme and to avoid contact sports such as rugby. We also suggested that annual bone densitometry examination would be useful in monitoring his progress. This result satisfied the mother as she could now take an informed decision, even though it was disappointing to the boy.

The bone densitometry result however did not necessarily indicate underlying OI and raised further questions on sensitivity and specificity of low BMD in identifying family members with OI. An extensive literature search in 1992 showed that very little was known about bone densitometry in OI as most of the OI research focused on the genetic abnormalities of type 1 collagen. We therefore studied systematically the bone mineral density in families by investigating all the OI in Cornwall.

By reviewing the records of the Departments of Orthopaedics and Rheumatology at the Royal Cornwall Hospital, Truro, which serves the county of Cornwall, and confirming with the Brittle Bone Society, we identified 10 families with OI in Cornwall. There were 21 clinically affected people. With a catchment population of 360,000, the prevalence of OI in Cornwall was established to be at least 5.8 per 100,000. All family members were invited for an interview, clinical examination and bone mineral density (BMD) studies. The family and fracture history was ascertained and the examination included musculoskeletal examination, scleral color, presence of deafness, dentinogenesis imperfecta and hypermobility.

All the family members from the 10 families (43 total individuals) were divided into three groups. Group A consisted of 21 patients with osteogenesis imperfecta by clinical criteria (nine males aged 9 to 79 and 12 females aged 5 to 66). Group B consisted of 14 clinically unaffected siblings or first degree relatives (eight males aged 7 to 63 and six females aged 15 to 41) and group C consisted of eight clinically unaffected parents or partners (four males aged 34 to 62 and four females aged 37 to 63).

The patients with OI were clinically classified according to Sillence second (table 1). Thirteen of the 21 patients were Sillence type I, 7 were Sillence type IV and 1 was Sillence type III. All subjects from the three groups underwent bone densitometry examination of the lumbar spine (Hologic QDR 1000). Because of the spectrum of age, the spinal BMD in each individual was compared with age matched normal controls (Hologic reference range) and expressed as a Z score, which is the number of standard deviations away from the expected mean for that age.

The mean Z scores for spine (L1 to L4) were for Group A −2.66 (SD 0.79, range −4 to −1.48), for Group B −0.87 (SD 1.43, range −2.81 to 1.86) and for Group C 0.05 (SD 0.52, range −0.6 to 0.99) (fig 1). All patients with osteogenesis imperfecta (Group A) had negative Z scores. Only one patient had Z score between −1 to −1.5, 7 patients had Z scores between −1.5 to −2, 13 patients had Z scores worse than −2. Six of 14 clinically unaffected first degree relatives from group B had Z scores worse than −1.5. All the unaffected partners who formed group C had Z scores within +/−1.

In patients with OI, the Z scores did not show any significant differences in various Sillence groups, though the numbers were small.

We decided to test the hypothesis that the individuals from group B (clinically unaffected first degree relatives of patients with OI) with lumbar spine Z scores lower than −1.5 were in fact “subclinical” cases of OI. A large three generation family with OI (fig 2) and also the family discussed above were invited for further genetic and biochemical diagnostic tests.

After an informed consent, blood samples and dermal punch biopsy samples were collected from all available members of these two families. In the family shown in figure 2, the genetic linkage analysis showed that all subjects with a history of fractures had inherited the mutant COL1A1 allele but two subjects without fractures and normal bone density had not. The 15 year old (fig 2, star) without fractures but significantly reduced bone mass (lumbar spinal Z score −1.5) had inherited the mutant allele. DNA from the cultured fibroblasts showed that a null allele at COL1A1 was the cause of OI in this family and the individual with low bone mass but no history of fractures...
had inherited the abnormality from her mother. There were clear reductions, compared with controls in the ratio of type-I:type-III collagen exported from skin fibroblasts cultured from several affected members from this family.

However, in the family described earlier in the case report, the results of genetic linkage analysis and collagen biochemistry were inconclusive.

Discussion

In its most obvious form with multiple fractures, musculoskeletal deformities, blue sclerae and strong family history, OI is an easy clinical diagnosis. However, diagnosing OI can be difficult in two clinical scenarios. At one extreme, a young child with no family history of OI but sustaining multiple fractures with reportedly “mild” trauma raises the issue of differentiating between “non-accidental” injury (child abuse) and OI. At the other extreme, a person with a family history of OI but who has not sustained any fractures raises the question whether they have inherited the disease or not.

The problem of differentiating between child abuse and OI has been discussed elsewhere and is not the focus of this Masterclass. We want to discuss the question of diagnosing OI in a clinically asymptomatic person.

People with mild (Sillence type I) OI may not fracture till late in life and it is known that female patients with OI have a higher risk of fractures before menarche and after menopause presumably because of the protective effect of oestrogen on skeleton during the reproductive years. Absence of fractures until late adulthood therefore cannot rule out the diagnosis of OI. Positive family history, clinical clues on examination and awareness of the possibility can help clinicians reach correct diagnosis. When the clinical diagnosis is in doubt, bone densitometry, genetic tests as well
as specialised collagen biochemical investigations could help to reach a conclusion (fig 3).

**HISTORY AND PHYSICAL EXAMINATION IN THE DIAGNOSIS OF OI**

The importance of detailed history taking and physical examination cannot be overstated. With rare exception, OI is transmitted in an autosomal dominant fashion. Family history of fractures with minor trauma as well as fracture rates (number of fractures per year) are important clues to the diagnosis of OI, which are unfortunately missed too often. Physical examination by a medical geneticist is thought to be as diagnostic as more expensive specialised biochemical tests on collagen from cultured fibroblasts. Steiner carried out studies on collagen synthesis in 48 children to distinguish child abuse from OI. Only six children had biochemical evidence of OI and in five of those six, the diagnosis was strongly suspected clinically by physical examination alone. The sixth child had clear signs of abuse and the biochemical tests were thought to be false positive. Diagnosis of OI was not subsequently made in any child in whom it was not clinically suspected or in whom biochemical findings were normal. They concluded, “comprehensive clinical evaluation by a knowledgeable physician is still adequate for the diagnosis (of OI)”. It is however noteworthy that all subjects in this study had sustained multiple fractures and it is uncertain whether physical examination alone could diagnose OI in an asymptomatic individual who has sustained no fractures but has a family history of OI.

**ROUTINE BIOCHEMISTRY AND RADIOLOGY IN OI**

The routine biochemical tests such as serum calcium, phosphorus, alkaline phosphate, vitamin D level and urinary calcium excretion are normal in patients with OI. However, these and other investigations remain an important part of a comprehensive examination of patients with unexplained osteoporosis and can reveal other metabolic causes unsuspected by clinical examination alone. Chenes studied mineral homeostasis in 47 children with OI during periods of clinical stability and controlled calcium intake. They found evidence of hypercalciuria in 36% children and the group with hypercalciuria was significantly shorter and had a greater lifelong fracture rate. It therefore appears that hypercalciuria occurs frequently in children with OI and its magnitude reflects the severity of the disease.

Patients with mild OI can have normal looking bones on radiography and therefore plain radiographs cannot rule out the diagnosis of OI. In type I OI, radiographic findings can vary from being “normal” or showing osteopenia, to gross abnormalities such as cod fish or biconcave vertebra, tumoral callus at fracture site and basilar invagination. Type II or the lethal form has radiological findings, which are generally characteristic of OI. These infants have long bones with multiple fractures that result in accordion or concertina appearance. The cranial vault is extremely under-osseified. Thin slender “beaded” ribs with multiple fractures and vertebral body compression fractures are also seen. Type III patients are severely affected with OI and have progressive musculoskeletal abnormalities. Wormian bones, under-osseified calvarium, angular deformities of long bones in extremities have all been described. A progressive cystic change in the epi-metaphyses is seen in some patients with type III, described as “popcorn” calcification. Type IV patients often have radiological features similar to type I but can also approach type III in severity.

**BONE HISTOMORPHOMETRY AND BONE BIOCHEMISTRY IN OI**

Both, bone biochemistry and histomorphometry tests show evidence of reduced bone formation and increased bone resorption in patients with OI (table 2).

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Salient features</th>
<th>Comment</th>
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<tbody>
<tr>
<td>Clinical</td>
<td>H/O repeated fractures with minimal trauma, positive family history, blue sclera, hypermobility, dentinogenesis imperfecta, pre-senile hearing loss, short stature, long bone deformities</td>
<td>Mild cases have subtle signs and family history may be missed unless specifically asked for</td>
</tr>
<tr>
<td>Genetic</td>
<td>1 Linkage analysis: 2 Direct mutation detection</td>
<td>Large family with clear separation in normal and abnormal subjects is needed for linkage analysis. Mutation analysis is labour intensive, expensive and not 100% sensitive</td>
</tr>
<tr>
<td>Routine and “Bone” Biochemistry</td>
<td>1 Routine investigations (serum calcium, phosphorus, alkaline phosphatase etc.), 2 Biochemical markers of bone turnover: serum osteocalcin, urinary collagen cross links, n-telopeptides, etc</td>
<td>Routine bone biochemistry is always normal, except increased urinary calcium excretion is in nearly half. Bone turnover (osteocalcin) and resorption (collagen cross links) is increased</td>
</tr>
<tr>
<td>Specialised collagen biochemistry</td>
<td>Fibroblast culture and collagen biochemistry</td>
<td>Studies on collagen produced by cultured fibroblasts are “gold standard” but can be normal in 10%–15%</td>
</tr>
<tr>
<td>Bone histomorphometry</td>
<td>Double tetracycline labelled iliac crest biopsy</td>
<td>Reduced trabecular and cortical volume, reduction in new bone formation</td>
</tr>
<tr>
<td>Radiological</td>
<td>Osteopenia, under-osseified calvarium, concertina appearance of long bones, basilar invagination, cod-fish vertebra, wormian bones, popcorn ossification</td>
<td>Plain radiographs can be normal and hence cannot be used for “ruling out” OI</td>
</tr>
<tr>
<td>Bone densitometry</td>
<td>Lumbar spine, femoral neck and whole body bone densitometry by dual energy x ray absorptiometry</td>
<td>Very useful test. Most patients have low BMD, but sensitivity unclear</td>
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</table>
In a histomorphometry study on iliac crest biopsies from nine children with OI, Baron 1 showed that the inability of osteoblasts to synthesise normal quantity of bone was compensated by increase in the number of osteoblasts. They also found that there was a high bone turnover (as shown by increased hydroxyproline excretion) with a persistently negative bone balance. Ste-Marie 2 confirmed the reduced bone formation in a histomorphometric study on 11 patients though they did not find increased resorption. Glorieux 3 analysed iliac crest bone biopsies from 44 OI patients and compared them with 36 age matched controls. They showed that there was an increase in the osteoclastic activity (reduced cortical width and trabecular thickness) and a reduction in new bone formation rate.

The increased bone turnover as well as increased bone resorption rate has been confirmed by bone biochemical tests. In a study on 17 pre-pubertal OI patients with no recent fractures, plasma osteocalcin (marker of bone turnover) was significantly increased as compared with age matched controls. 4 In another study, bone resorption measured by urinary collagen cross linked peptides was above 75th percentile of controls. 5

As the results of the histomorphometric or the bone biochemical tests are non-specific, these tests cannot be used for diagnostic purposes in patients with OI.

BONE DENSITOMETRY IN OI
Since 1992, few studies have examined the role of BMD measurements in the diagnosis of OI but it is still controversial whether all patients with OI have osteoporosis. There are several problems with the diagnosis of “osteoporosis” in children as the WHO definition of “more than −2.5 SD below peak bone mass (T score)” cannot be applied to children who have not yet to attain the peak bone mass. Also, patients with OI have small stature and therefore the results of BMD need to be “adjusted” for body size. There are no “normal” data available for the femoral neck BMD in children and hence the Z and T scores in studies are usually calculated based on a very small sample of healthy children. Apart from our own experience, 6 only one other study has looked at the BMD measurements in asymptomatic OI patients. In a study on nine children, Zionts 7 reported that children with mild OI had significant reduction in spinal and hip BMD as compared with age and sex matched controls. All but two patients with OI had sustained multiple fractures and the controls also had sustained a single upper extremity fracture, though with significant trauma. In the two OI patients who had never sustained a fracture, the diagnosis of OI was based on other clinical signs such as blue sclera. These two children had spinal BMD that was 17% and 26% lower than their case controls. As the OI children were not compared with a larger control group, Z scores are not available.

During a cross sectional study on BMD in 132 healthy school children aged 5 to 13, Davie 8 assessed nine children with type I OI. The vertebral area, BMC and BMD were all significantly low in OI patients compared with healthy subjects and the mean spinal Z score was −2.52. However, two of the nine OI children had had lumbar spine or femoral neck BMD within “normal range”. The percentage of healthy children with BMD worse than 2 SD below the mean for their age was not given. A more recent study by Moore 9 examined paediatric patients referred to their hospital with multiple fractures with no known cause. A detailed history and examination led to two age and fracture rates matched groups: 12 patients with OI and 14 patients with “not OI”. To detect the ability of DXA to differentiate between OI and “not OI”, they compared the spine and whole body BMD in these two groups. The OI group had a significantly lower BMD in spine (p<0.002) and whole body (p<0.0004). When boundaries of −2 Z scores were used, the spinal BMD gave a sensitivity of 91.7% and the whole body BMD gave a specificity of 100%. These figures suggest that spinal and whole body BMD measurements could be useful in differentiating between OI and child abuse.

Even though children with OI have low BMD, the rate of bone accrual may not be reduced. Reinus 10 retrospectively evaluated vertebral mineralisation rates (change in BMD Z scores per year) in 27 children with mild OI. None of the subjects had clinical or radiographic evidence of vertebral fractures. They found that paediatric patients with OI mineralise their vertebrae at rates similar to healthy children. However, the OI children still lagged behind in overall mineralisation, as the process could not keep up with the rapid increases in the vertebral volume with growth.

GENETIC AND SPECIALISED COLLAGEN BIOCHEMICAL TESTS TO DIAGNOSE OI
The genetic techniques to diagnose OI remain expensive, insensitive and are not readily available. Two types of gene-based diagnoses are used based either on genetic linkage or direct mutation detection—each with its advantages and limitations. Genetic linkage analysis requires a clear family history with family members divided unambiguously in “affected” and “unaffected”. A mutant allele is first identified in an affected member, confirmed by its presence in other affected members and absence in unaffected members. 11 The asymptomatic person is then tested for the presence or absence of this allele by analysis of genetic markers. However, two genes, COL1A1 and COL1A2 encode the three chains of type 1 collagen and mutant allele in any one gene can lead to OI.

Direct detection of mutation does not require extensive family history and the method concentrates on the search for mutation to one area of the gene. The success rate at best is around 50%, and the costs remain prohibitive because of the labour intensive test.

Another diagnostic tool is to study the biochemical abnormalities in the type 1 collagen produced by cultured fibroblasts from

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[1-11] References not visible in the text.
dermal punch biopsies. Various types of collagen genes represent about 5% of the proteins synthesised by fibroblasts. Labelling of the cells with radioactively labelled proline can enrich the signal from collagen relative to other synthesised proteins. The synthesised procollagen molecules are separated on the basis of molecular weight by SDS-polyacrylamide gel electrophoresis. A second procollagen aliquot is treated with pepsin that removes the amino and carboxy terminal peptides before analysis on the SDS-polyacrylamide gel electrophoresis. Examining the distribution of labelled proteins between cultured medium (secreted) and the cells can monitor the efficiency of collagen secretion. The most common biochemical abnormality seen in the fibroblasts from type I OI patients is the underproduction of the structurally normal collagen. Other clinical types of OI (table 1) may show qualitative defects in which populations of structurally abnormal and normal molecules are synthesised. However, cells from 10% to 15% of people with non-lethal forms of OI seem to synthesise normal amount of collagen the structure of which seems normal. Also, the whole process including growing fibroblasts in culture typically takes around three months. Hence this investigation cannot be used in settings where a quick decision is to be made between OI and child abuse. Despite these drawbacks, this technique is the current “gold standard” laboratory test for the diagnosis of OI and is being used in some parts of the United States for collagen defects screening. The use of genetic testing or specialised collagen biochemistry tests in daily clinical practice are not indicated considering the costs involved. However, in difficult cases with diagnostic dilemma, they could be used after consultation with specialists.

IDIOPATHIC JUVENILE OSTEOPOROSIS AS A DIFFERENTIAL DIAGNOSIS OF OI

As the name suggests, idiopathic juvenile osteoporosis (IJO) is a rare disorder of childhood and is of unknown aetiology. No genetic abnormality has been found in these cases, though minor non-glucocorticoid or defects in collagen gene control could conceivably cause IJO. It almost always involves spine and can also cause fractures of long bones leading to difficulty in walking. Positive family history is not a feature of IJO, and in fact such history should raise the possibility of OI. The largest series of 21 patients of IJO by Smith failed to show any abnormalities of collagen biochemistry and serum concentrations of vitamin D metabolites though on bone histological examination, excessive osteocytes were seen in the trabecular and cortical bone. Bone densitometry studies in IJO show uniformly low bone density at the lumbar spine. In contrast with OI, most cases show a dramatic recovery by adolescence (puberty) with spontaneous reconstruction of biconcave vertebral deformities.

CONCLUSIONS

Diagnosis of OI in an asymptomatic person with a positive family history can be difficult. Clinical signs such as blue sclera and dentino-osseous imperfection even in the absence of fractures are diagnostic and signs such as short stature and hypermobility would increase the likelihood of the diagnosis. Bone densitometry is emerging as the investigation of choice because of its non-invasive nature and easy availability. The sensitivity and specificity of low BMD is still unclear, but a normal or above normal bone density would be helpful to assure the patient and the doctor. If the clinical examination and the bone densitometry results are equivocal, specialised tests in collagen biochemistry or the genetic investigations should be considered after consultations with a specialist.

We would like to thank Dr Bryan Sykes, Dr Roger Smith and their coworkers at the Institute of Molecular Medicine, Oxford, where the genetic linkage analysis and collagen biochemistry work was done in 1994. We would like to thank Dr Robert Steiner of the Oregon Health Sciences University, Portland, OR, for his helpful comments on the manuscript.