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G E N E T I C C O U N S E L L I N G I N S E V E R E

O S T E O G E N E S I S I M P E R F E C T A

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To Philip and Alexandra

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ABSTRACT

Osteogenesis imperfecta (OI) is a heterogeneous group of disorders of connective tissue which principally manifest as osteoporosis and bone fragility. It is likely that monogenic defects of collagen underlie these conditions.

The present study addresses the problem of giving genetic counselling to parents of a 'sporadic' case of severe OI, either of the perinatally lethal or severe deforming variety. Such cases are likely to represent either fresh dominant mutations with no recurrence risk to sibs or autosomal recessive inheritance, with a 25% recurrence risk. Occasionally, parental gonadal mosaicism for a dominant mutation may also account for recurrences in sibs.

The main existing clinical and genetic classification of OI by David Sillence and his colleagues is extremely useful, but has limitations, due to heterogeneity within types and clinical overlap between types. Severe progressively deforming OI can be autosomal recessively inherited (Sillence type III) or autosomal dominantly determined (Sillence type IV); sporadic cases cannot be precisely allocated to either type. The present study overcomes this dilemma by pooling together a type III/IV group and determining the empirical recurrence risk to sibs. This is 4.4% (6 affected sibs out of 135 sibs of 104 cases). In these families, the parents are unrelated. In an additional 4 families, the parents are consanguineous and as there is other

evidence for autosomal recessive inheritance in these families, their disease is presumed to be recessively determined.

A source of confusion between the perinatally lethal (Sillence type II) and the severe deforming (Sillence type III/IV) categories is that the time of death may overlap. The present study suggests that a solution to this dilemma is to classify cases radiologically (rather than by time of death), as soon after birth as possible. In addition, the degree of radiological abnormality at birth can predict the prognosis, to some extent.

Originally, Sillence and his colleagues suggested that all 3 perinatally lethal types (IIA, IIB and IIC) were autosomal recessively inherited. The recurrences in sibs in the type II groups in the present series are: type IIA, no recurrences in the 38 sibs of 30 cases; type IIB, one affected sib of 13 sibs of 15 cases; type IIC, no affected sibs of the 3 sibs of 3 cases. For the type IIA group, the data support the suggestion of other authors that the majority of cases arise by new dominant mutations, and so the risk of recurrence to sibs is small. The occasional reports of sib recurrences probably can be attributed to parental germinal mosaicism. The present data and other evidence for types IIB and IIC OI means that autosomal recessive inheritance still cannot be ruled out.

Detailed clinical, radiological and family data are included in order to demonstrate the manifestations in the various types of OI to which the recurrence risk figures apply. No reliable

distinguishing features between the sporadic and familial cases of severe deforming OI (type III/IV) are found. It is not possible to pinpoint parents as being heterozygotes for recessive forms of OI on clinical grounds.

The outcome of collaborations with colleagues in molecular genetics and biochemistry are described.

STATEMENT BY THE AUTHOR

a) This thesis contains no material which has been accepted for the award of any other degree or diploma in any University. Neither does it contain, to the best of the author's knowledge and belief, any material previously published or written by another person, except where due reference is made in the text.

b) The author consents to the thesis being made available for photocopying and loan if applicable.

E. M. Thompson

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CHAPTER 1

INTRODUCTION

1.1 OI is an 'old' disease

Examples of osteogenesis imperfecta (OI) dating to ancient times are found in fact and fiction. Probably the oldest documented case is that of an Egyptian mummy from the 21st Dynasty, circa 1000 BC. The body, now housed in the British Museum, is that of an infant whose skeletal deformities are characteristic of OI (Gray, 1969). Wells (1965) described a deformed and fractured femur of an older child which was recovered from the site of a Saxon cemetary at Burgh Castle, Suffolk. The bone, estimated to be nearly 1500 years old, probably showed evidence of OI.

Another possible early description of OI concerns Ivar the Boneless, a mythical Danish warrior prince living in the 9th Century AD. He had to be carried into battle on a shield because he could not walk on his soft legs. He was the oldest son of Ragnar Ladbrock who had approached his wife's bed with unseemly haste one night, for which misdemeanour Ivar was conceived (Leader, BMJ 1957).

Some authors ascribe the first description of OI to Malebranche in 1678 (quoted by Seedorff, 1949) who described a male of 20 years with multiple fractures. An intriguing idea about aetiology was attributed to Muys in 1751 (quoted by Seedorff,

1949) and concerns 'a child who was born at terme to a woman who had seen an execution upon the wheel. The child had all its members broken, like those of a thief'. The more recent history of understanding of OI is given in section 1.6.

1.2 The problem in brief: genetic counselling in severe sporadic OI

Despite the immense progress in the understanding of OI, which is now considered to be a group of monogenic disorders involving the collagen genes, genetic counselling remains difficult in isolated cases with severe disease. Such cases are likely to represent either dominant new mutations with no recurrence risk to sibs, or autosomal recessive inheritance with a 25% recurrence risk. (Occasionally, parental gonadal mosaicism for a dominant mutation may also account for recurrences in sibs). At the present time, there is no reason to assume that any particular clinical sub-group has just one pattern of inheritance. It is this genetic counselling dilemma in single cases of severe OI which the present study addresses.

1.3 What is OI?

OI can be defined as a group of heritable disorders of connective tissue, involving bone, skin, ligaments, tendons, fascia, sclerae, middle and inner ear, teeth and the heart (McKusick, 1972). As its name suggests, the most dramatic and usually the most important clinical manifestation results from osteoporosis

leading to bone fragility. Fractures occur with minor trauma or even spontaneously. Appropriately, those affected often refer to the disorder as 'brittle bones'. Bone deformity is another sequela of osteoporosis; the long bones may become bowed and twisted and the base of the skull and vertebral bodies flattened (platybasia and platyspondyly, respectively). Other common manifestations include blue sclerae, dentinogenesis imperfecta leading to discoloured, fragile teeth which wear and chip readily, deafness, joint laxity and short stature. Multiple small bony islands of the skull (Wormian bones) are often seen radiographically. Skin laxity, tendency to bruising, herniae and cardiac valvular lesions also occur.

1.4 The differential diagnosis of OI

It is important to be aware of the differential diagnosis of OI in order to make the correct clinical diagnosis of OI and reduce the heterogeneity in genetic studies. The differential diagnosis of OI varies with age.

1.4.1 Differential diagnosis prenatally and at birth

1.4.1.1 Differential diagnosis of short-limbed dwarfism

Short-limbed dwarfism noted at birth or detected prenatally on ultrasonography, may be due not only to severe OI, but also to a variety of disorders such as hypophosphatasia, achondrogenesis, thanatophoric dysplasia, Jeune's asphyxiating thoracic dystrophy,

chondroectodermal dysplasia or achondroplasia. All of these have well recognised radiological characteristics (Wynne-Davies et al., 1985) and are not considered here in detail.

1.4.1.2 OI-like syndromes with additional anomalies presenting at birth

Reports exist of cases with 'OI' at birth but with additional defects, so that they may not represent true OI. Remigio and Grinvalsky (1970) described 2 sibs, a male and a female, both of whom had multiple fractures at birth, blue sclerae and Wormian bones, together with mucoid degeneration of the aortic media and heart valves. The boy also had long fingers, subluxation of one ocular lens and coarctation of the aorta. He died on day 32 of cardiac failure; his sister died at 16 hours and had suffered an intracerebral haemorrhage. There was no family history of OI and the parents were unrelated.

Buyse and Bull (1978) reported 3 sibs, a girl and 2 boys with characteristic features of 'OI congenita' at birth. In addition, all 3 had microcephaly and cataracts; other defects included single umbilical artery, cardiac abnormalities, low-set ears, small genitalia and enlarged cerebral ventricles. One was stillborn and the others died soon after birth. The parents were well and unrelated.

Saint-Martin et al. (1979) described 2 sons of unrelated parents. Both babies were stillborn and had curvature of long bones,

multiple fractures and absent ossification in the cranial vault. Additional manifestations which would be unusual in OI included micrognathia, cleft palate and syndactyly. One child had an omphalocoele.

Finally, McGillivray et al. (1985) described 2 male sibs of unrelated parents. The first was born preterm and had short-limbed dwarfism, osteoporosis and Wormian bones. Additional features were a horseshoe kidney, anal agenesis and ambiguous genitalia. The second sib was diagnosed with detailed ultrasound scanning during the second trimester and the pregnancy was terminated. Similar abnormalities to those in the brother were present.

1.4.2 Differential diagnosis in infancy

During infancy, non-accidental injury (NAI) can present with multiple or recurrent fractures. Differentiation from OI can be difficult, especially if other signs of OI or a positive family history are lacking; blueness of the sclerae is an unhelpful sign in early infancy since it is commonly found in normal babies (Paterson, 1977, 1978; Smith et al., 1983).

NAI is, however, commoner than OI (Taitz, 1987) and there are some features which may help to differentiate the two conditions. These include other evidence of injury with NAI, such as bruising, torn frenulum of the lip or retinal haemorrhages. Fractures of the ribs and scapulae, and at the metaphyses of the

long bones may occur in NAI but are rare in OI, in which the diaphysis is usually involved (Carty, 1988). Delay in seeking medical attention may also suggest NAI. Even in mild OI, some degree of osteoporosis should be identifiable radiologically and Wormian bones are an almost constant finding in OI (Cremin et al., 1982).

Two rare disorders with fragile bones presenting in infancy are pyknodysostosis and osteopetrosis. Similarities with OI include Wormian bones, blue sclerae and scoliosis in the former and short stature in both. Unlike in OI, however, both show radiologically dense bones.

Idiopathic hyperphosphatasia (juvenile Paget's disease) is a very rare condition which presents in infancy with deformed, fragile bones and blue sclerae; the plasma alkaline phosphatase is raised.

1.4.3 Differential diagnosis in childhood and adolescence

During childhood and adolescence, any cause of osteoporosis may mimic OI. Idiopathic juvenile osteoporosis usually manifests in the prepubertal growth spurt and is often difficult to distinguish from OI especially if the latter is mild with normal sclerae and no family history of OI. Idiopathic osteoporosis is usually self-limiting and various radiological features may be associated with it, for example, a linear area of rarefaction in

the metaphysis (Smith et al., 1983). The presence of multiple Wormian bones would favour a diagnosis of OI.

1.4.4 Differential diagnosis in adult life

Occasionally, mild OI may be diagnosed for the first time in adult life. For example, osteoporosis may worsen and become clinically obvious in a post-menopausal woman with mild OI.

1.4.5 Disorders with unusual features which are probably part of the OI spectrum

Beighton (1981) reported on 20 members of a 3 generation South African family who had dentinogenesis imperfecta, blue sclerae and Wormian bones. Unusually, only one of the 20 had fractures. A similar, but sporadic case was reported by Crawfurd and Winter (1982).

Levin et al. (1985) described 12 individuals from 2 families and one sporadic case with osteoporosis and recurrent fractures, normal sclerae and teeth but with multilocular lesions which were radiolucent, radiopaque (or both) in the maxilla and mandible. In 1988, Levin et al. described a family with OI type I in which affected individuals had unusual dental abnormalities, which included oval pulp chambers with apical extensions into the coronal portions of the roots of the permanent teeth.

1.4.6 Disorders similar to, but probably distinct from OI

Other conditions with similar features to OI are more difficult to include as OI syndromes. For example, Hall and Shaw (1985) reported on a 12 year old boy with blue sclerae, multiple Wormian bones, deafness, osteoporosis and multiple fractures. In addition, there were bilateral triphalangeal thumbs, duplication of the terminal phalanx of the left ring finger, syndactyly, camptodactyly, poor callus formation around fractures and multiple pseudarthroses.

Another case was a girl described by Feingold et al. (1980) with short stature, bony abnormalities and excessive callus formation who died at 7 years. The authors were uncertain as to whether this could be classified as OI.

Beighton et al. (1985) described a consanguineous South African family of Indian origin in which 4 brothers and 2 cousins had 'severe OI together with blindness due to hyperplasia of the vitreous, corneal opacity and secondary glaucoma'. The sclerae were white. Robinow (1985), however, suggested that the family suffered from the autosomal recessive osteoporosis - pseudoglioma syndrome.

Heide (1981) also described an unusual, probably recessive disorder resembling OI in 3 sibs born to unrelated parents. The syndrome consisted of osteoporosis, fractures, Wormian bones, frontal bossing, macrocephaly, short fingers, joint laxity,

congenital blindness and oligophrenia. The latter two manifestations would be unusual in OI.

1.4.7 Co-existence of OI and other connective tissue disorders

Occasionally, patients with features of OI and other known syndromes have been described. Carey et al. (1968) reported a family with 23 cases of OI in 4 generations. The proband had features of both OI (fragile bones, blue sclerae, dentinogenesis imperfecta and deafness) and the Marfan syndrome (tall stature, arachnodactyly and aortic insufficiency). However, the authors could not rule out inheritance of the Marfan syndrome from the mother and of OI from the father. Another case of OI was said to have features of the Marfan syndrome (Meigel et al., 1974). The evidence for the latter was minimal however, consisting of a narrow thorax, thin limbs and mild arachnodactyly. The patient, an 8 year old boy, had recurrent fractures and was a sporadic case in his family. His parents were first cousins.

Sippola and Prockop (1983) studied a patient who combined features of mild OI and Ehlers-Danlos syndrome with blue sclerae, bilateral hip dislocations, joint laxity and Wormian bones. The patient had not had fractures but other members of his family had. Biering and Iversen (1955) described a boy with blue sclerae and fractures who also had marked joint hyperextensibility and loose wrinkled skin, and suggested that he had both OI and Ehlers-Danlos syndrome. Cutis laxa, however, is

another possible diagnosis.

1.4.8 Differential diagnosis of non-skeletal manifestations of OI

Extra-skeletal manifestations of OI may be the presenting feature. These include blue sclerae, discoloured teeth, deafness or joint laxity. The London Dysmorphology Database (Winter et al., 1984) lists 31 disorders (many of which are very rare) which may be associated with blue sclerae. Most are clearly distinguishable from OI; others show more overlap. For example Greenfield et al. (1973) described keratoconus, blue sclerae, middle ear bony conduction defect and spondylolisthesis in a brother and sister born to consanguineous parents and reviewed 11 similar cases from the literature. Walker (1971) described blue sclerae, myopia, occasional keratoconus, arachnodactyly, loose-jointedness and progressive sensorineural deafness in at least 3 generations of a family. MacLean et al. (1986) reported on a father and daughter with blue sclerae, Wormian bones, mandibular hypoplasia, shallow glenoid fossae and campomelia. They called the disorder 'the Grant syndrome' after the family.In all 3 reports, the main differentiating feature from OI was lack of fractures.

There are several abnormalities of teeth which resemble those in OI; these have been variously classified. Shields et al. (1973) defined two types of dentin dysplasia and three types of dentinogenesis imperfecta (DI), all of which are autosomal

dominantly inherited. Type I DI occurs in OI; type II DI, which is also called hereditary opalescent dentine, occurs without bone disease and is linked to Gc (Ball et al., 1982). (The latter is discussed in section 6.7.8). Amelogenesis imperfecta is a group of disorders of enamel rather than dentin (McKusick, 1988).

1.5 Variability - a hallmark of OI

A striking feature of OI is the wide spectrum of clinical manifestation. At one extreme, severity may be so great as to lead to preterm delivery of a stillborn infant; at the other, affected individuals may lead a normal life.

Biochemically, much evidence has accumulated that defects in collagen underlie OI. Heterogeneity is also apparent at this level and a variety of abnormalities in collagen genes and in collagen protein synthesis have been described (see section 1.9). Use of the term the 'Brittle Bone Syndromes' (Smith et al., 1983) seems justified and the likening of OI to the thalassaemia syndromes and haemoglobinopathies appropriate (Byers et al., 1984).

1.6 OI as a problem of classification - a historical review

The heterogeneity of OI is reflected in a bewildering profusion of synonyms and clinical classifications. Of interest in this regard is the history of understanding of OI. Early descriptions

focussed on anatomical and clinical aspects, followed later by details of gross pathology and histopathology. Genetic classifications began to emerge during the last 40 years and biochemical studies involving collagen have proliferated since around 1975. Some milestones in the development of understanding of knowledge about OI are given in appendix 1.1, with associated synonyms.

The very early history of OI is outlined in section 1.1. The first documentation of hereditary bone fragility was made by Ekman in 1788. The account concerned a family with 'osteomalacia congenita' affecting members of three generations. Interestingly, no mention was made of blue sclerae or deafness. Early descriptions concentrated on bone fragility; extra-skeletal manifestations were gradually added to the phenotype. The first suggestion that the disorder is a 'generalised hypoplasia of the mesenchyme' was made by Eddowes in 1900 and was supported by the work of Bauer in 1920. Attempts at clinical classification began with Looser's (1906) division of OI into congenita and tarda groups, according to the time of the first fractures (see table 1.1). Seedorff (1949) suggested that the clinical features of fractures, blue sclerae and deafness were inherited independently by 5 closely linked dominant genes. For example, cases of severe OI congenita could have inherited 3 bone fragility genes and the gene for blue sclerae, whereas the tarda cases could have 1 or 2 bone fragility genes, the gene for blue sclerae and in some cases the gene for presenile hearing loss. However, it is now generally accepted that in a given case, a single gene defect is

Table 1.1

SOME CLASSIFICATIONS OF OI AND PROPOSED MODE OF INHERITANCE

Looser (1906)

- OI congenita (fractures at birth)
- OI tarda (fractures after birth)

Seedorff (1949)

- OI congenita (fractures before birth)
- OI tarda
  - gravis (fractures at birth)
  - levis (fractures later after birth)

Fairbank (1951)

- Thick bone
- Slender bone
- Cystic bone

Cocchi (1964)	Mode of inheritance
Trias der fragilitas ossium hereditaria (fractures, blue sclerae, deafness)	AD
Osteogenesis imperfecta letalis Vrolik (lethal form with fractures)	AR
Osteopsathyrosis Ekman-Lobstein (fractures without other symptoms)	AR] AD] forms

Ibsen (1967)

Bone fragility and blue sclerae	AD
Lethal OI congenita (lethal by 2 yr)	AR
Bone fragility without other symptoms	AD
Bone fragility, normal sclerae	AR

Bauze et al. (1975)

- Mild
- Moderate
- Severe

Sillence et al. (1979a & b (see table 1.2)

AD - autosomal dominant

AR - autosomal recessive

more likely to underlie the various connective tissue manifestations of OI (McKusick, 1972).

A popular early view held by workers from the United Kingdom (Bell, 1928), Japan (Komai et al., 1956), Italy (Caniggia et al., 1958) and Sweden (Smars, 1961) was that all the clinical features of OI were accounted for by variable expressivity of only one autosomal dominant gene. More recently, other authors (Cocchi, 1964; Ibsen 1967; Sillence et al., 1979a and b) concluded that there were several genetically separate OI syndromes with overlapping clinical features (see tables 1.1 and 1.2). The existence of several dominant and recessive OI syndromes was suggested.

1.7 The Sillence classification

The most useful and widely known classification of OI to emerge is the one devised by David Sillence and his colleagues in 1979 (Sillence et al., 1979a and b). Based on clinical and genetic criteria, the authors divided OI into four main types (table 1.2): Types I and IV were autosomal dominantly inherited; sclerae were blue in type I and normal in type IV. Deformity was generally mild in type I but in type IV deformity was variable and could be severe. Types I and IV OI were subdivided later into A and B groups, based on the absence or presence of dentinogenesis imperfecta (DI), respectively (Sillence, 1981); this followed observations that DI segregates in some families but not in others (Levin et al., 1978). Type II OI, the

Table 1.2

CLASSIFICATION OF OI AND PROPOSED MODE OF INHERITANCE ACCORDING TO SILLENCE ET AL., 1979b*

Type	Clinical features	Mode of inheritance
I	Mild deformity Blue sclerae Presenile hearing loss in some	Autosomal dominant
IA	Normal teeth	
IB	Dentinogenesis imperfecta	
II	Perinatally lethal	Autosomal recessive
IIA	Thick femurs, continuously beaded ribs	
IIB	Thick femurs, discontinuously beaded ribs or no beads	
IIC	Irregularity of bone	
III	Progressively-deforming	Autosomal recessive
	Sclerae blue at birth and fade or normal from birth. Dentinogenesis imperfecta in some	
IV	Variable deformity	Autosomal dominant
	Normal sclerae	
IVA	Normal teeth	
IVB	Dentinogenesis imperfecta	

* Types I and IV subdivided into A and B groups by Sillence et al., 1981 (based on work by Levin et al., 1978, 1980).

Type II subdivided into A, B and C groups by Sillence et al., 1984.

'perinatally lethal' form, was subsequently subdivided into three radiological sub-groups by Sillence et al. in 1984. The authors suggested 'that most cases of OI type II represent autosomal recessive traits'. Patients with type III OI developed severe progressive deformity and had pale blue or normal sclerae. It is of interest that other authors have also noted that patients with white or normal sclerae tended to have severe deformity of long bones and the spine, fractures which occurred earlier and more often, dentinogenesis imperfecta and severe disability, in contrast to those with blue sclerae who tended to have a milder disease and were more likely to have affected relatives (Bell, 1928; Smars, 1961; Bauze et al., 1975).

Although all 21 patients with type III OI in Sillence and his colleagues' original paper were sporadic (1979b), the authors suggested that autosomal recessive inheritance was likely, based on consanguinity of parents in 2 families and on previous reports of affected sib pairs. Whilst recognising that some cases may be due to autosomal dominant new mutation, type III OI was shown as autosomal recessive in their table 4, and this has become widely quoted (Smith, 1983; McKusick, 1988). Subsequently, the authors saw 5 families with either multiple affected sibs or parental consanguinity (Sillence et al., 1979a; Sillence, 1981). More recently Sillence et al. (1986) have described 8 sibships in 7 families with multiple affected sibs (7 sibships) or consanguineous parents (1 sibship); 2 were being re-reported. Having originally classified type III OI on clinical grounds of severe progressively deforming OI, they now use the criterion of

being recessive to redefine the range of severity in type III OI, so that 'the OI type III phenotype does not necessarily equate with progressively deforming OI, and probably only a proportion of cases with severe deformity and normal sclerae have OI type III'.

Classifications are useful if they provide guidelines for genetic counselling, prognosis and treatment. To date, no classification of OI satisfactorily fulfils these functions. It may well be that classifications of OI based on clinical criteria alone will never do so, and that clinical criteria combined with information about defects of the collagen genes or the protein itself will be more useful in the future.

1.8 Limitations of the Sillence classification for genetic counselling

The Sillence classification provided a useful focus on genetic sub-groups of OI. Genetic counselling is straightforward in cases with a family history, but for single cases, particularly those with severe disease, the classification has limitations, due to heterogeneity within types and clinical overlap between types. Indeed, at the 3rd International Conference on OI (Pavia, September, 1987) Sillence summarised 361 families with OI studied to date and noted that 112 had OI type I, 68 OI type II, 8 OI type III, 87 OI type IV; the remaining 86 sporadic cases were not able to be classified into any of these groups because they were too young or because type III and IV OI could not be

distinguished (Sillence, 1988).

1.8.1 Severe progressively-deforming OI

Strong evidence exists for heterogeneity within the severe deforming group. Whilst there are instances of probable autosomal recessive severe deforming OI, with reports of affected sibs and of single cases in a sibship or cousins with consanguinous parents (see table 1.3), it is noteworthy that several studies record a paucity of affected sibs (Young and Harper, 1980; Wynne-Davies and Gormley, 1981; Cohen et al., 1984) or note that the majority of severe OI are sporadic (Smith et al., 1975). Furthermore, Sillence and colleagues recently noted heterogeneity in the phenotype of cases with autosomal recessive OI (Sillence et al., 1986) as described in section 1.7.

Single cases of type III and IV cannot be reliably distinguished because of overlapping clinical features between the two types and there are no clinical or radiological features which allow identification of an isolated case of recessive type III OI (Sillence, 1982). In other words, the phenotype does not necessarily predict the inheritance pattern in individual cases, which makes genetic counselling impossible in sporadic cases of severe OI.

Table 1.3

FAMILIES REPORTED WITH PROBABLE AUTOSOMAL RECESSIVE SEVERE DEFORMING OI (SILLENCE TYPE III OI)

Affected sibs

Glanzmann	1944*
Kaplan and Baldino	1953* (C)
Adatia	1957*
Lievre	1959 (C)
Awwaad and Reda	1960* (C)
Horan and Beighton	1975 (**)
Sillence et al.	1979a, 1986
Aylsworth et al.	1984
Robinson et al.	1987
Brons et al.	1988

Single cases with consanguineous parents

Rohwedder	1953*
Maloney	1969 (affected cases in 2 branches of family)
Nicholls et al.	1984
Sillence et al.	1979b, 1986

* Indicates that these cases could possibly be classified as Sillence type IIB, since X-rays taken in the first week were not presented. In the others prolonged survival or perinatal X-rays suggest type III OI. The importance of X-rays taken in the first week for classification is discussed in section 4.1.2.

(C) parents consanguineous

(**) two sisters married an uncle and a nephew

1.8.2. Perinatally lethal OI

1.8.2.1 Mode of inheritance

In their original study, Sillence et al concluded that 'some, if not all' cases of type II OI showed autosomal recessive inheritance (Sillence et al., 1979b). Likewise, when type II OI was later subdivided into 3 forms, designated A, B and C, segregation analysis suggested that all 3 of these subgroups were the result of autosomal recessive inheritance (Sillence et al., 1984). Although a number of sib pairs with perinatally lethal OI have been reported (see table 1.4), several studies have noted a deficiency of affected sibs of probands (Spranger et al., 1982; Cohen et al., 1984). A postal survey conducted in the United Kingdom in 1979-1980 produced information on 79 cases born with multiple fractures. Of these, 15 had thick bones and died; none of their sibs were affected (Young and Harper, 1980). This seemed to confirm the impression that, in the United Kingdom at least, affected sib pairs with perinatally lethal OI were very uncommon, suggesting that the majority of cases arise as a result of new autosomal dominant mutation rather than from autosomal recessive inheritance.

1.8.2.2 Overlap of type II with type III OI

In addition to the confusion over the likely mode of inheritance of perinatally lethal OI, there have been suggestions that the distinction between types II (particularly IIB) and type III OI is invalid. This will be discussed in section 4.1.2.3.

Table 1.4

FAMILIES REPORTED WITH MORE THAN ONE SIB WITH SILLENCE TYPE II OI

Type of OI	Author(s)	No. affected in sibship	Parental consanguinity
IIA	Dinno et al., 1982	2	No
	Shapiro et al., 1982*	2	No
	Le Freche et al., 1977**	2	No
IIB	Hein, 1928	1	Yes
	Goldfarb and Ford, 1954	2	No
	Zeitoun et al., 1963***	2	Yes
	Chawla, 1964	4	No
	Braga & Passarge, 1981	3	No
	Stephens et al., 1983	2	No
	Patel et al., 1983****	2	?
	Elejalde & Elejalde, 1983*****	2	No
	Ghosh et al., 1984	3	Yes
	Sillence et al., 1984	2	No
	Brons et al., 1988	2	Yes
IIC	Sillence et al., 1984	3	Yes
	Danks, 1975	2	Yes
	Brons et al., 1988	2	No

* Byers et al. (1988a) reported 5 sibships with recurrent type II OI. Gonadal mosaicism (rather than recessive inheritance) was likely in 3 of these, including 2 sibships with type IIA OI, one of which was described by Shapiro et al., 1982.

** Le Freche et al. described stillborn female twins whose radiographs had the type IIA OI appearance. The authors stated that the twins were monozygotic but the grounds for this assumption were not given.

*** Could possibly be classified as type III OI, as the first radiograph shown was taken at 26 days.

**** The first affected sib, in addition to OI, had cleft lip and palate, and anencephaly.

***** The first affected sib also had polydactyly, which is a common finding in Negro people, the race of this family. The second affected sib had amniotic bands leading to finger amputations.

1.9 The biochemical basis of OI

1.9.1 Defects in collagen cause OI

As long ago as 1900, Eddowes suggested that OI is due to 'hypoplasia of mesenchyme' (Eddowes, 1900). A half a century later, McKusick proposed that defects in collagen metabolism were associated with inherited connective tissue disease (McKusick, 1972). In 1975, Penttinen et al. produced the first evidence of this in noting reduced type I collagen production by cultured cells from patients with various forms of OI compared to control cells. Since then, remarkable progress has been made in studying the biochemical basis of disorders of connective tissue and it is now clear that the majority of cases of OI are attributable to genetic defects in type I collagen.

The following sections (1.9.1 - 1.9.4) are taken from reviews by Prockop and Kivirikko (1984), Cheah (1985), Prockop (1985), Prockop et al. (1979) and Byers and Bonadio (1985).

1.9.2 The collagen family

Collagen is among the most abundant proteins in the body and it is one of the largest. It forms the major structural component of the extracellular matrix. To date, some 13 different types of collagens have been identified (Shows et al., 1989).

Collagenous molecules contain triple helical regions characterised by the presence of a basic repeating unit of amino acids consisting of -Gly-X-Y-. A variety of amino acids may occupy the X and Y positions, but proline and hydroxyproline often occupy the X and Y positions respectively. This triple unit is essential for the formation of a semi-rigid triple-helical molecule composed of three chains. The basis for categorizing the different collagen types is the presence and structure of both globular (non-collagenous) domains and interchain disulphide bonding.

Types I, II and III collagens form fibrils and so are referred to as fibrillar collagens. Type I collagen is the major constituent of bone, skin, tendons, ligaments and dentin. Type II collagen is the predominant collagen of cartilage. Type III is a less abundant collagen but is usually found in association with type I. Type IV collagen is the most common constituent of basement membranes. Type V is found in association with blood vessels and smooth muscle cells. The other collagens are found in small amounts in specific tissues.

1.9.3 Structure and function of type I collagen

Type I collagen accounts for about 90% of collagen in the body (Eyre, 1981). It is comprised of 2 identical polypeptide chains called α1(I) and a third chain with a slightly different amino acid sequence, called α2(I). The 3 chains are wrapped around each other in a triple helix, similar to a 3-stranded rope. The

major biological property of the protein is that it spontaneously self-assembles under physiological conditions into long, thin fibrils which have about the same tensile strength as steel wires.

In the triple helical region there are 338 tandem repeats of the Gly-X-Y tripeptide. Glycine is the smallest amino acid and therefore can occupy the axial position. As mentioned above, X and Y are often proline and hydroxyproline which are rigid, cyclic amino acids that limit rotation of the polypeptide backbone and thus contribute to the stability of the triple helix. The remaining X and Y positions are occupied mainly be charged and hydrophobic amino acids. These occur in clusters along the surface of the molecule and direct the self-assembly of the collagen molecule into a quarter-stagger array which accounts for the characteristic cross-striations of collagen fibrils. After fibrils are assembled, covalent cross-links are formed among adjacent molecules which give the fibrils the required tensile strength.

1.9.4 Biosynthesis of type I collagen

Although the structure of this collagen is relatively simple, its biosynthesis is extremely complex (table 1.5). The molecule is first synthesised as a larger precursor called procollagen, which is secreted into the extracellular matrix and which must be cleaved at both ends to produce collagen. The enzymes procollagen N-proteinase and procollagen C-proteinase cleave the

Table 1.5

BIOSYNTHESIS OF TYPE I COLLAGEN

INTRACELLULAR

COLA1 and COL1A2 genes transcribed

MRNA's translated on ribosomes on rough ER
Post-translational processing
'Signal' peptides cleaved from amino-termini
prolyl and lysyl residues converted to hydroxy forms
hydroxylysyl residues substituted with galactose
or glucosyl galactose.
Manose rich oligosaccharide added to carboxy-terminal propeptide.

Chains assemble and are disulfide linked at carboxy-terminal propeptides
Protein folds into triple helix, propagating from the carboxyl to the amino-terminus
Secretion of procollagen to extracellular matrix

EXTRACELLULAR

Procollagen molecule cleaved at each end to form **collagen**
Self-assembly of collagen **fibrils**
Cross-linkage between fibrils

amino- and carboxyl-terminal propeptides, respectively. If the amino-propeptides are not cleaved off, fibrils can form but they are thin and irregular and become inadequately cross-linked. If the carboxyl-terminal propeptide is not cleaved, the large carboxyl-terminal propeptides prevent the protein from assembling into fibrils. Cleavage of procollagen and assembly of collagen into fibrils may occur within crypts or folds of fibroblasts (just outside the cells) or may occur at some distance from them.

The intracellular part of the process is also complex. The pro α-chains of procollagen are synthesized by translation of the appropriate mRNA's on ribosomes attached to the rough endoplasmic reticulum (ER). As newly-assembled chains pass into the cisternae of the rough ER, a series of post-translational processing steps occur. 'Signal' peptides are cleaved from the amino-termini of all chains, prolyl and some lysyl residues in the Y-position are converted to their hydroxy from by enzymes and hydroxylysyl residues are substituted with galactose or glucosyl-galactose. A mannose-rich oligosaccharide is added to the carboxyl-terminal propeptides. After the chains are assembled, carboxyl-terminal propeptides associate and are disulfide linked. Post-translational modifications of prolyly and lysyl residues continue. Once about 100 hydroxyprolyl residues have been made, the protein folds into a triple helix, beginning at the carboxyl-terminal ends of the chains, propagating the helix towards the amino-terminus. About 8 enzymes are involved in the intracellular processing and assembly of procollagen and they

modify over 100 sites per chain.

The procollagen molecule is then secreted into the extracellular matrix and cleaved at both ends to form collagen. Formation of fibrils then occurs and these become cross-linked. Cross-linkage is initiated by oxidative de-amination of selective lysine and hydroxylysine residues followed by complex interactions of the resulting aldehyde groups with side-chains of amino acids on adjacent molecules.

1.9.5 Genes for type I collagen

The $\alpha 1$ and $\alpha 2$ chains for human type I procollagen are encoded at the unlinked loci COL1A1 and COL1A2 on chromosomes 17 and 7 respectively (Heurre et al., 1982; Henderson et al., 1983). The human COL1A1 and COL2A1 genes were cloned only recently (Myers et al., 1981, 1983; Dalgleish et al., 1982; Chu et al., 1984). The former is approximately 18,000 bases and the latter approximately 38,000 bases. Both contain 51 exons, but the COL1A1 gene has smaller introns. A distinctive feature is the presence of 54 base-pair (bp) exons which code for 18 amino acids with the sequence Gly-X-Y (Yamada et al., 1980; Tate et al., 1982). A few exons are 45 bp and a few are 108 bp (twice 54). It has been suggested that collagen genes arose by duplication and recombination of an ancestral 54 bp gene (Yamada et al., 1980, 1984; Chu et al., 1984). The 54 bp exon is common to the fibrillar collagens (Cheah, 1985) but it is not seen in some other collagens, e.g. type IX (quoted in Prockop, 1985) so that

the true primordial origin of this large gene family remains uncertain.

1.9.6. Clinical correlations of biochemical defects in OI

The first structural mutation in one of the constituent chains of type I procollagen was demonstrated in 1981, in a baby with type IIA OI (Barsh and Byers, 1981). Since then, there has been a proliferation of work on the biochemical basis of OI, so that a picture is emerging to account for its clinical heterogeneity. Reviews on this subject have been prepared by Pope et al. (1983), Byers and Bonadio (1985), Prockop and Kivirikko (1984), Cheah (1985), Prockop (1985), Sykes (1985), Sykes and Smith (1985), Tsipouras and Ramirez (1987), Sykes (1987) and Byers et al. (1988a).

1.9.6.1 Autosomal dominant OI - Sillence types I and IV

1.9.6.1.1 Mutations in the α1(I) chain

In 1977, Bryan Sykes and his colleagues noted that skin of patients with probable type I OI (and of others with severe OI) contained a decreased proportion of type I compared to type III collagen which they proposed was due to decreased synthesis of type I collagen by the cells. Studies of synthesis of type I procollagen by Barsh et al. (1982) and Fraser et al. (1983) showed that production of type I procollagen was about half the normal level whilst that of type III procollagen was normal in

patients with type I OI. In addition, it was demonstrated that synthesis of pro α1(I) chains was about half the normal level while synthesis of pro α2(I) chains appeared normal (Barsh et al., 1982). This decreased synthesis of proα1(I) chains was due to lowered levels of mRNA (Rowe et al., 1985). A heterozygous non-functioning allele for pro α1(I) was suggested to be the cause of this defect (Byers and Bonadio, 1985).

Not all mutations in type I OI are of this 'null allele' type. A patient with mild autosomal dominant OI, who was originally studied by Nicholls et al. (1984b), synthesised type I collagen molecules which contained a cysteine residue in the carboxyl-terminus of the α1(I) chain (Steinmann et al., 1986). At first, it was thought that the cysteine was within the triple helical domain (where cysteine residues are not normally found) and previously, the presence of cysteine in the triple helix had been associated only with perinatally lethal disease. Subsequently, however, it was demonstrated that there was a substitution of cysteine for glycine which in fact lay just outside the triple helical domain, in the carboxyl-terminus (Cohn et al., 1988). Another patient with moderately severe OI also had a cysteine for glycine substitution, but in this case, the substitution was within the triple helical domain, but was at the amino-terminal end (de Vries and de Wet, 1986). The significance of these findings will be discussed in section 1.9.6.5.3.

1.9.6.1.2 Mutations in the α2(I) chain

Defects in the α2(I) chain may also produce a similar phenotype. Byers et al. (1983b) demonstrated a mutant α2(I) chain in a woman with mild to moderate OI who had blue sclerae. Another boy and his mother with 'atypical' OI and blue sclerae were found to have a 19 base pair deletion in one COL1A2 gene (Sippola et al., 1984; Kuivaniemi et al., 1988). Wenstrup et al. (1988) described yet another mutation in the α2(I) chain of a mother and son with mild to moderate OI and blue or blue-grey sclerae (which the authors called type IV OI). The mutation was a substitution of arginine for glycine at position 1012, the last triple helical glycine in the α2(I) chain. This resulted from a single nucleotide change in one COL1A2 gene. The type I molecules which contained the mutation were over-modified. This can be explained by the fact that chains which are not in a triple helical structure are available for post-translational modifications, but once a stable triple helix is formed, post-translational modifications cease. In the present example, type I collagen molecules which have incorporated a chain carrying the mutation were over-modified because the mutation presumably impairs helix formation at its origin (helix formation proceeds from the carboxyl-terminus, that is, at amino acid 1014, to the amino-terminus). The type I collagen molecules were of normal thermal stability however, unlike those in most perinatally lethal OI patients (see section 1.9.6.2).

1.9.6.1.3 Linkage studies

Further evidence that defects in the chains of type I collagen underlie autosomal dominantly inherited OI has come from linkage studies. In 1983, Tsipouras et al. demonstrated linkage of a polymorphic marker for the COL1A2 gene to the disease locus in a 4 generation family with mild autosomal dominant OI. As additional markers at COL1A2 were discovered and more families were tested, discordant and concordant families with Sillence types I and IV OI emerged (Tsipouras et al., 1984; Grobler-Rabie et al., 1985; Wallis et al., 1986; Falk et al., 1986).

When markers at the COL1A1 locus were tested in addition to those at COL1A2, Sykes et al. (1986) found no examples of discordance at both loci in 11 families with Sillence types I and IV OI. More recently, a further 38 pedigrees were analysed in a collaborative study (Sykes et al., in press). All 8 families with Sillence type IV OI segregated with COL1A2. On the other hand, Sillence type I OI segregated with both COL1A1 (17 pedigrees) and COL1A2 (7 pedigrees). The concordant locus was uncertain in the remaining 6 type I pedigrees, but again, no family was shown to be discordant at both loci. Interestingly, presence or absence of presenile hearing loss was the best predictor of the mutant locus in type I OI families, with 13 of 17 COL1A1 segregants and none of 7 COL1A2 segregants showing this feature.

It is possible and indeed likely that the mutation in each family

is different, as is evident from the examples cited. Nevertheless, prenatal diagnosis or predictive testing in asymptomatic individuals at risk can be carried out using the linkage information in suitable families. (Sykes and Ogilvie, 1988).

1.9.6.2 Perinatally lethal OI - Sillence type II

1.9.6.2.1 Heterozygosity for a deletion in the α1(I) chain

Biochemical studies are mostly compatible with Sillence type II OI arising from new dominant mutations in the genes of type I collagen (Byers et al., 1988a,c). The best characterised example concerns a baby with probable type IIA OI. The infant was originally described by Heller et al. (1975) and Penttinen et al. (1975); reduced collagen synthesis was noted. Fibroblasts cultured from the baby were found to synthesise two distinct pro α1(I) chains, one normal and one abnormal (Barsh and Byers, 1981). Subsequently, a deletion of about 500 base pairs was shown in one pro α1(I) allele (Chu et al., 1983). The reading frame was retained, however, allowing synthesis of a shortened pro α1(I) chain, which could form a triple helix, albeit of greatly reduced stability (Williams and Prockop, 1983; Barsh et al., 1985; Chu et al., 1985). Prockop (1984) referred to this phenomenon of 'wastage' of the normal chains as 'protein suicide'.

Another baby with perinatal OI with a de novo deletion in one pro α1(I) chain has been studied (Dalgleish, personal communication, 1989). The deletion involves 3 residues in the triple helical domain of one pro α1(I) chain, consistent with a 9 base pair deletion in one COL1A1 allele.

1.9.6.2.2 Heterozygosity for a substitution in the α1(I) chain

The most common type of mutation described to date in perinatally lethal OI involves an abnormal α1(I) chain which contains a single amino acid substitution in the triple helical domain. Such mutants have been described in 3 babies with probable type IIA OI (Steinmann et al., 1982, 1984, 1988; Cohn et al., 1986; Bateman et al., 1987a; Vogel et al., 1987) and in 4 others (including 2 half-sibs) with unspecified perinatally lethal disease (Bateman et al., 1987b, 1988; Constantinou et al., 1989; Cohn et al., 1989). These have mostly arisen apparently 'de novo' in the proband (the exception being the half-sibs studied by Cohn et al., 1989 who are described in section 1.9.6.2.8).

In the case of Steinmann et al., the mother had the Marfan syndrome. Collagen produced by her cells did not, however, show the substitution mutation.

An unusual feature in the case of Constantinou et al. was that although the type I procollagen produced by the proband's parents' fibroblasts did not contain the substitution mutation,

the mother's type I procollagen was partly overmodified and had a lower thermostability. The mother was asymptomatic but was somewhat short and she had slightly blue sclerae. The authors concluded that the substitution was a fresh dominant mutation in the proband, but that a mutant type I procollagen allele inherited from the mother may have contributed to the lethal phenotype. These substitution mutants are considered further in section 1.9.6.5.

1.9.6.2.3 Heterozygosity for an insertion in the α1(I) chain

A third type of mutation in perinatally lethal OI concerns a baby with type IIA OI. Studies on tissues from the baby showed that there was an insertion of 50-70 amino acids in the triple helical domain of half the α1(I) chains synthesised (Byers et al., 1988b). This is consistent with a duplication of a segment of about 600 base pairs in one COL1A1 allele. Unlike the deletion and substitution mutation cases, the chains were not overmodified for reasons which are not immediately clear.

1.9.6.2.4 Heterozygosity for a deletion in the α2(I) chain

A fourth type of mutation in perinatally lethal OI involves a heterozygous deletion in the pro α2(I) chain (Willing et al., 1988). A 4.5 kilobase pair deletion in a paternally-derived COL1A2 allele was demonstrated in the proband. This removed 7 exons which code for residues 586-765 of the triple helical domain. Although the abnormal chains were incorporated into the

triple helix, procollagen molecules containing these chains were not secreted. Neither parent carried the deletion.

1.9.6.2.5 Genetic compounds

Some cases involve possible genetic compounds. A deletion from the triple helical domain of all the proα2(I) chains together with a reduced rate of synthesis of proα2(I) chains in the same patient was reported by de Wet et al. (1983). The phenotypically normal father also demonstrated a reduced rate of synthesis of proα2(I) chains. The authors suggested that the child inherited the gene for reduced rate of synthesis from the father and that the gene for the shortened proα2(I) chains arose as a new mutation. Further work on the latter showed that the mutation was in fact a single base substitution which caused efficient splicing of mRNA from the last codon of exon 27 to the first codon of exon 29 of COL1A2, allowing synthesis of a shortened but in-frame proα2(I) chain (de Wet et al., 1986; Tromp and Prockop, 1988).

Knisely et al. (1988) reported a baby with radiological type IIA OI who had an abnormal karyotype, 46XY, inv(7)(p13q22), which he inherited from his healthy mother. The authors suggested that since the breakpoint lies in the region of the COL1A2 gene, the proband could represent a compound heterozygote for a mutation in COL1A2 together with another unidentified abnormal allele. The latter could have been a fresh mutation or inherited from the healthy father.

Pope et al. (1984) found that 6 babies with perinatally lethal OI had a 300 base pair deletion in an αI-like collagen gene. The healthy parents of 4 of these were tested and one of each pair was also found to carry the deletion. Three of the 4 families were Asian. The authors proposed that the probands represented genetic compounds. Subsequently, however, it was suggested that the deletion (which is in fact in the gene for type II collagen) is nothing more than a length polymorphism, commonly seen in normal Asian Indian and West Indian populations (Sykes and Ogilvie, (1984); Sykes et al., (1985)).

1.9.6.2.6 Heterozygosity for abnormal α(I) chains - mutation not defined

In other cases, the precise mutation has not been defined, but heterozygosity for abnormal α chains has been deduced from the finding of delayed mobility of half the α1 or α2 chains (Bateman et al., 1984; Bonadio et al., 1985; Bonadio and Byers., 1985; Byers et al., 1988c). This results from increased post-translational modification which occurs if formation of the triple helix is impaired.

1.9.6.2.7 Homozygous mutants

Only one report found homozygous mutants (Uitto et al., 1983). These concerned 2 patients with type II OI who appeared to synthesise only lengthened proα1(I) chains.

1.9.6.2.8 Gonadal mosaicism

The evidence to date strongly supports the notion that most cases of perinatally lethal OI result from new dominant mutations. This does not, however, exclude a risk of recurrence. Byers et al. (1988c) in studying 3 families with recurrent perintally lethal OI in a sibship, found that the data were compatible with the affected infant being heterozygous for a mutation in a type I collagen gene whereas each parent was homozygous for normal collagen genes in somatic cells. This suggests that the recurrences can be accounted for by gonadal mosaicism in one parent in each family. In one of these families, the healthy mother had 3 affected babies by 2 unrelated spouses (Horwitz et al., 1985) which makes gonadal mosaicism in the mother highly likely. Similarly, Cohn et al. (1989) described a father of 2 offspring with type II OI who had different mothers. The mutation in the offspring was identified as a point mutation in the 1(I) chain which led to an aspartic acid for glycine substitution at position 883 of the triple helix. The mutation disrupted a BglI recognition site, providing a method to assay for the presence of the abnormal allele in family members. The mutation was absent in somatic DNA from the father and other family members. In contrast, the father's sperm exhibited two DNA populations, one with and one without this mutation, providing direct evidence for gonadal mosaicism.

1.9.6.2.9 Mouse mutants

Support for the concept that the mutations described above are in fact the cause of the disease comes from the work of Stacey et al. (1988). They produced mutant pro α1(I) mouse genes containing either a cysteine or an arginine substitution for glycine at position 859 of the triple helical domain. This was introduced into transgenic mouse embryos and resulted in a dramatic lethal phenotype which clinically and biochemically resembled the lethal perinatal OI phenotype in humans. In addition, as little as 10% mutant gene expression could disrupt normal collagen function.

1.9.6.3.0 Concluding comment

There is increasing biochemical and molecular evidence that most type II OI cases arise as fresh dominant mutations. For future research, it would be important for biochemical studies to be undertaken on families with affected sibs with type II OI, and for clinical and radiological details to accompany scientific reports, to further facilitate identification of any clinical and biochemical correlations.

1.9.6.3 Severe deforming recessive OI-Sillence type III

1.9.6.3.1 Homozygosity for a deletion in the α2(I) chain

Biochemical studies on well defined cases of type III OI are few

in number. The best characterised example is described by Nicholls et al (1979, 1984a). The patient secreted a procollagen which did not contain α2(I) chains. Proα2(I) chains appeared transiently within cultured skin fibroblasts in diminished quantities but were not incorporated into the collagen triple helix (Deak et al., 1982, 1983). Nuclease S1 mapping and Southern blotting located an 18 base pair deletion in the carboxyl-propeptide of the proα2(I) chain (Dickson et al., 1984). The patient was a homozygote for this defect and his consanguineous clinically normal parents were both heterozygotes.

1.9.6.3.2 Recessive OI unlinked to COL1A1 or COL1A2

Aitchison et al. (1988) reported on a child with severe deforming OI. He is case 90 in the present study. The child was born to consanguineous parents and the mother had had two affected sibs. Her parents were also related. Surprisingly, the disease gene appeared to be unlinked to either the COL1A1 or COL1A2 locus, since the patient was heterozygous for DNA markers linked to COL1A1 and COL1A2, whereas homozygosity for one locus would be expected. Although possible explanations include recombination between the gene and the marker, or that recessive inheritance is not operating, the most likely explanation is that the disease in this case is not due to a structural mutation in either locus. Instead, it is possible that the patient is homozygous for a defect in one of the enzymes which processes type I collagen. Although this is the first report of its kind, similar results have been described by Wallis et al. (1989) and Daw et al. (1988)

in three and one family with recessive OI, respectively.

1.9.6.4 Severe deforming sporadic OI - ?Sillence type III or IV

As mentioned previously, it is impossible to identify clinically a sporadic case of severe deforming OI as due to recessive or dominant inheritance. Tenni et al. (1988) studied several patients in this category. The cells of 3 patients synthesised 2 forms of α1(I) procollagens, one of normal electrophoretic mobility and the other more slowly migrating. (Although the healthy parents were not studied, the results suggest the possibility of a new dominant mutation in the probands). Further studies in one of these patients led the authors to suspect a heterozygous mutation in one pro α1(I) chain near the carboxyl-terminus of the triple helix or in the carboxyl-propeptide. The precise mutation was not identified, but interestingly, the extent of overmodification of the abnormal chains was not great and it also decreased with age, possibly owing to a normal diminution with age of the activities of the enzymes involved. An unusual aspect of this case was that proteoglycans from the cultured skin fibroblasts were abnormal in molecular size and composition. The explanation for this was unknown. In addition, hydroxyapatite crystals were deposited in the dermis, perhaps resulting from interaction between collagen and proteoglycans.

Francis et al. (1988) also described abnormalities of type I collagen formation, secretion or α chain migration in 11 of 13

patients with severe deforming OI, 9 of whom are in the present study (sporadic cases 15, 30, 45, 46, 48, 57, 60 and sibs 102 and 103). In one of these (case 57), the secreted α1(I) band split into a doublet, representing one normal and one abnormal chain. Again, this may be evidence for a heterozygous mutation.

1.9.6.5 Does the mutation predict the phenotype?

At the present time it is possible to explain in part the degree of severity of a particular OI phenotype on the basis of the underlying mutation (Sykes, 1987; Cohn and Byers, 1988; Byers et al., 1988a). The severity depends on the type of mutation, whether it is in the α1 or α2 chain, and its position in the chain.

1.9.6.5.1 'Included' and 'excluded' mutants

Some mutations allow the abnormal α chain to be incorporated into the procollagen molecule whereas others do not. Sykes (1985) referred to these as 'included' and 'excluded' mutants respectively, and pointed out that the former are likely to have a more deleterious effect on the phenotype than the latter. Examples are the case studied by Chu et al. (1983) in which a shortened proα1(I) chain was incorporated into the triple helix, thus rendering it very unstable, and resulting in its degradation (an 'included' mutant). The baby had lethal (type IIA) OI. By contrast the 'null allele' mutant described by Barsh et al. (1982) had mild OI (an 'excluded' mutant). The patient's

cells produced only normal α1 chains, albeit at half the normal level. A simple analogy is that a small wall made of normal bricks is stronger than a normally-sized wall made of faulty bricks!

1.9.6.5.2 Mutations in the α1(I) chain are usually more deleterious than those in α2(I)

Mutations in the α1 chains seem in general to be more harmful than those in α2. There may be several reasons for this. First, if α2(I) chains are absent, α1(I)$_3$ trimers can form which are similar to type I collagen. Survival without α1(I) chains is not possible, as demonstrated in mice homozygous for an abnormal α1(I) gene (Schnieke et al., 1983). Secondly, if an abnormal chain which is incorporated into the type I procollagen molecule (an 'included' mutant) involves the pro α1(I) chain, three-quarters of the procollagen molecules will be abnormal, whereas only one half will be abnormal if the mutation is in the pro α2(I) chain, owing to the fact that the procollagen I molecule is a heterotrimer of α1 and α2 chains in the ratio of 2:1.

1.9.6.5.3 The position of a mutation affects the phenotype

At least seven babies (2 of whom were half sibs) with type II OI have been described with single amino acid substitutions in the triple helical domain of the 1014 residue pro α1(I) chain, at positions 988 (Cohn et al., 1986), 748 (Vogel et al., 1987), 904 (Constantinou et al., 1989), 391 (Bateman et al., 1987a), 664

(Bateman et al., 1987b, 1988) and 883 (Cohn et al., 1988). All 6 mutations involve a substitution for a glycine residue; in the first 3, the substitute was cysteine, whilst in the next two, it was arginine and in the last, aspartic acid. The abnormal α1 chains are incorporated into the procollagen molecule, but show increased post-translational modification and the abnormal molecules have lowered melting temperature, secretion is delayed and degradation of the molecule is increased. The substitution for glycine, the only amino acid small enough to occupy the axial position in the triple helix, apparently slows the folding of the triple helix or reduces its stability beyond the mutation. Since the formation of the triple helix proceeds from the carboxyl terminus towards the amino terminus, it seems that substitutions of glycine to cysteine near the carboxyl terminus in the α1(I) chain (positions 988, 904 and 748 noted above) have lethal effects. Conversely, a patient with a glycine to cysteine substitution at position 526 of the pro α1(I) chain (that is, nearer the amino-terminus) survived but had severe bone deformity (Cohn and Byers, 1988). Arginine seems to be an even more damaging substitute than cysteine, with a lethal phenotype resulting from a mutation not only at position 664 but also at position 391 (Bateman et al., 1987a).

On the other hand, the less deleterious effect of a cysteine for glycine substitution found in a patient with mild autosomal dominant OI (Nicholls et al., 1984; Cohn et al., 1988; see section 1.9.6.1.1) may be explained by its location outside the triple helical domain. Similarly, another patient with moderately

severe OI with a cysteine for glycine substitution within the triple helical domain may have been spared a lethal phenotype because the mutation occurred near the amino-terminus, (de Vries and de Wet, 1986) which may have been less disruptive to helix formation.

These examples provide some evidence for the suggestion that there is a phenotypic gradient of decreasing severity as a given mutation passes from the carboxyl- to the amino-terminus of the triple helix of the α1(I) chain (Bonadio and Byers, 1985; Byers et al., 1988a).

The same explanations do not seem to hold true, however, for substitutions in the α2(I) chain, which were demonstrated in collagens produced by cells of a family with mild dominant OI (Wenstrup et al., 1988). In this case, an arginine residue replaced a glycine residue at the carboxyl-terminal end of the triple helix. Perhaps this is another example of a mutation being less deleterious when it is in the α2(I) chain, than if it were in α1(I).

In conclusion, it is now possible to make some correlations between the clinical phenotype and the biochemical basis of OI. Clearly, the rate of advancement of knowledge in this field during the last 24 years has been phenomenal (indeed, this summary of the literature to the end of 1988 will probably be outdated by the time it comes to press!). For the future, it would be particularly useful if biochemical analysis in each

individual sporadic case of lethal and severe deforming OI could identify those in whom the disease is autosomal recessively inherited, so that a precise risk could be given to individual families and a reliable prenatal diagnosis could be offered for future pregnancies at high risk. In those cases in whom a fresh mutation was suspected however, the possibility of parental gonadal mosaicism would need to be borne in mind.

CHAPTER 2

AIMS OF THE STUDY

2.1 Determination of empirical recurrence risks

The primary aim of the study was to assess the empirical recurrence risks in a large number of patients with the progressively-deforming and perinatally lethal forms of OI, in order to provide much-needed data for purposes of genetic counselling.

Such data could also allow conclusions to be drawn regarding the likely mode of inheritance of these various forms of severe OI.

2.2 Assessment of prognosis

Because of the overlapping phenotype between types II and III, and types III and IV OI it was aimed to determine what factors, if any, could predict prognosis at birth.

2.3 Search for factors to distinguish the autosomal recessive cases from true sporadic cases

Although previous studies had not identified any clinical or radiological factors which might predict precisely which sporadic severe cases were due to autosomal recessive inheritance, it was thought that careful study of a large group of patients might

reveal some previously unrecognised useful distinguishing clinical or radiological manifestations.

2.4 Clinical assessement of individual cases

It was aimed that the author would personally examine as many cases and their families as possible in order to:

1. Confirm the diagnosis of OI.

2. Assess the severity of the disease.

3. Classify cases according to the Sillence classification if possible.

4. See if there were any commonly occurring associated manifestations.

5. Assess first degree relatives (parents and sibs) for any evidence of OI or associated connective tissue manifestations such as blue sclerae, joint or skin laxity, dental anomalies, hearing loss or short stature.

6. Obtain detailed pedigree information.

2.5 Radiological assessment

In addition, it was essential to obtain all previous radiographs of affected individuals in order to:

1. Confirm the diagnosis of OI.

2. Confirm the presence of fractures at birth. (The relevance of this is discussed in section 3.1

3. Assess for any radiological manifestations which might distinguish any particular subgroups of cases.

4. Assess for presence and degree of progressive bone deformity.

It was proposed that all radiographs would be assessed by a Consultant Radiologist (Dr Christine Hall) and the author. Selected radiographs, including all taken perinatally of each patient would be copied and retained to provide proof of the diagnosis of OI and of the fractures at birth and to demonstrate any particular features.

2.6 Biochemical analysis

Collaboration was established with Dr Bryan Sykes, John Radcliffe Hospital, Oxford in order that analysis of the type I collagen structural genes in some cases with OI types II and III

could be undertaken. The goals were two-fold. First, analysis of the DNA would be undertaken to look for large rearrangements of COL1A1 in probands. Secondly, segregation analysis in suitable families would be carried out using DNA markers for COL1A1 and COL1A2. Since the families consisted of normal parents who had had one or occasionally more than one affected child, the aim was to see if, by comparing haplotypes for COL1A1 and COL1A2 markers of affected and normal sibs, exclusion of autosomal recessive inheritance at one or both loci was possible.

Collaboration was also arranged with Dr Martin Francis at the Nuffield Orthopaedic Centre, Oxford, so that collagens produced by cultured skin fibroblasts of some patients with severe progressively deforming OI could be studied.

CHAPTER 3

METHODS

3.1 Ascertainment of cases

The two main groups of patients to be studied were;

a) Survivors of the perinatal period with severe progressively-deforming OI who were born to normal parents.

b) Patients with perinatally lethal OI, defined as stillborn babies or those who died within one week of birth.

In order to maximise the case numbers different sources of ascertainment for the two groups are required, as will become apparent. Therefore, the study was divided into two sections. The author, in collaboration with Dr Ian Young (Consultant Clinical Geneticist, Leicester Royal Infirmary), Dr Christine Hall (Consultant Radiologist, The Hospital for Sick Children, Great Ormond Street, London) and Professor Marcus Pembrey (Professor of Paediatric Genetics, Mothercare Department of Paediatric Genetics, Institute of Child Health, London) conducted the study of the perinatal survivors. Dr Ian Young, with the same collaborators, including the author, coordinated the study of the cases of perinatally lethal OI.

3.1.1 Ascertainment of patients who survived the perinatal period

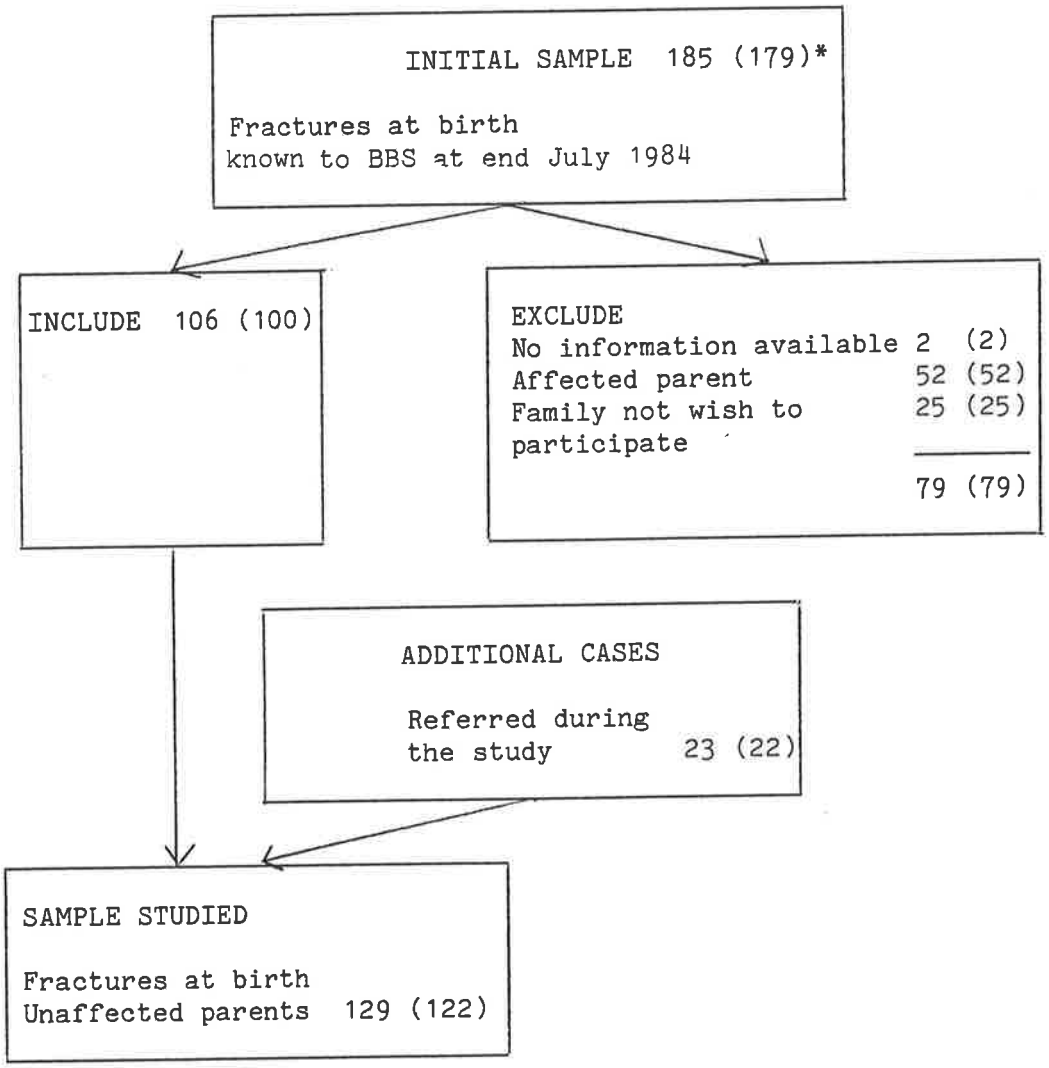
There is no single criterion which will include all patients with severe OI. Nevertheless, the majority of patients with severe OI will have fractures at birth (Beighton et al., 1983), making this the best inclusion criterion for this group of patients.

These patients were ascertained largely through the Brittle Bone Society (BBS), a support group in the United Kingdom for patients with OI and their families, founded in 1968. Patients known to the BBS include the majority of those with severe OI for two reasons. First, such patients and their families require much support in terms of physical aids and advice on management of daily activities. The BBS has a reputation for providing generously for these needs. Secondly, the BBS has developed an informal network of communication, so that new cases are rapidly brought to their attention and offered their services. On joining the BBS (which does not require payment), patients or their families complete a short questionnaire on certain clinical data, including whether or not fractures were present at birth.

The ascertainment of these cases is summarised in table 3.1. The initial sample of patients includes all patients known to the BBS to the end of July 1984, who had fractures at birth. These numbered 185 cases from 179 families. Two patients were excluded as no information about their pedigree was available. Fifty-two cases with proven autosomal dominant OI, that is with an affected

Table 3.1

ASCERTAINMENT OF PERINATAL SURVIVORS



* Cases (families)

parent, were excluded. The remaining families were approached by the committee of the BBS. Families of 25 patients (all single cases) did not wish to participate and so had to be excluded as there was no other avenue of access to information about them, since the BBS questionnaire does not include the names of the patient's general practitioner or hospital doctors. The 100 families of 106 cases who agreed to participate in the study were contacted by the author. During the course of the study, to June 1985, a further 23 cases (from 22 families) came to light and were studied. These were referred by clinical geneticists who knew of our interest in OI and 10 of these cases were born during the time of the study. Thus, the group studied who were born with fractures, whose parents were unaffected, number 129 cases from 122 families (table 3.1).

3.1.2 Ascertainment of patients who died perinatally

Ascertainment of these cases was through the records of the clinical genetics units throughout Great Britain which were invited to participate by providing details of families referred with a history of perinatally lethal OI. A total of 60 cases from 57 families were ascertained. Only 2 of these families were known to the Brittle Bone Society, which emphasises the need for separate ascertainment of perinatally lethal cases and cases surviving the perinatal period.

3.2 Collection of clinical data

3.2.1 Collection of clinical data on patients who survived the perinatal period (table 3.2)

The group studied numbered 129 patients from 122 families.

3.2.1.1 Families interviewed by the author

A total of 92 families were seen personally by the author, mostly during a home visit. In these 92 families there were 98 affected individuals. The interview with the family followed a standardised procedure. The protocol is shown in appendix 3.1 and is described as follows:

a. Medical history of the affected individual

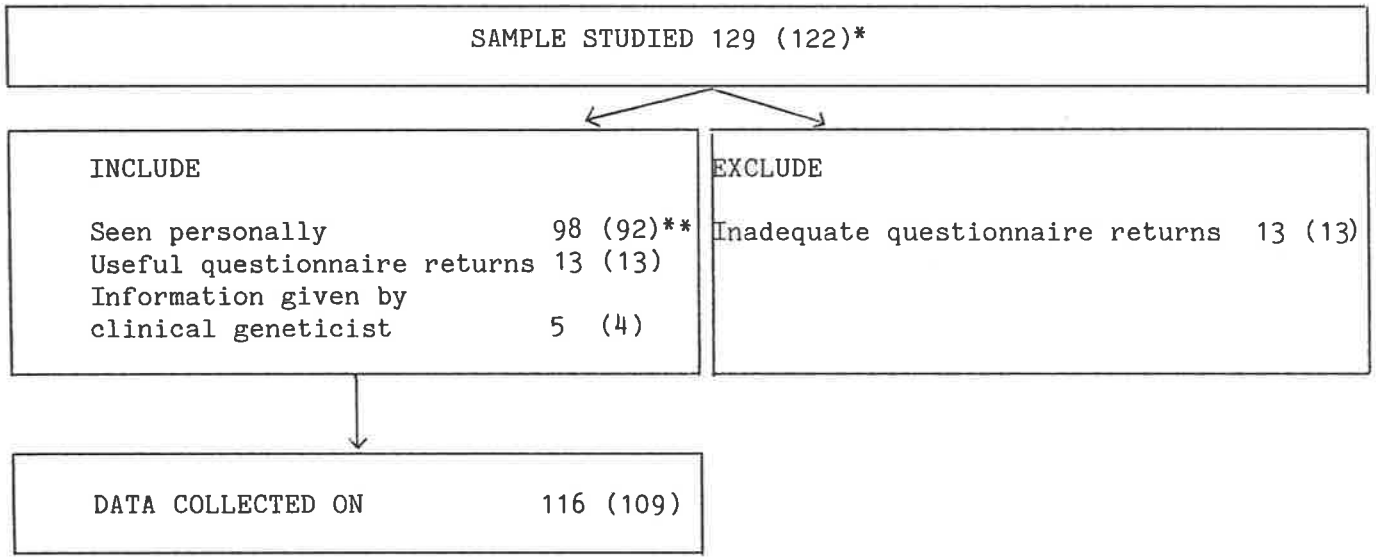
The information was usually obtained from the mother of the affected individual and included details about the pregnancy and delivery, and the number of fractures found to be present at birth. Further information included:

- fracture history, documenting the number of fractures since birth, their sites and frequency and the degree of trauma which caused them.

- scleral colour at birth and subsequently and any day to day variations.

Table 3.2

COLLECTION OF DATA ON PERINATAL SURVIVORS, WHO HAD FRACTURES AT BIRTH AND NORMAL PARENTS



* Cases (families)

** The 92 families of 98 cases were interviewed by the author but only 80 affected individuals were examined, since 18 had died.

- other manifestations of OI namely any unusual tendency to skin bruising, skin or joint laxity, joint dislocations, frequent epistaxis, history of cardiac murmurs, visual or auditory defects (and by whom these had been identified), any tendency towards excessive sweating, abnormalities in the colour or strength of the teeth, physical handicap, herniae, other general health problems, especially respiratory infections or significant constipation, and time of menarche when appropriate.

- other anomalies

- operative orthopaedic treatment which might have modified skeletal deformity.

b. Examination of the affected individual

Of the 98 cases, 80 were examined by the author and photographed when possible. The remaining 18 had previously died. For many of these, a photograph was available.

The aspects examined were as follows: (see also appendix 3.1)

Severity of disease

- height or length, and occipito-frontal head circumference.

- limb deformity. The upper and lower segments of all 4 limbs were examined and recorded as either straight, mildly bowed, moderately bowed or severely bowed.

- chest and spinal deformity.

Sclerae

- scleral colour was measured using a colour chart (appendix 3.2). An individual sclera varies somewhat in colour, and the darkest colour of the sclerae was scored as either white or one of four shades of blue (very pale blue, pale blue, moderate blue or deep blue). Sclerae which were white or very pale blue were designated normal, as has been done previously (Sillence et al., 1979b).

Other manifestations of OI were noted

- evidence of dentinogenesis imperfecta (discoloured or worn teeth).

- joint laxity. Initially it was proposed to score individual joints on both active and passive motion, and to obtain a total score. However, this proved to be unrealistic, as it was too time-consuming and many individual joints were difficult to score because of bone deformity. Therefore, joint laxity was scored for the small joints of the hand and for the larger joints (wrists, elbows, knees, ankles). A

positive score for the hand joints was obtained if several joints were hyperextensible on passive movement. Hyperextensibility was judged to be present if the interphalangeal joints extended beyond 180°, the first metacarpophalangeal (MP) joint extended beyond 90°, the 2nd to 5th MP joints extended beyond 200° or if the thumb could be flexed so that it came to rest on the forearm with ease. A positive score for each large joint was obtained if the wrist and ankle extended beyond 90° and the elbow and knee extended beyond 180°, respectively, on passive movement.

- skin laxity and texture were studied on the dorsum and palm of the right hand.

- skin bruising.

- cardiac murmurs.

- facial features of OI (broad forehead, flat maxillae, triangular-shaped face, prominent jaw).

- high-pitched nasal voice.

Other features examined

- evidence of strabismus.

- evidence of puberty. (It was often inappropriate to examine for this in the home).

All of this data is presented in appendix 6.1. The notes for appendix 6.1 contain further information on methods of data collection.

c. Assessment of parents and sibs

Particular note was taken of ethnicity, age at the birth of the affected child, delays in conceiving greater than one year, parental consanguinity, maternal miscarriages and any stillborn sibs of the affected individual.

Note was made of any history of fractures and the trauma which caused them, joint dislocations, tendency to bruising or hearing loss in parents and sibs. Where possible, parents and full sibs were examined for evidence of OI, in particular, note was taken of their height, scleral colour, any skin or joint laxity or dental defects.

d. Pedigree

A detailed pedigree was constructed including information on 1st, 2nd and 3rd degree relatives where possible, particularly noting any history of fractures after mild trauma, blue sclerae, hearing loss, short stature or dental defects.

e. Social history

Initially it was thought that information regarding the social impact of the disease on the family would be of interest. However, it proved to be difficult to elicit such information in a meaningful way in the long schedule outlined.

Further information was obtained from hospital records where necessary.

3.2.1.2 Families who were sent a questionnaire

26 families who were relatively inaccessible were sent a postal questionnaire (appendix 3.3) and 13 provided useful returns including a photograph of the affected individual. The 13 who either did not return the questionnaire or gave inadequate information are excluded (table 3.2).

3.2.1.3 Families in whom the information was provided by a clinical geneticist

The information on five cases from four families was provided by a consultant clinical geneticist (table 3.2).

3.2.2 Patients with perinatally lethal OI

Home visits were carried out by the author to 9 families (of 11 cases) and by Dr Ian Young to 17 families (of 17 cases) in order

to obtain a history of the pregnancy, to construct pedigrees and examine first degree relatives, as described above in section 3.2.1. When this was not possible (for the other 29 families of 34 cases) details were obtained directly from the records of the referral centres and attempts were made to update the pedigree by correspondence with the family's general practitioner. None of the affected babies were personally examined by the author, but attempts were made to obtain post-mortem reports and photographs.

3.3 Collection of radiological data

3.3.1 Collection of radiological data on patients who survived the perinatal period

Ideally, it might have been desirable to obtain a recent full skeletal survey on all cases but this was thought to be unsuitable for two reasons. First, many patients have had numerous radiographs taken in the past (some have had over 200) and any further unnecessary radiography should be avoided. Secondly, most patients are severely handicapped by their disease and would find an additional trip to a hospital an added burden.

In all cases, therefore, exhaustive attempts were made to obtain all the previous radiographs of each affected individual. Radiographs were available in 97 cases and in 40 cases radiographs taken in the first week of life were also available.

All radiographs were examined by Dr Christine Hall (Consultant

Radiologist, The Hospital for Sick Children, Great Ormond Street) and the author.

The diagnosis of OI was confirmed by the presence of osteoporosis, recent or old fractures and skeletal deformity.

Radiographs taken in the first week of life were examined for the number of fractures. These films, together with all those of the perinatally lethal cases, were presented to Dr Hall for classification into mild Sillence type I OI, the type III-like appearance, IIA, IIB or IIC. The classification was based on the degree of shortening and modelling of the long bones, especially the femora, the shape of the ribs, the presence and degree of platyspondyly and the degree of skull mineralisation.

Radiographs taken after the first week of life were examined and a record was made of the following features:

General - osteoporosis

Long bones - deformity: mild, moderate, severe
- modelling: normal, abnormal, slender bones
- cystic epiphyseal changes

Chest deformity

Pelvis - protrusio acetabulae
- other deformities

Spine - platyspondyly

- kyphoscoliosis

Skull - Wormian bones

- deformity, for example, platybasia

The ages at which these particular defects were noted was recorded. The record form used is shown in appendix 3.4.

A representative set of radiographs from each patient was copied and retained by the author. These included a film of one or more long bones, the spine, pelvis, chest and skull where possible. This was done in order to keep a record of proof of the diagnosis of OI and to record any particular or unusual features such as pseudoarthrosis or to show any notable changes during the patient's life, such as the progression of deformity in a limb or in the spine.

3.3.2 Collection of radiological data on patients who died perinatally

Radiographs were available in all but 10 cases, and were examined by Drs Christine Hall, Ian Young and the author on 2 occasions, and were classified as described above. For the 10 cases whose radiographs were no longer available, it was confirmed that a radiograph had been taken to verify the diagnosis of OI, but the radiograph itself was now unavailable for review.

3.4 Analysis of data

First, the patients are classified into Sillence types. This is presented in chapter 4. In chapter 5, the ratios of affected to unaffected sibs are given, together with data about the families which is relevant to the discussion on recurrence risks which follows. Chapter 6 is concerned with the clinical findings in the patients. Each manifestation is presented and discussed as a unit. This chapter includes a discussion about prognosis based on radiological findings at birth. Finally, in chapter 7, postnatal radiological manifestations in perinatal survivors are presented and discussed.

The raw data for chapters 5, 6 and 7 are presented in appendices 5.1, 6.1 and 7.1. This will facilitate further analysis on the patients in future which would be required if, for example, new biochemical findings were made in the cases.

Part of the work, on prognosis and recurrence risks, has been published previously (Thompson et al., 1986 and 1987; Young et al., 1987).

3.5 Biochemical analysis

3.5.1 Structural gene analysis and segregation analysis

Collaboration was established with Dr Bryan Sykes (John Radcliffe

Hospital, Oxford). In his laboratory, structural and segregation analyses of the type I collagen structural genes were carried out on some of the cases and their families from the present study. The work is described in full in the BA (Hons) dissertation by Miss Katherine Aitchison (1987). The work is outlined in brief here but will not be discussed further.

3.5.1.1 Structural gene analysis in perinatally lethal and severe deforming OI

In 5 Sillence type II OI cases and 9 Sillence type III cases (see table 3.3), structural gene analysis was carried out in respect of COL1A1. The probe used was CG103 which was orginally isolated from a library of human genomic DNA in cosmid pHC79. Probe CG103 spans COL1A1. (At that time, a probe which spanned the entire length of COL1A2 was not available). The restriction enzymes HindIII and BamHI recognise 5 and 4 cutting sites respectively within COL1A1. Use of both enzymes meant that it was possible to detect any gross rearrangement (deletion, duplication or insertion) within the COL1A1 coding sequence, with about a 100 base pair resolution. In one case (no. 120) the enzymes RsaI, PvuII and EcoRI were also used.

In summary, no structural rearrangements were detected in the cases studied. This does not exclude the possibility of rearrangements involving less than 100 base pairs or of point mutations in COL1A1.

Table 3.3

CASES ON WHOM BIOCHEMICAL ANALYSES WERE PERFORMED BY AITCHISON (1987)

Case no. in present study	Ref. no. of Aitch., 1987	Type of OI	Structural analysis	Segregation analysis
120	844	IIA	+	-
128	213	IIA	+	-
146	113	IIA	+	-
159	1386	IIB	+	-
160	1381	IIB	+	-
90	p1	III	+	+
99	p3	III	+	+
100	p5	III	-	+
102, 103 (sibs)	p2	III	+ (both sibs)	+
104	p4	III	+	+
47	p9	III/IV	+	+
48	p8	III/IV	+	+
54	p7	III/IV	+	+
69	p6	III/IV	+	+

III - probably recessive

III/IV - sporadic case

3.5.1.2 Segregation analysis in severe deforming OI

In five families with probable recessive Sillence type III OI and in 4 with a sporadic case of severe deforming OI, segregation analysis was carried out (see table 3.3). In only one family with type III OI, were both affected sibs available to give a blood sample (cases 102 and 103). Their parents were not available, however. In the other families, both parents, the affected child and a normal sib were usually available to give a blood sample.

A variety of probes for both COL1A1 and COL1A2 which recognise dimorphic restriction enzyme cutting sites were used; these result in 4 and 8 possible haplotypes for COL1A1 or COL1A2, respectively. The markers used are highly reliable linkage markers for COL1A1 or COL1A2; the maximum recombination distance for markers for COL1A1 and the gene is 44 kb (between an MspI dimorphic site and the 3' end of COL1A1). The markers for COL1A2 are all intragenic.

The aim of the exercise was to see whether, by comparing the haplotypes for COL1A1 and COL1A2 markers of affected and normal sibs, exclusion of autosomal recessive inheritance at one or both loci was possible.

For one consanguineous pedigree (of case 90), in which the disease was highly likely to be autosomal recessively inherited, the affected child was heterozygous for markers for both COL1A1

and COL1A2, whereas homozygosity at one locus would be expected. The likelihood is that the disease is linked to a third locus, perhaps one which codes for an enzyme involved in the processing of collagen. This example was mentioned in section 1.9.6.3.2 and is published in full (Aitchison et al., 1988).

For the other cases, the results were inconclusive, mainly due to lack of availability of key family members.

3.5.2 Analysis of collagens produced by cultured skin fibroblasts in severe deforming OI

The studies by Francis et al. (1988) on 9 cases from this series are mentioned in section 1.9.6.4. The work was presented as a poster at the 'Bone and Teeth' meeting, Cardiff, September 1988 and will be published in due course.

3.6 Follow-up information for patients and their physicians

At the end of the study, the patients were sent a letter explaining the outcome of the study, in terms of the likelihood of recurrence in sibs and offspring, relevant to their particular type of OI. A copy of the letter was sent to the patient's general practitioner and other physicians, whose names had been recorded during the study. The mode of ascertainment had not allowed general practitioners to be contacted prior to the study and so it was important to inform them that the study had taken place and of its findings.

CHAPTER 4

CLASSIFICATION OF PATIENTS

Because the classification of OI is complicated, it is very important to define precisely both clinically and radiologically the patient groups to which the recurrence risk figures apply.

4.1 Patients who survived the perinatal period (first week of life)

Information on 116 cases from 109 families was obtained (table 3.2).

4.1.1 Identification of probable cases of type I OI new mutations

It is well known that patients with the milder type I OI may present with fractures at birth (Sillence et al., 1979b). Eleven such cases (nos. 109-119) could be identified on the basis that they had little or no bony deformity associated with mild radiological changes, and minimal handicap. The height of these patients (N=10) was below average, but all were taller than -6.2 SD below the mean, with an average of -4.0 SD (range -2.6 SD to -6.2 SD). All but one of these were seen personally by the author. In nine cases the mother reported that at birth the number of fractures present was one (2 cases), 2 (4 cases), 3 (1 case), 4 (1 case), 14 (1 case). In 2 cases the number of

fractures at birth was unknown (see appendix 6.1 for case identification). Neonatal radiographs of two of these patients were available and confirmed the presence of 2 and 4 fractures respectively and showed good length and modelling of long bones, and mild bowing only of femora and tibiae in one (figure 4.1).

These 11 cases with probable type I OI form a separate group in the analysis of recurrence risks, on the basis that they represent known new dominant mutations of type I OI and their inclusion with the other perinatal survivors with severe progressively deforming OI would falsely lower the recurrence risks.

This left 105 cases from 98 families with severe OI, who were born with fractures to normal parents, and who survived the first week of life.

4.1.2 Radiographic appearance in the first week of life

Of these remaining 105 cases from 98 families, radiographs taken in the first week of life were available in 38 cases and comprised a complete 'babygram' in 32 and incomplete radiographs in 6. These revealed one of two main patterns:

4.1.2.1 Type III/IV OI pattern

Thirty five of the 40 had the following pattern, shown in figure 4.2 (for case identification, see appendix 6.1): The femora were

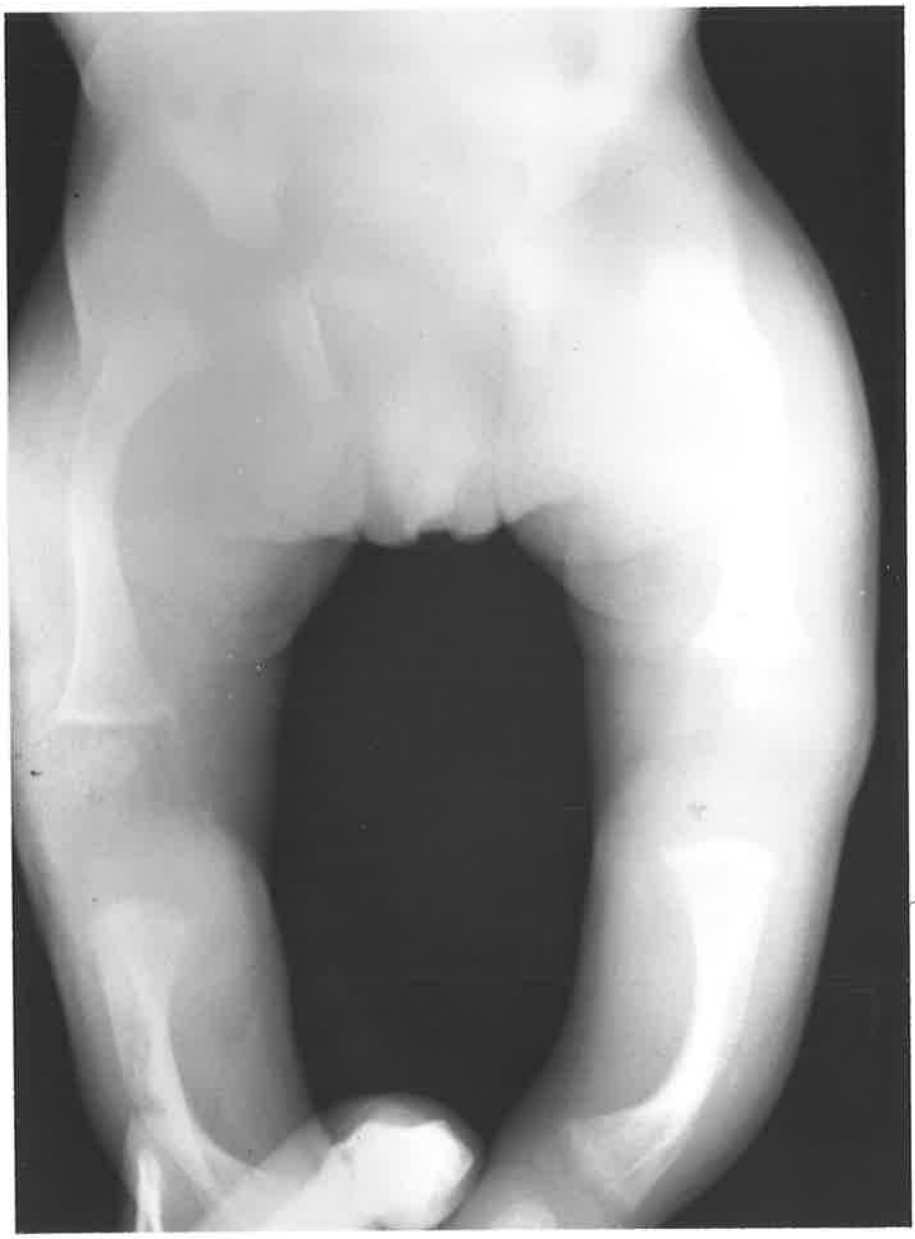


Figure 4.1

Radiograph taken on day 1 of case 109 (probable type I OI).

Note good length and modelling of long bones and only mild bowing.

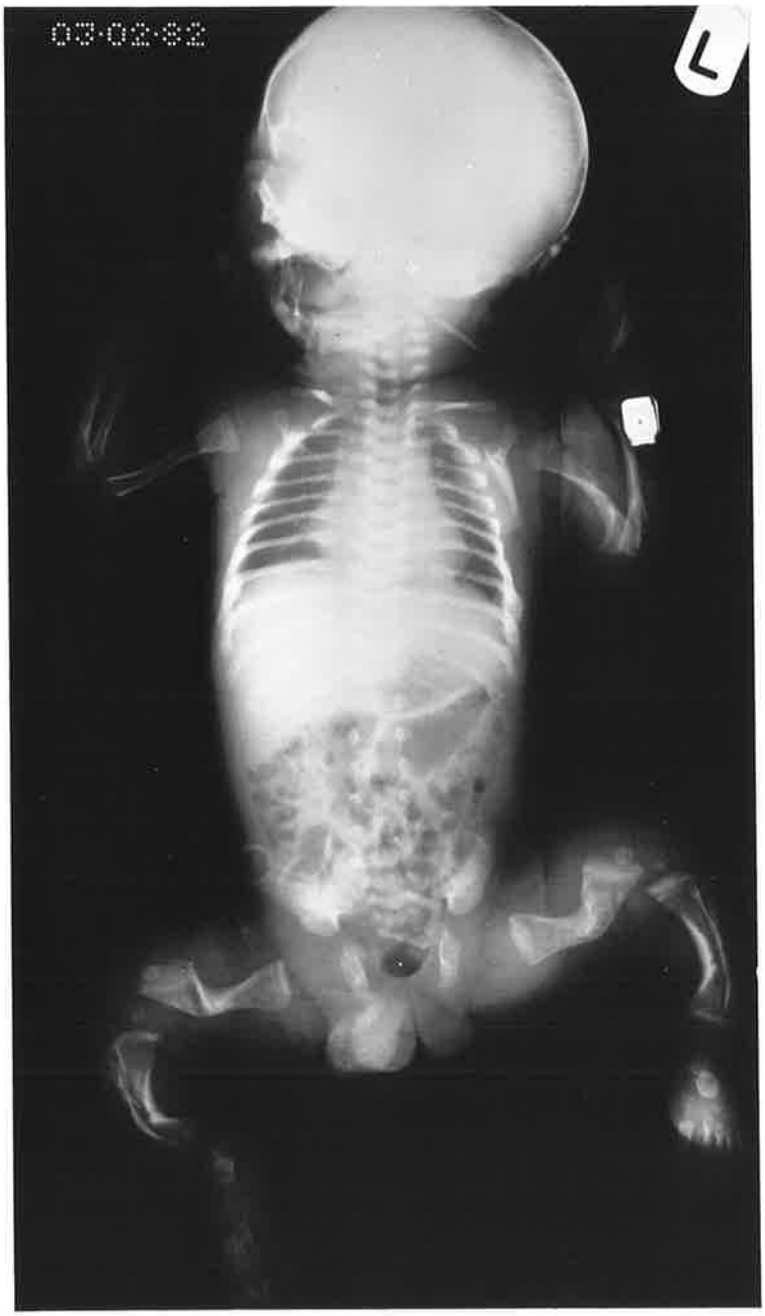


Figure 4.2

Radiograph taken on day 1 of case 4 (sporadic type III/IV OI).

Note moderate shortening of long bones. The femora show some modelling with thin, angulated diaphyses and flared metaphyses. The ribs are slender, the humeri are well modelled and the skull shows good mineralisation.

shortened with flared metaphyses probably due to multiple small fractures. The diaphyses were thin and showed a moderate degree of modelling and often mid-shaft angulation. The overall appearance of the femur was like an apple core. In nine of these cases, the length of the femora was a little greater than in the rest, but overall the changes were more severe than in type I OI. After about one month, the femur in some cases developed an unmodelled rectangular appearance, presumably due to callus formation around the mid-shaft. This will be discussed in section 4.1.2.3. The ribs were generally slender with or without acute fractures. In 1 of 32 cases (no. 90) there were multiple discontinuous 'beads' on the ribs, presumably due to fractures with callus formation. The humeri were usually well-modelled. The skull was usually well-mineralised, revealing multiple Wormian bones. The vertebral bodies were usually of normal height but showed platyspondyly in 5 of 32 cases (nos. 53, 72, 90, 91 and 96). The tibiae were usually angulated at the junction of the upper 2/3 and lower 1/3, except in one child (case 32) who was a breech presentation with extended legs; his tibiae were straight.

The number of discrete fractures was often very difficult to count accurately, but ranged in number from 1 to 30, with a mean of about 9.

Seven of these 35 patients died; 5 died under 6 months, the other 2 died at 14 months and 46 months. Neonatal radiographs were available for only one pair of affected sibs (cases 95 and 96)

and both showed the same radiological pattern, as described above.

This radiological picture is similar to that described by Sillence for type III OI (Sillence et al., 1979b; Sillence et al., 1986), but it is likely that the sporadic cases represent a heterogeneous type III/IV group, consisting of a mixture of recessive (type III) and new dominant (type IV) OI cases. (The disease in affected sibs however probably does correspond to recessive Sillence type III OI).

4.1.2.2 Type IIB OI pattern in perinatal survivors

Three cases (nos. 162-164) showed the radiographic pattern of OI type IIB (figure 4.3): The femora were broad, rectangular and crumpled with little or no modelling. The ribs were thin with multiple discontinuous beads. The humeri showed some modelling. The spine showed platyspondyly in 2 out of 3 cases. The tibiae were angulated and the skull showed some mineralisation.

These three patients died at 4 weeks, 6 weeks and 26 months respectively.

The radiological features at birth are summarised in table 4.1 and the case numbers in each category are presented in table 4.2.

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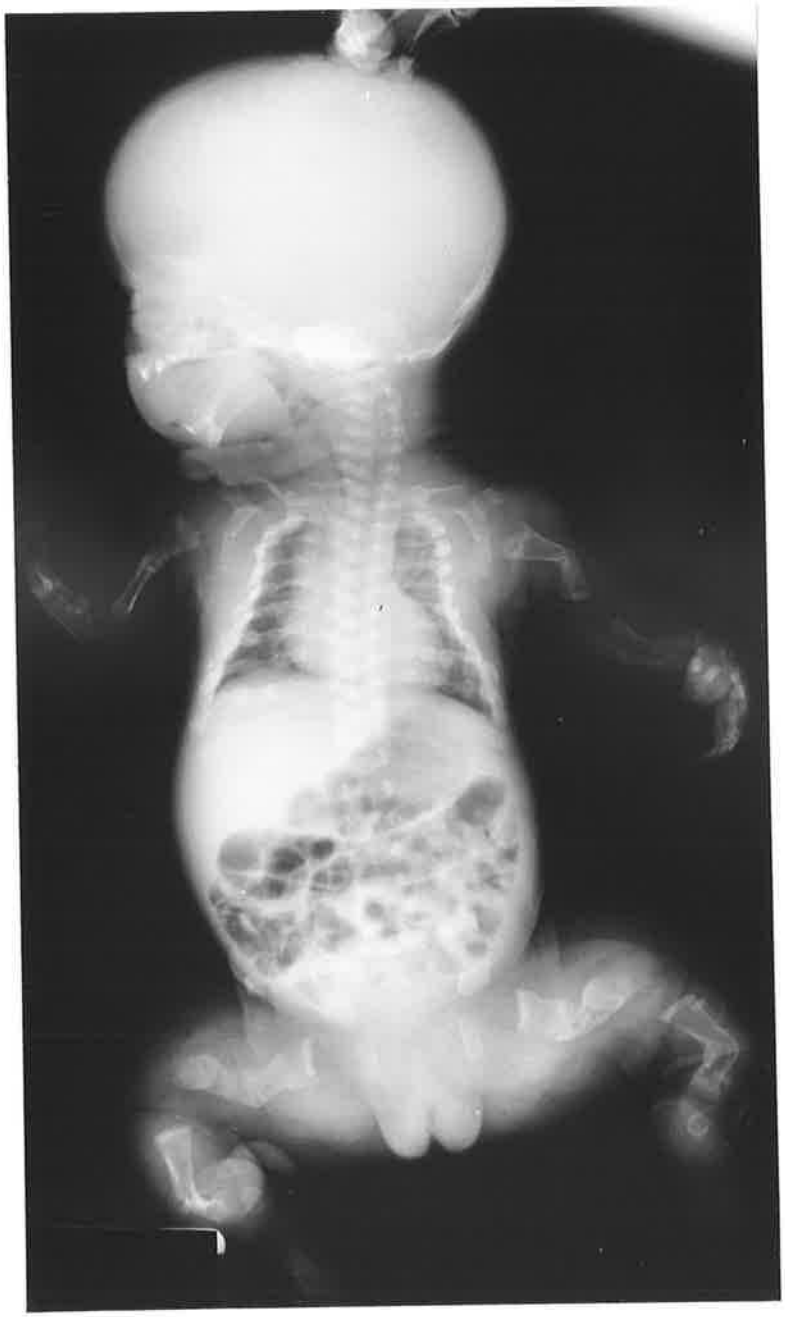


Figure 4.3

Radiograph taken on day 1 of case 162 (type IIB OI) who died at 26 months.

Note the broad, short, unmodelled femora, the thin discontinuously beaded ribs and the shortened but partially modelled humeri. The skull shows some mineralisation. Platyspondyly is not evident in this view.

Table 4.1

RADIOLOGICAL FEATURES AT BIRTH

Type of CI	Femora	Ribs	Humeri	Skull mineralisation	Height of vertebral bodies	Tibiae
I	Well-modelled Normal length ± mild bowing	Normal or a little thin	Like femora	Normal	Normal	Normal or mild bowing
IIA	Very short, broad, un-unmodelled	Broad, continuous beads	Like femora	Absent	Flat	Angulated
IIB	Very short, broad, unmodelled	Thin ± discontinuous beads	Some modelling	Near normal	Near normal	Angulated
IIC*	Short, poorly modelled, multiple angulations	Thin, irregularly shaped	Like femora	Poor	Near Normal	Angulated
III/IV	Moderately short, diaphyses thin & angulated with some modelling, metaphyses flared. Overall, femur looks like an 'apple core'	Thin, rarely with discontinuous beads	Well-modelled	Normal	Usually normal	Angulated

* Hallmarks: irregularity of bone shape, speckled calcification, and long, down-pointing ischia.

Table 4.2

CLASSIFICATION OF CASES OF OI, BORN WITH FRACTURES TO UNAFFECTED PARENTS, BASED ON RADIOGRAPHIC APPEARANCE WITHIN THE FIRST WEEK OF LIFE

Cases	Perinatal survivors (lived beyond first week). No. cases	Perinatal lethals (stillborn or died in first week). No. cases	Total
TOTAL	116	60	
Radiographic appearance at birth			
Type I new mutation	11	0	11
Type IIA	0	30	30
Type IIB	3*	12	15
Type IIC	0	3	3
Type III/IV	35] Severe, progressively	5	107**
Original X-ray at birth now unavailable	67] deforming OI	10	

* The patients died at 4 weeks, 6 weeks, and 26 months, but had the typical IIB X-ray pattern at birth.

** Total no. of type III/IV is 35 + 7 + 65 = 107. In addition, is an affected fetus (case 97), the sib of cases 95 and 96 (see section 4.3.2).

4.1.2.3 The importance of examining radiographs soon after birth for classification of cases of types IIB and III/IV OI

As mentioned above in section 4.1.2.1, the well-modelled femur of a recessive type III or sporadic type III/IV OI patient in some cases developed a thick unmodelled rectangular appearance after about one month (figures 4.4a and b). The importance of this is that the femur comes to resemble that seen in type IIB OI at birth, so that the time at which the radiograph is taken is critical for the accuracy of classification. This change in the femora was noted to occur in 16 sporadic cases and 2 with probable recessive OI. The earliest time it was observed was at 4 weeks in 2 cases, one with sporadic OI (case 64) and one probable recessive case (case 90). Table 4.3 indicates the ages at which the femora first appeared broad and unmodelled. It should be noted that this reflects the availability of radiographs at various ages, to some extent. On the other hand, 4 weeks is likely to be the earliest age at which the femora can appear unmodelled, since radiographs available at 13 days (case 91), 17 days (case 3), 2 1/2 weeks (case 32), 3 1/2 weeks (case 59) and 4 weeks (case 31) showed normal femoral modelling.

In summary, it is important to classify the type of OI on the basis of radiographs taken before 4 weeks of age, preferably within the first week of life, so as to avoid confusion between types IIB and III/IV OI.

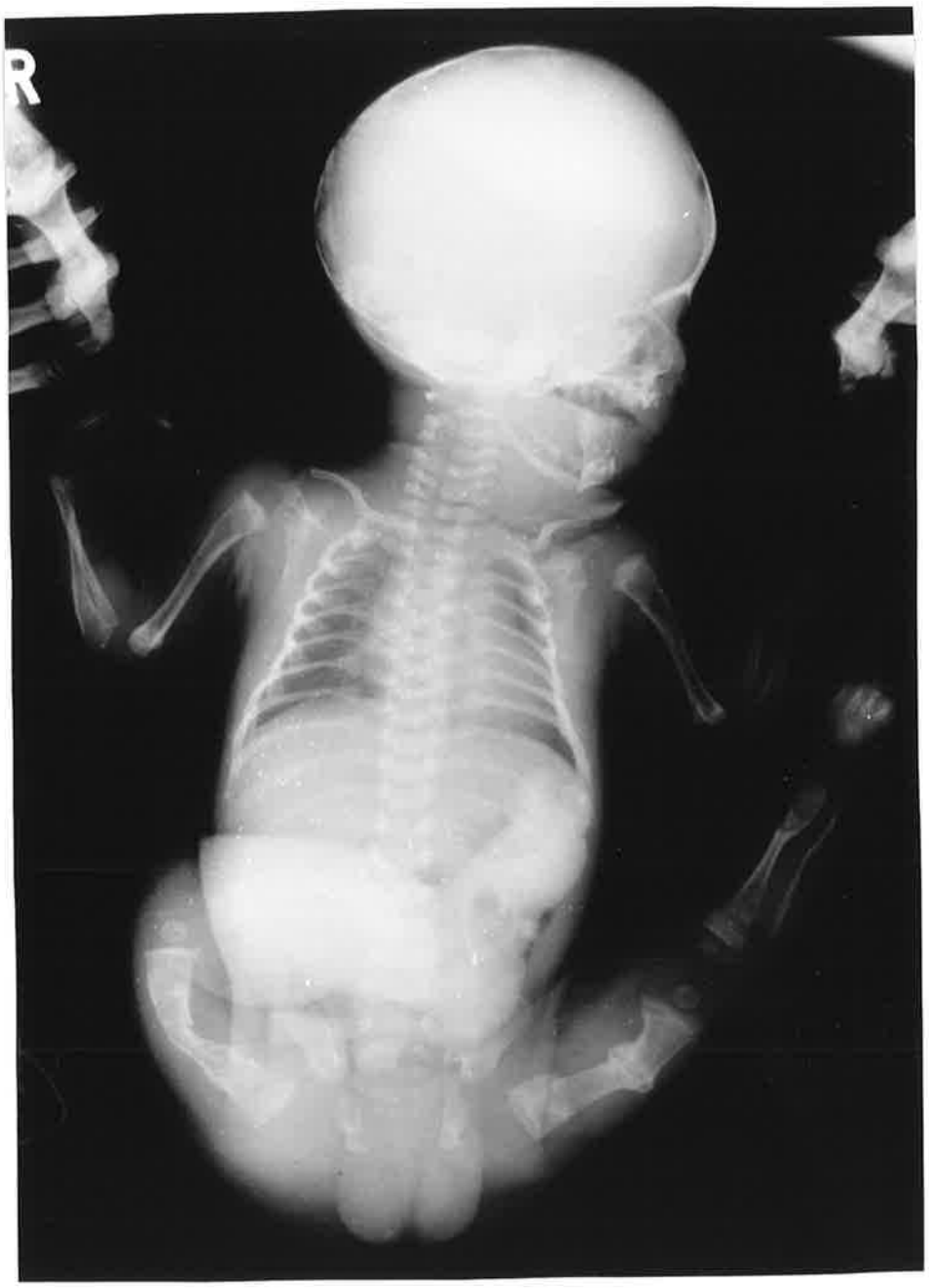


Figure 4.4(a)

Radiograph taken on day 1 of case 64 (sporadic type III/IV OI).

Note that the appearance is similar to that of case 4 (figure 4.2) and that the femora are well modelled.

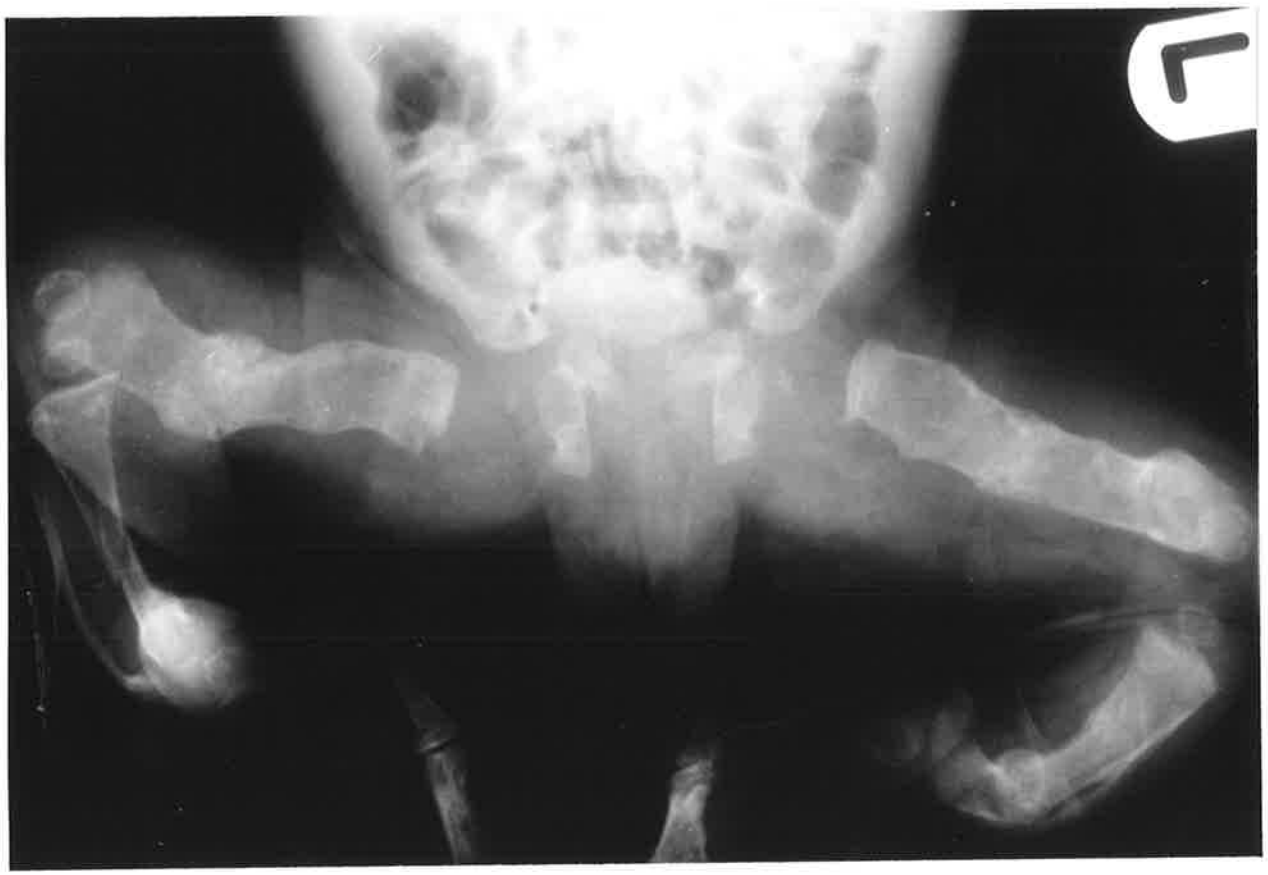


Figure 4.4(b)

Radiograph taken at 4 weeks of age of case 64.

Note that the femora have become thick, unmodelled and rectangular in shape.

Table 4.3

AGE AT WHICH THE FEMORA FIRST APPEARED BROAD AND UNMODELLED IN TYPE III AND TYPE III/IV SPORADIC OI CASES IN WHOM THE RADIOGRAPHS AT BIRTH SHOWED FEMORAL MODELLING

Type of OI	Age at which femora first appeared broad	Case no.
III (n = 2)	4 weeks	90
	2 years	95
III/IV sporadic (n = 16)	4 weeks	64
	6½ weeks	63
	8 weeks	72
	13 weeks	31
	14 weeks	20, 29
	4 months	3
	5 months	34, 32
	6 months	10
	12 months	11
	13 months	21
	19 months	55, 62
	25 months	4
	32 months	51

4.2 Patients with perinatally lethal OI (stillborn or died within one week of birth)

The 60 cases from 57 families were classified according to radiological appearance where possible (tables 4.1 and 4.2), as defined by Sillence et al. (1984).

4.2.1 Type IIA OI

30 cases (nos. 120-149) were classified as type IIA OI. Radiologically the features were as follows (figure 4.5):

The femora were short, broad, rectangular and crumpled and in general the lack of modelling was even more marked than in type IIB OI. The ribs were thick and continuously beaded, particularly evident in ribs 7 to 10. The humeri, like the femora, were short, broad and poorly modelled. The skull showed almost complete lack of mineralisation. The spine showed platyspondyly usually involving more than half of the thoracic and lumbar vertebral bodies. In 3 of the cases (nos. 133, 143 and 146), the ribs, whilst showing almost continuous beading, were thinner than in type IIA, but thicker than in type IIB (figure 4.6).

Notably, 4 of the cases were fetuses terminated between 17 and 23 weeks gestation and in them the type IIA radiographic pattern was readily apparent on films taken after delivery, even at that early stage (figure 4.7). Figure 4.8 shows a normal fetus, for comparison.

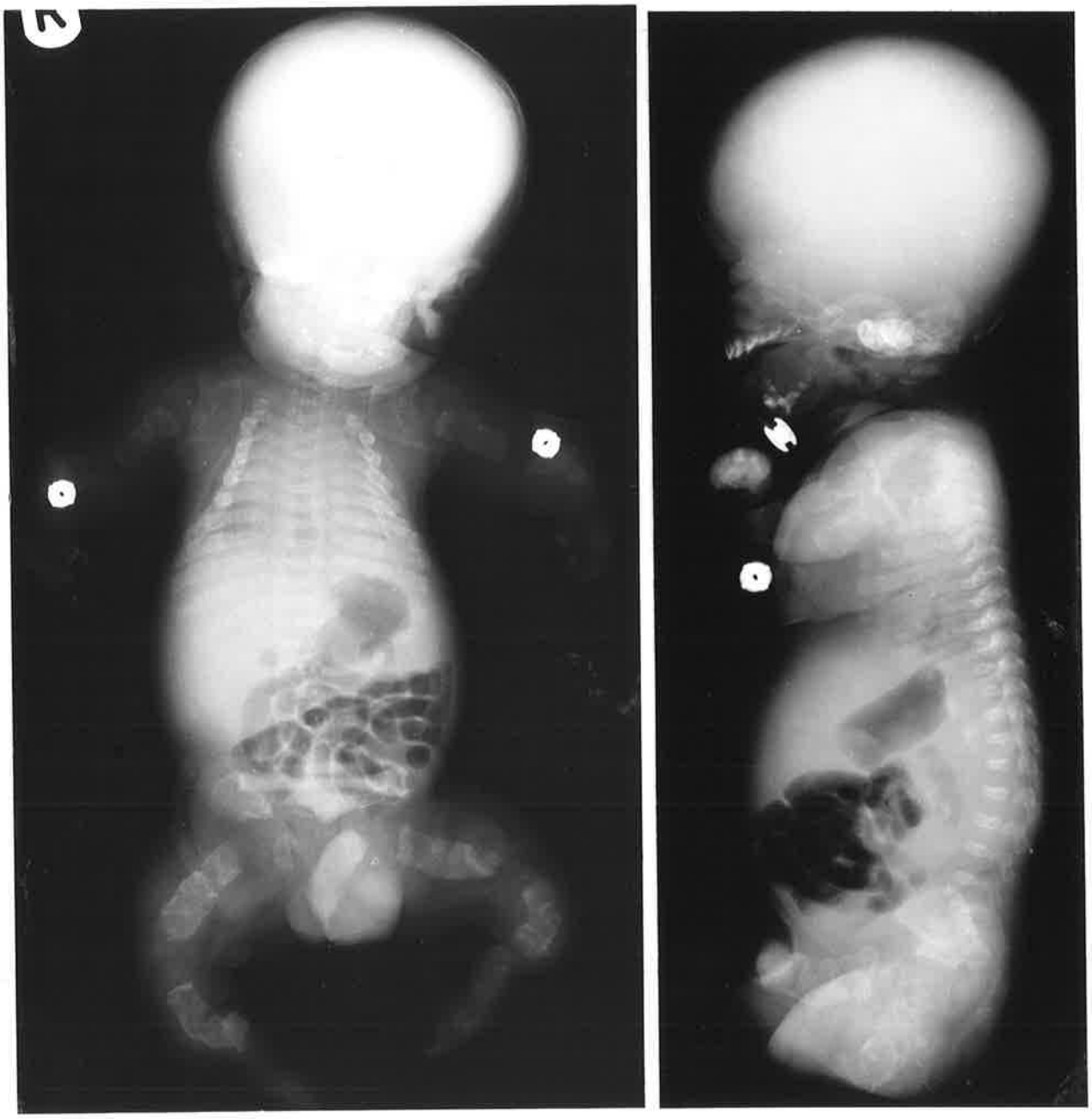


Figure 4.5

Radiographs of case 138 (type IIA OI).

Note the broad short rectangular femora, the thickened continuously beaded ribs, the short broad humeri, platyspondyly and very poor skull mineralisation.

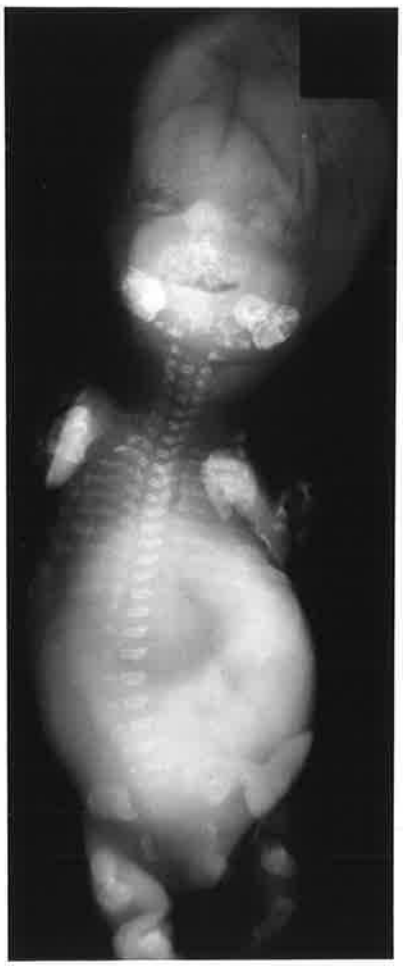


Figure 4.6

Radiograph of case 143 (type IIA OI).

Note the similarity to case 138 in figure 4.5, but that although the ribs appear to be continuously beaded, they look thinner. This baby was born at 31 weeks gestation; case 138 was born at 39 weeks.

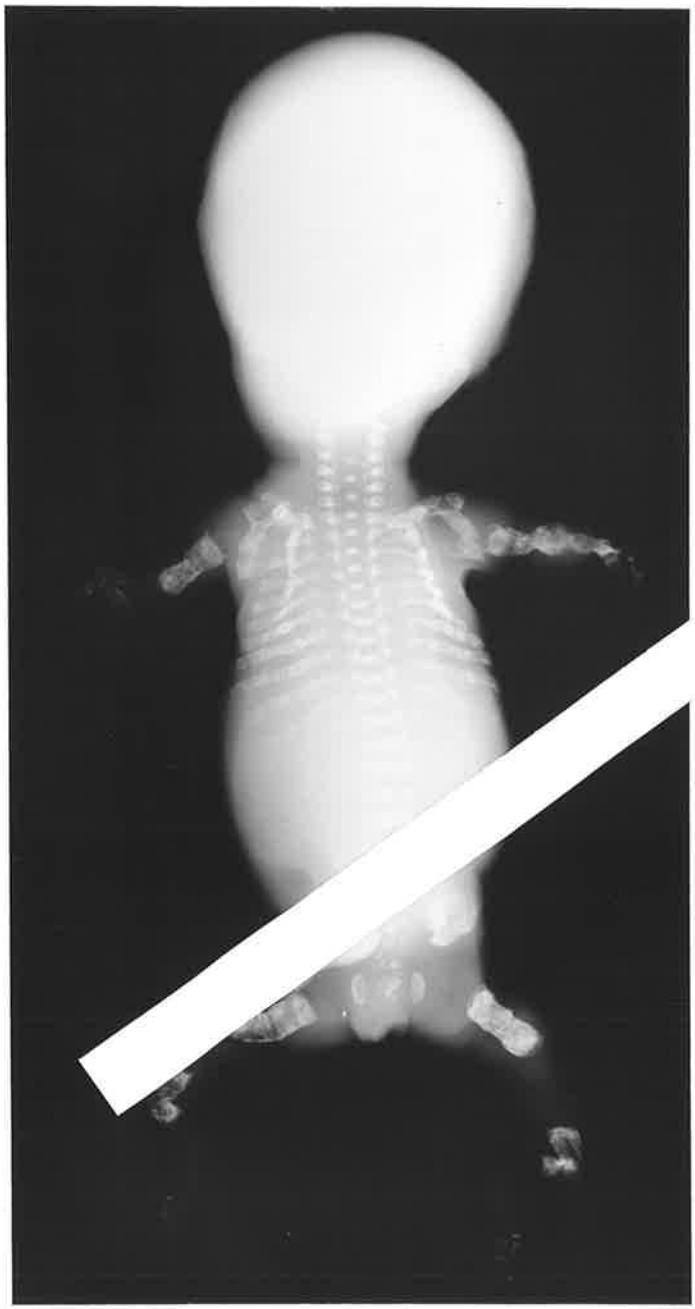


Figure 4.7

Radiograph of case 121 (a 2nd trimester fetus with type IIA OI).

Note the thickened unmodelled femora and humeri, the thickened continuously beaded ribs and absent skull mineralisation.

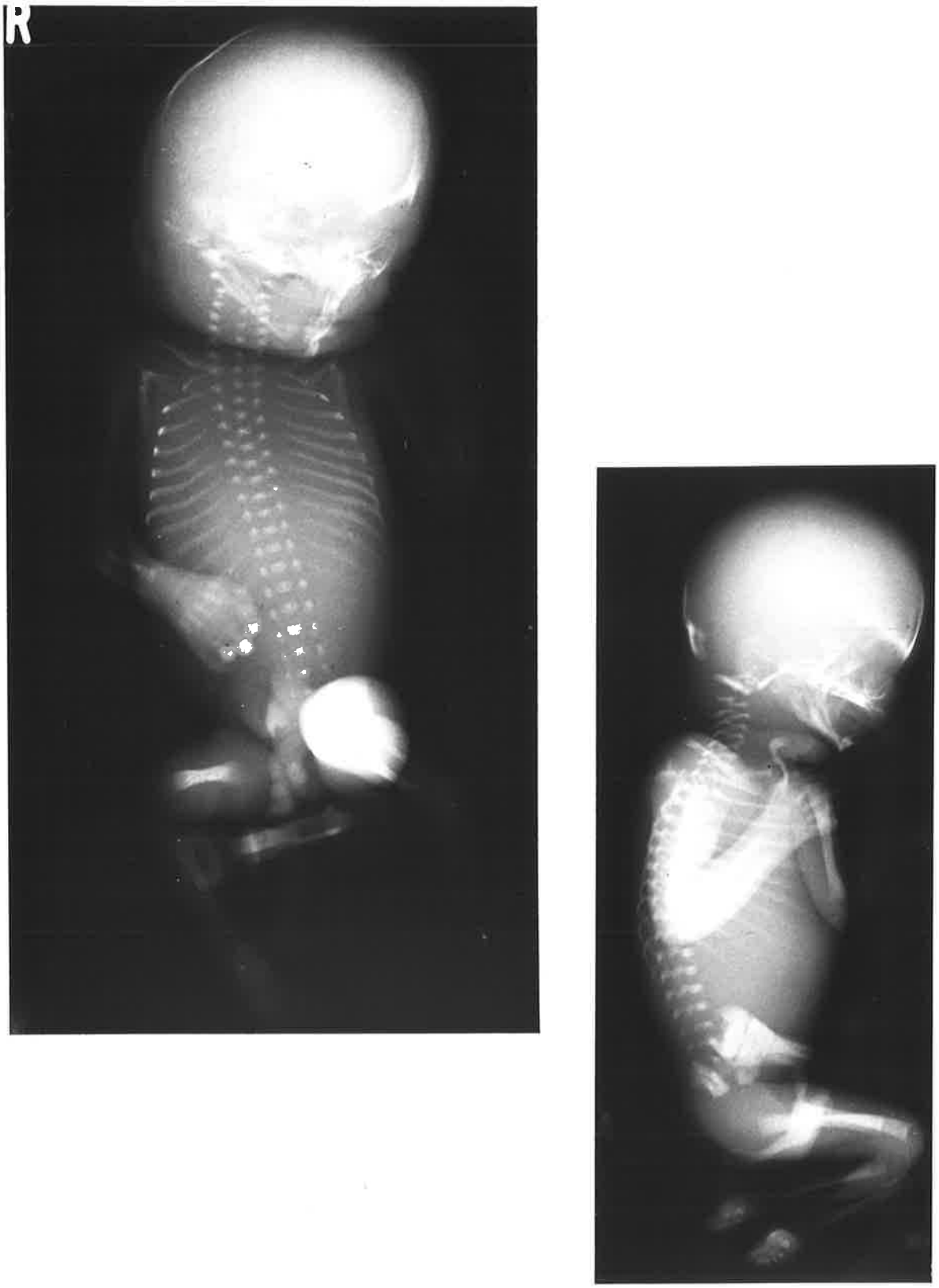


Figure 4.8

Radiographs of a normal fetus (one of twins) at 17 weeks gestation.

4.2.2 Type IIB OI

Seventeen cases were initially classified as type IIB (Thompson et al., 1986). Subsequently, we realised that only 12 of these (cases 150-161 who were from 11 families) conformed to the radiological criteria for IIB in the original description of Sillence et al. (1984), noted in section 4.1.2.2 above. The femora were short, broad, rectangular and crumpled with poor or absent modelling, as in type IIA, but in type IIB the femora tended to be a little longer. All of the 12 cases had thin ribs; in 11, multiple discontinuous beads were present. One case (no. 161), an 18 week terminated fetus, had no rib beading. The thin ribs in type IIB OI contrast with the thick continuously beaded ribs seen in type IIA. In addition, it was noted that in type IIB bone modelling was generally better than in IIA. The humeri were better modelled, the skull mineralisation was better, although still incomplete and the vertebral bodies tended to be of more normal height, although platyspondyly of some vertebrae was noted. All cases of type IIB OI had angulated tibiae. Figure 4.9 shows the findings in a baby with perinatally lethal type IIB OI. Comparison with the radiograph of a baby with type IIB who survived the perinatal period (figure 4.3) shows no major differences between the two.

There was one affected sib pair, and they had the same radiological appearance (figures 4.9 and 4.10).

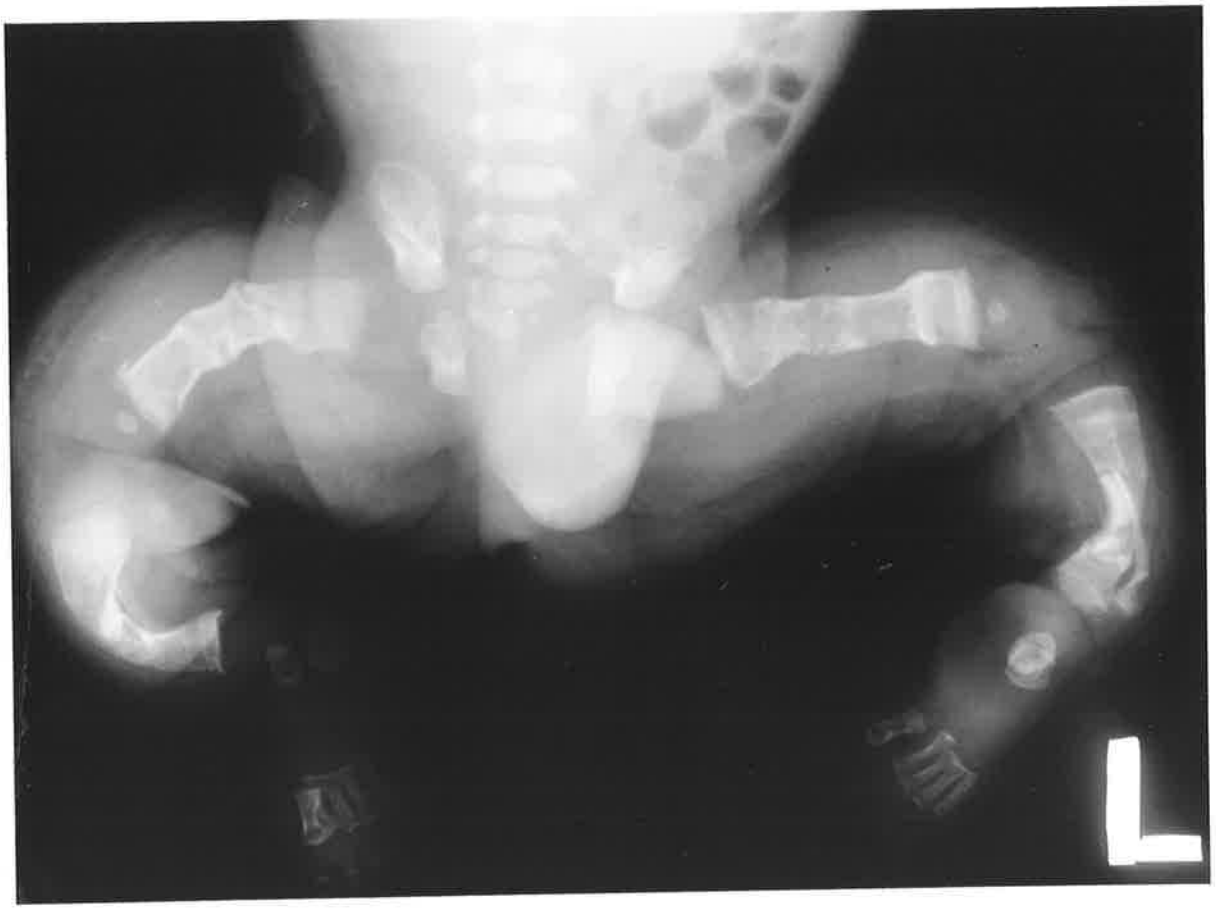


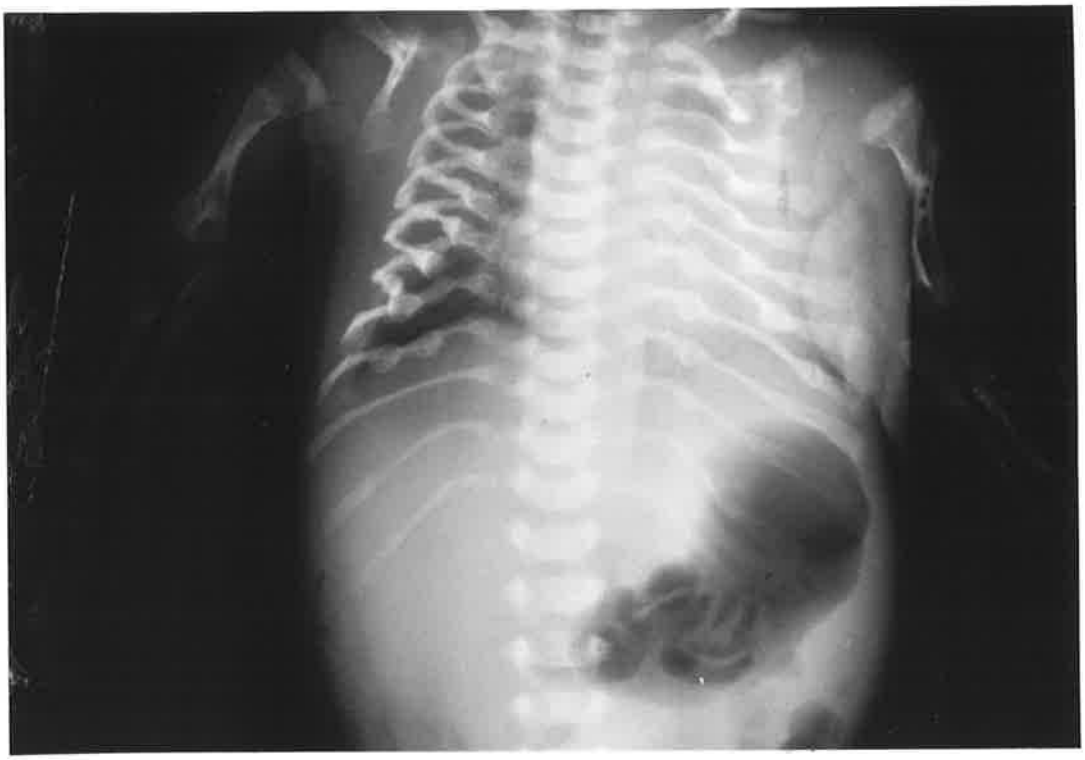
Figure 4.9(a)

Radiographs of case 151 (type IIB OI) who died at 8 hours.

Note the broad short rectangular femora (figure 4.9(a) and the thin ribs with multiple discontinuous beads (figure 4.9(b)). The skull is fairly well mineralised revealing multiple Wormian bones (figure 4.9(c), see over).

Figure 4.9(b)

See legend for figure 4.9(a).



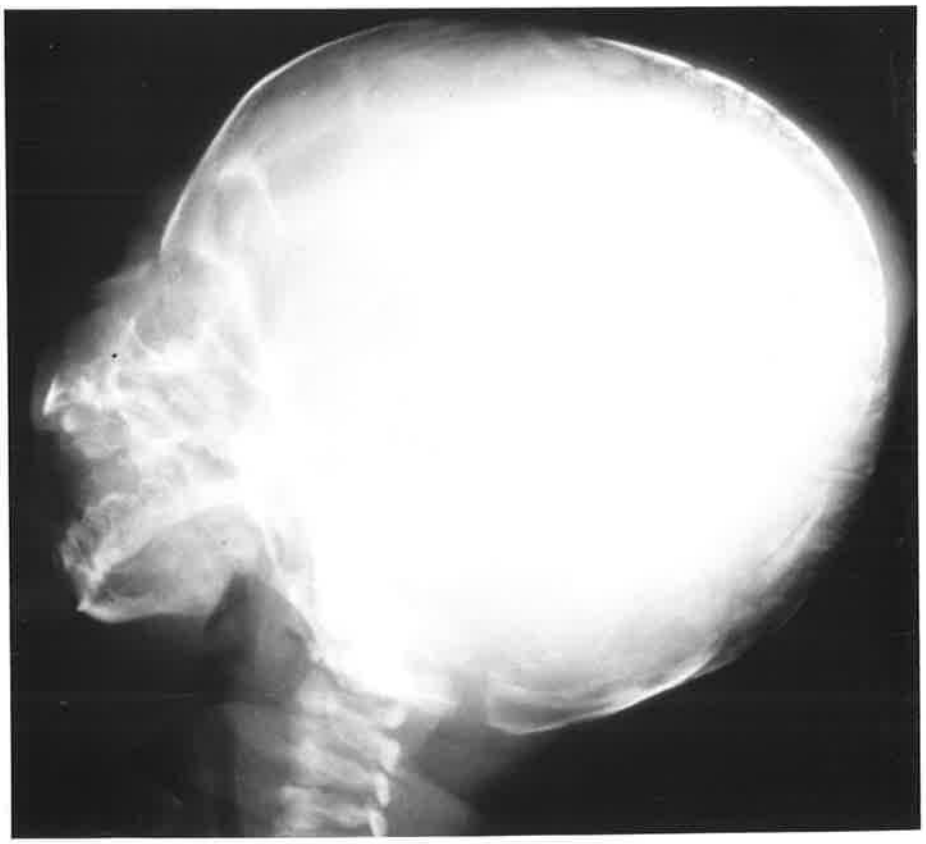


Figure 4.9(c)

See legend for figure 4.9(a).

In 2 of the single cases, cases 160 and 153 (delivered at 23 and 36 weeks gestation respectively) the thickness of the ribs was intermediate between types IIB and IIA OI; since the other features were more typical of IIB, they were classified as such (figure 4.11). Another single case (no. 158, born at 34 weeks gestation) had longer femora than the others, but these were completely unmodelled and the other features were consistent with IIB.

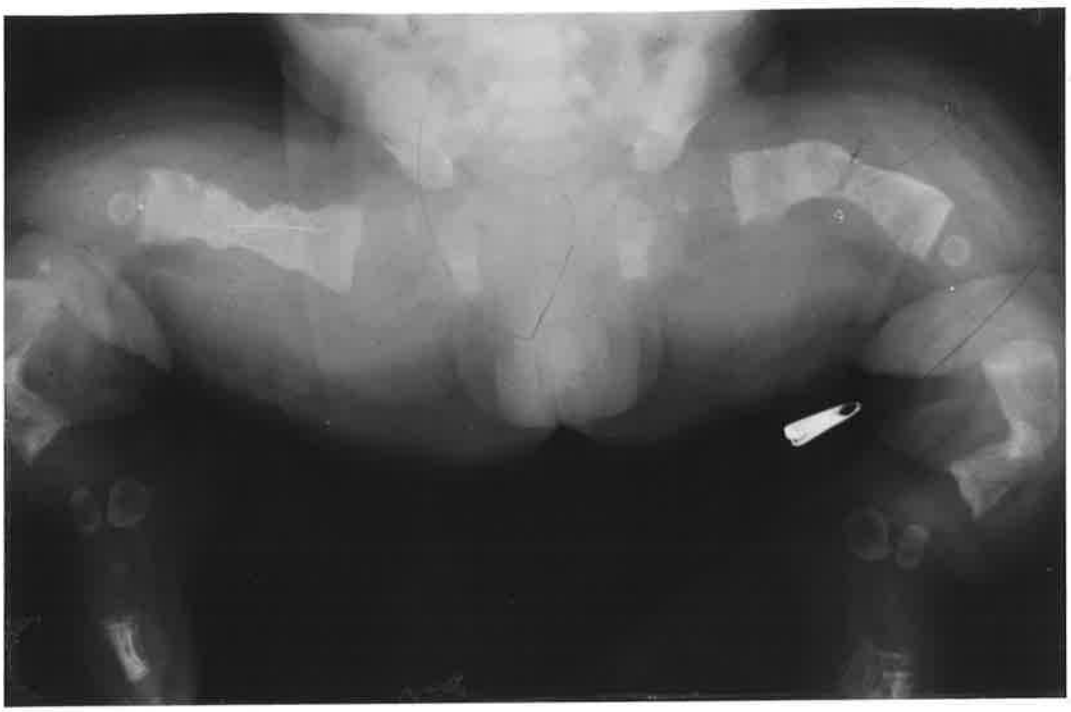
Although classification was difficult in one fetus (case 160 mentioned above), in another fetus (case 161, terminated at 18 weeks gestation), the type IIB OI radiological pattern was recognisable on radiographs taken after delivery (figure 4.12).

4.2.3 Type III/IV OI

The other 5 babies (from three families) who died perinatally and who were originally classified as type IIB OI (Thompson et al., 1986) in fact had the radiological appearance of type III/IV, described above in section 4.1.2.1. The radiograph of one of these five is shown in figure 4.13. Two of the five were single cases in their family (cases 88 and 89) and three were sibs (cases 106, 107 and 108). It is noteworthy that these sibs all showed the same type III/IV appearance radiologically (figure 4.14). The index case (no. 106)in this family was liveborn at 38 weeks and lived for 10 hours; the disease was detected prenatally by ultrasonography in his two affected sibs and radiological confirmation of their diagnosis was made after termination of

Figure 4.10

Radiographs of the legs and chest of case 150. The child died at 48 hours and has the same radiographic appearance as her brother, case 151 (figure 4.9).



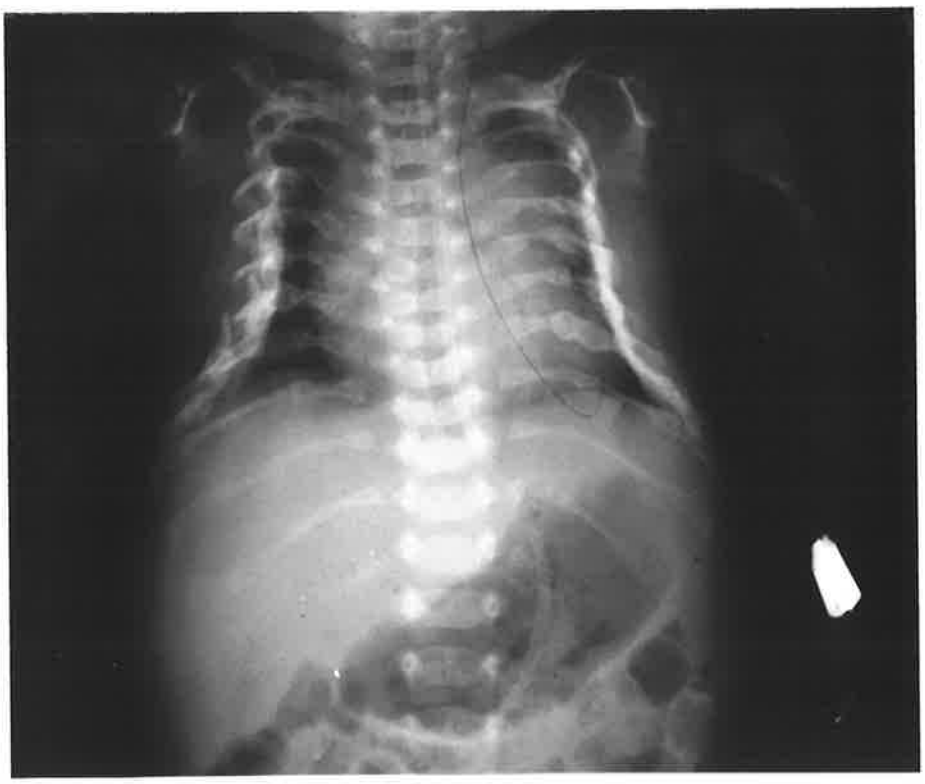


Figure 4.11(a)

Radiograph of cases 160 (figure 4.11(a)) and 153 (figure 4.11(b)), both with type IIB OI.

Note that the overall appearances are consistent with type IIB OI but the ribs are of intermediate thickness.

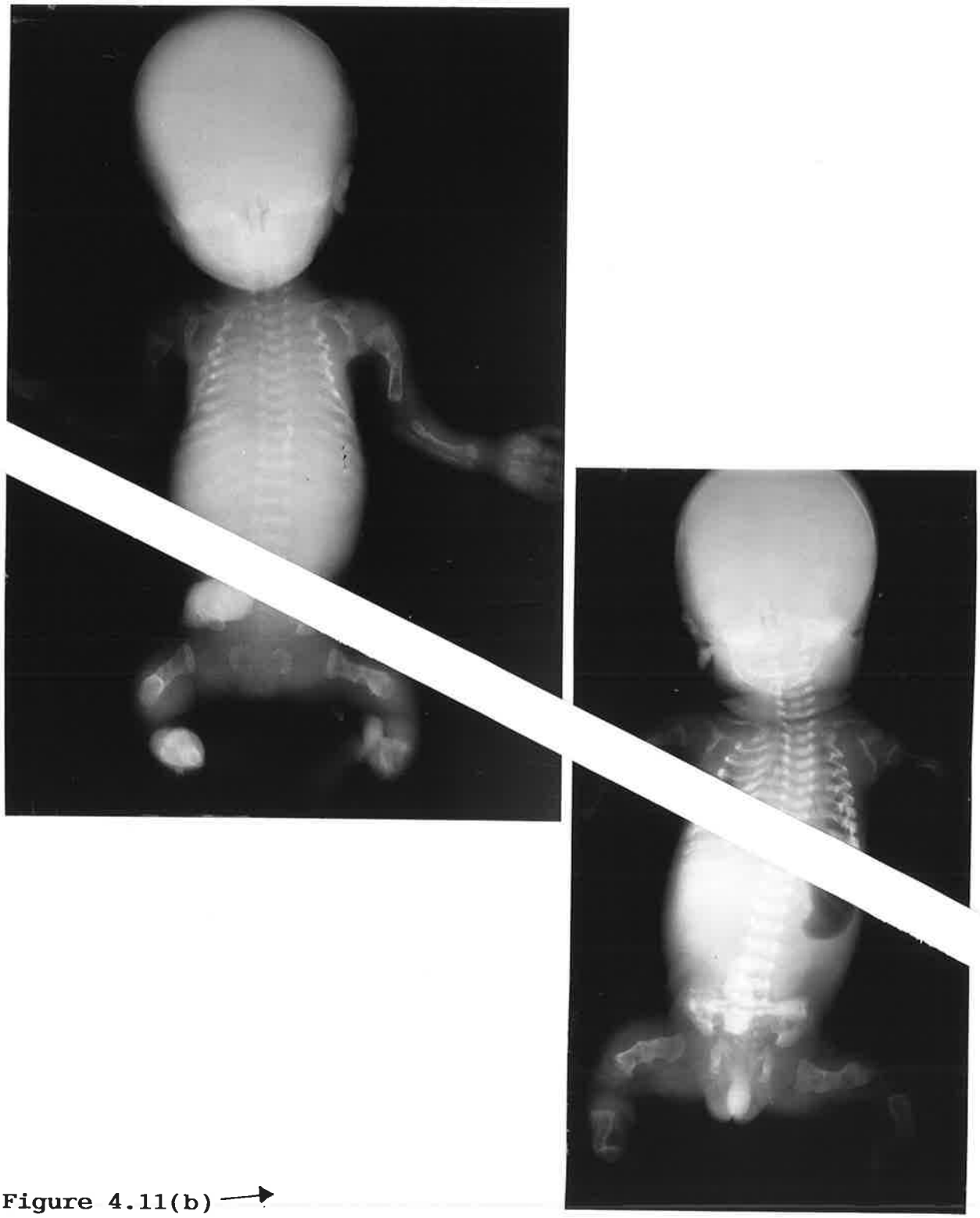


Figure 4.11(b) →

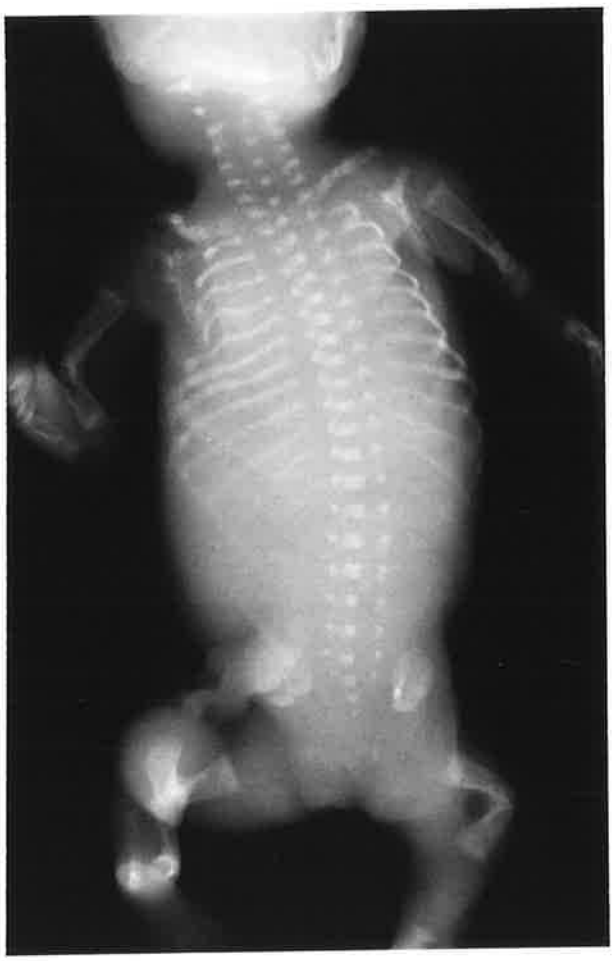


Figure 4.12

Radiograph of case 161, a fetus with type IIB OI, terminated at 18 weeks gestation.

Note the unmodelled femora and thin ribs.

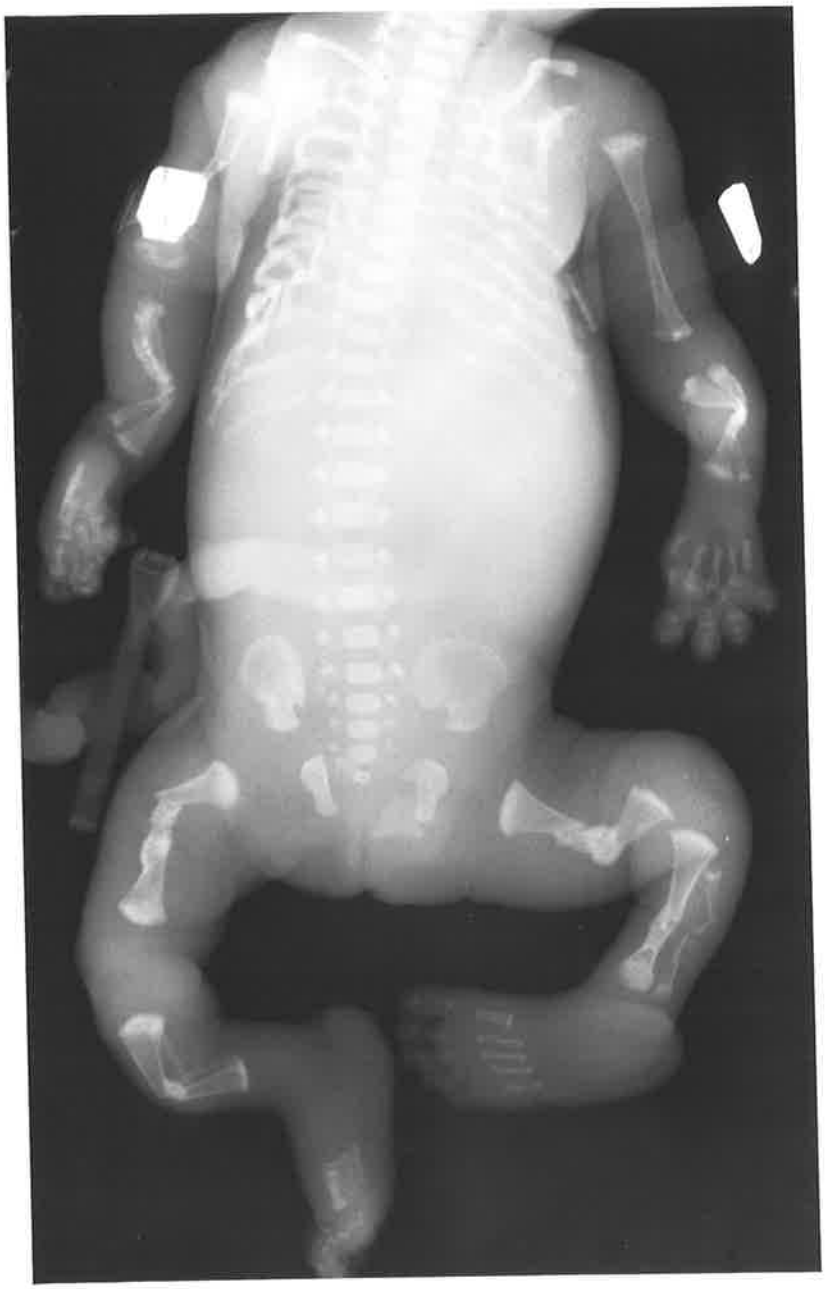


Figure 4.13

Radiograph of case 88 (sporadic perinatally lethal type III/IV OI) stillborn at 30 weeks gestation.

Note that the length and modelling of the long bones are much better than in type IIB OI. The ribs are thin and discontinuously beaded. The appearance is more like that of type III/IV than IIB OI.

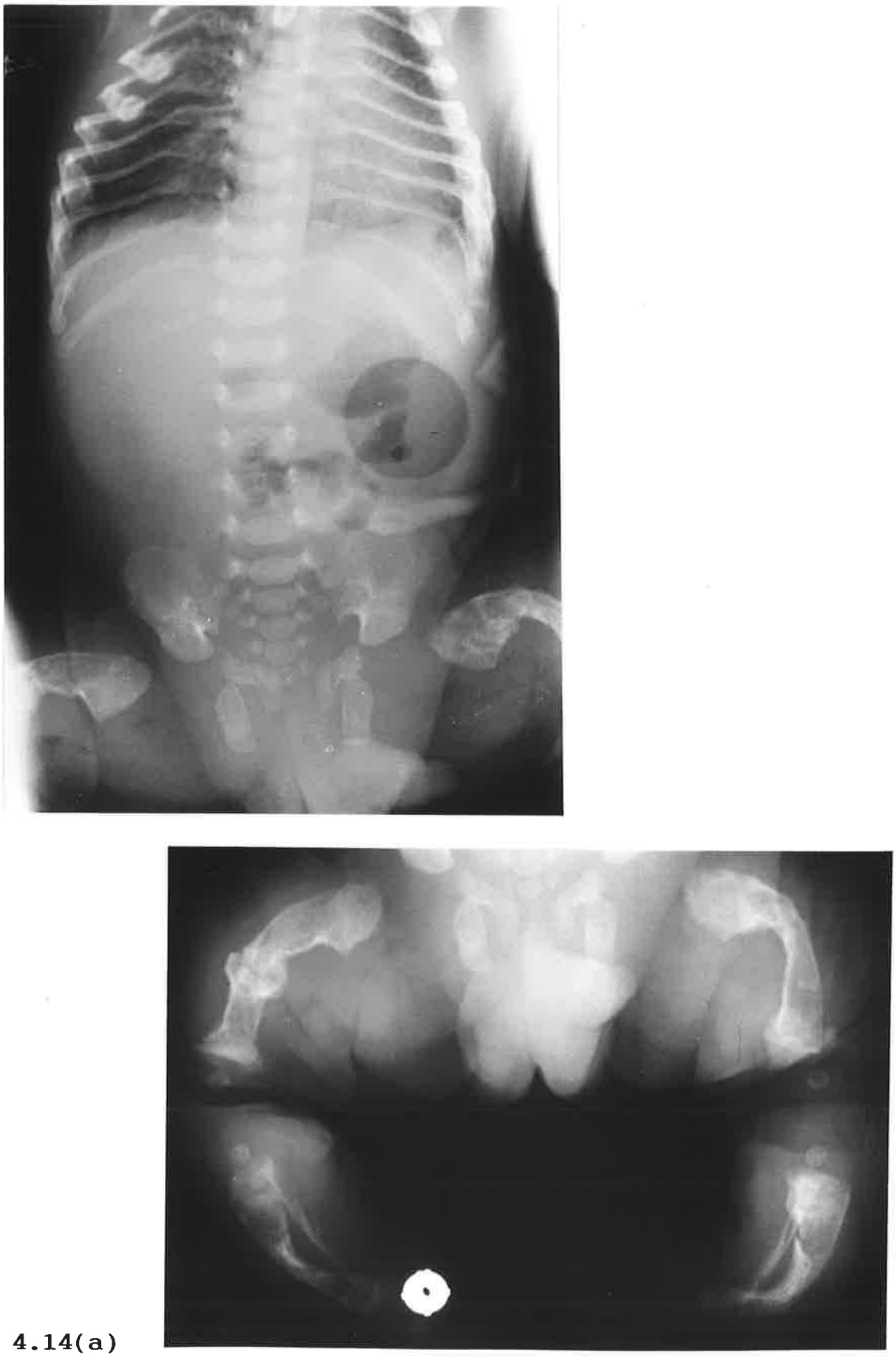


Figure 4.14(a)

Radiographs taken at birth at 38 weeks gestation of case 106, who died at 10 hours. The length and modelling of the femora are more in keeping with type III/IV OI than type IIB.

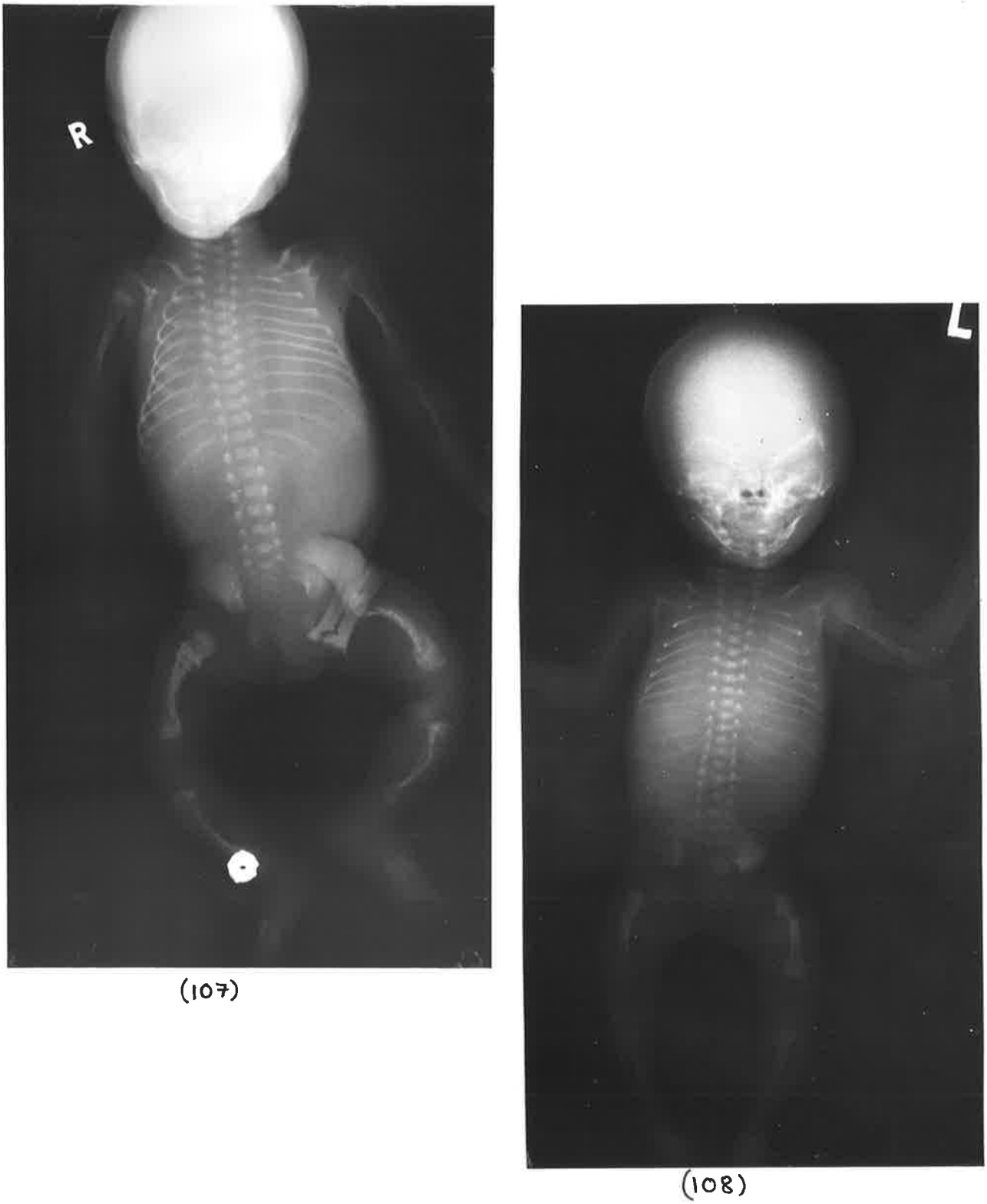


Figure 4.14(b)

Radiographs of the 2 affected sibs of case 106, terminated at 27 weeks (case 107) and 20 weeks (case 108). There is good modelling of the femora and other long bones, in keeping with type III/IV OI.

pregnancy at 27 and 20 weeks, respectively.

4.2.4 Type IIC OI

Three cases (nos. 165-167) were classified as type IIC OI, on the basis of the following (figure 4.15):

The femora and humeri were short, broad, unmodelled and irregular in shape. The ribs were thin, irregularly shaped and discontinuously beaded. The skull showed poor or absent mineralisation. The vertebral bodies were of near-normal height. In the pelvis, the ilia were flared and the ischia were long and pointed downwards. It was noted that the hallmarks of this type of OI appear to be irregularity of bone shape, including that of the scapulae, and speckled calcification.

One of the cases (no. 166) was a nineteen week gestation terminated fetus and again the type IIC appearance is recognisable even at this time.

4.2.5 Unclassifiable perinatally lethal OI

In ten cases (nos. 168-177), the diagnosis of OI was originally made on radiological appearance, but as the radiograph was no longer available, classification was impossible.

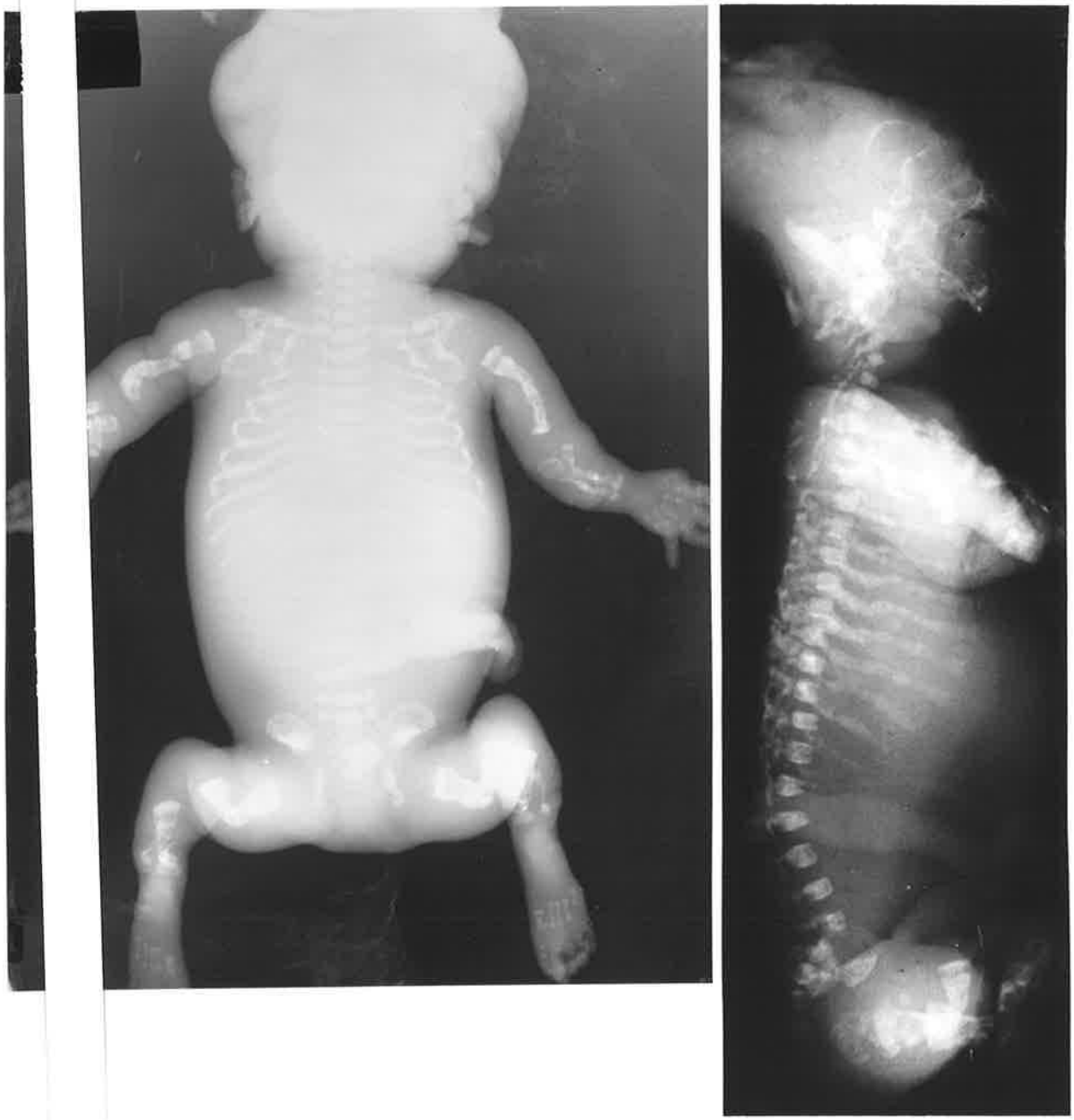


Fig 4.15

Rad aphs of case 167 (type IIC OI) who was stillborn at 28 wee

Not e shortening of long bones, especially the femora which sho ltiple angulations, the thin irregular ribs, the absence of l mineralisation, the fairly normal vertebral body height and e long downward-pointing ischia. The bone shape is gene ly irregular with speckled calcification.

4.3 Grouping of patients for analysis of recurrence risks

4.3.1 Introduction

For the assessment of recurrence risks in sibs, it is considered logical to group the patients according to radiological appearance at birth, rather than to classify them as perinatally lethal cases versus perinatal survivors, since consistency in radiological appearance is more likely to indicate a true subgroup, than is age of death. Thus sib recurrences will be considered for each of the radiological groups delineated in the preceding sections. This presents no problem for types IIA, IIC and the unclassifiable perinatally lethal cases, since they were all perinatally lethal. Patients with types III/IV and IIB OI were represented in both the perinatally lethal or surviving categories. In addition, for 67 perinatal survivors, the neonatal radiograph was no longer available and these require placement into an appropriate group.

4.3.2 Type III/IV OI

One hundred and five cases survived the perinatal period. In 38 of these, neonatal radiographs were available; 35 showed the type III/IV radiological appearance and three showed the type IIB appearance. In 67 perinatal survivors, the neonatal radiograph was no longer available. They all had moderate or severe progressively deforming OI and the clinical manifestations and

course of their disease did not clearly differ from the 35 whose neonatal radiographs showed the type III/IV pattern. Therefore, it is considered reasonable to include in the sib recurrence analysis for type III/IV OI the 67 perinatal survivors with no neonatal radiographs. In addition, the five cases with perinatally lethal disease showing the type III/IV appearance are included in this group. Thus there are a total of 35 + 67 + 5 = 107 cases (nos. 1-96 and 98-108) with type III/IV OI. Case 97 is an affected fetus, the sib of cases 95 and 96. The radiograph of the fetus was unavailable. It could be argued that since three of 38 perinatal survivors showed the IIB OI pattern, some of the 67 with no neonatal radiograph available could have type IIB. However, type IIB is a much more severe clinical disease than type III/IV and is very likely to lead to death in infancy. The maximum age of death of a child with IIB OI was 26 months, and of the 67 perinatal survivors with no radiograph, only 9 had died before 26 months. Extrapolating from the fact that 3/38 with neonatal radiographs were shown to have type IIB, then about one other (3/38 x 9 = 0.71) could be expected to in fact have type IIB OI. (Note: In Thompson et al., 1987, the total number of cases with newborn radiographs available is given as 40, not 38. This is because the two with type I OI were included).

4.3.3 Type IIB OI

There were three perinatal survivors and 12 perinatally lethal cases (from 11 families) with the type IIB OI pattern, a total of 15 cases (from 14 families).

Addendum to chapter 4: Recently, Williams et al. (1989) reported on clinical features in cases 95, 96 and 97 (three sibs with OI). Herein and previously (Thompson et al., 1987) we classified them as type III OI. Williams et al. have classified cases 96 and 97 as type IIB and case 95 as type III, apparently on the basis of survival rather than immediate postnatal radiographic appearance which was, in fact, identical in cases 95 and 96 and showed femoral modelling consistent with type III OI. The radiograph of the fetus (case 97) was unavailable; in their article, Williams et al. show figure 4 as the radiograph of case 97 but it is in fact that of case 95 (Thompson et al., 1989).

CHAPTER 5

ANALYSIS OF RECURRENCE RISKS

5.1 Introduction

In this section, the number of recurrences of OI in sibs is shown. The sibships are presented and any factors which could bias the number of sibs counted as affected are considered. These include both the spontaneous miscarriage rate and any delays in conceiving reported by the mothers, which could possibly represent early loss of affected fetuses. Parental consanguinity, ethnic origins and parental ages at the birth of the affected child are other factors which may have a bearing on recurrence rates. The raw data is shown in Appendix 5.1. Detailed clinical assessment of parents and normal sibs is presented in order to demonstrate that they are indeed not affected with OI. The extended families of the OI patients are also considered to see if any other relatives had OI. This information is then synthesised into a discussion on the recurrence risks.

The reader will note that in this and the following chapters, data pertaining to the type I new mutation cases is generally presented last. Whilst this may seem numerically unpleasing, it is done to emphasise that the study primarily concerns severe OI. It also seemed logical to follow a progression from the most severe type II category through types III/IV and III, to type I,

the mildest form of OI, of which there were relatively few (11) cases.

5.2 Results

5.2.1 Number of recurrences in sibs (table 5.1)

Type IIA OI

The 30 cases with type IIA OI had 38 sibs, all normal.

Type IIB OI

The 15 cases with type IIB OI had 13 sibs, one of whom was affected, giving an empirical recurrence risk of 7.7%. The family with two affected sibs was ascertained only once, so the older one was taken as the proband and the affected sib was counted only once.

Type IIC OI

There were 3 cases with type IIC OI who had three sibs, all normal.

Unclassifiable perinatally lethal (PNL) OI

The 10 babies in this group had 22 sibs, all normal.

Table 5.1

RECURRENCES IN SIBS

Type of OI	Cases	Total sibs	Affected sibs	Recurrences	Comment
I	11	13	0	0	-
IIA	30	38	0	0	Rare reports of affected sibs suggests a small recurrence risk should be quoted (see section 5.3.2.1)
IIB	15	13	1	7.7%	Overall evidence suggests higher recurrence risk in these rare forms (see section 5.3.2.2)
IIC	3	3	0	0	
III/IV All cases.	107	146	10	6.9%	-
Parents non-consanguineous	104	135	6	4.4%	-
All perinatally lethal cases	60	80	3	3.8%	This provides a recurrence risk figure if a perinatally lethal case is unclassifiable

Type III/IV OI

The 107 cases with type III/IV OI came from 98 families. Again, each family was ascertained only once, and one affected child (usually the older) was taken as the proband and other affected sibs were counted only once. There were therefore 98 probands, who had 146 sibs of whom 10 were affected, giving an empirical recurrence risk of 6.9%.

Type I OI

The 11 cases had 13 sibs, all unaffected.

5.2.2 The sibships (appendix 5.1)

The sibships are presented in appendix 5.1 For types I, IIA, IIC and unclassifiable perinatally lethal OI there were 11, 30, 3 and 10 sibships respectively, all with one affected child. For type IIB OI, there was 1 sibship with 2 affected sibs and 13 with one affected child. For type III/IV OI, there were 98 sibships; in 8 there were affected sibs (6 with 2 affected sibs and 2 with 3 affected sibs) and in the remaining 90 families, the proband had no affected sibs. One of the 90 single cases in a sibship was born to related parents, who were first-cousin Pakistanis (figure 5.1). Two of the mother's sibs were said to have 'brittle bones' and were born in Pakistan. They died at one week and one year, respectively. The mother of the proband, a well-educated woman, stated that the phenotype of the proband and her sibs were very similar. It is presumed that this case, together with the cases

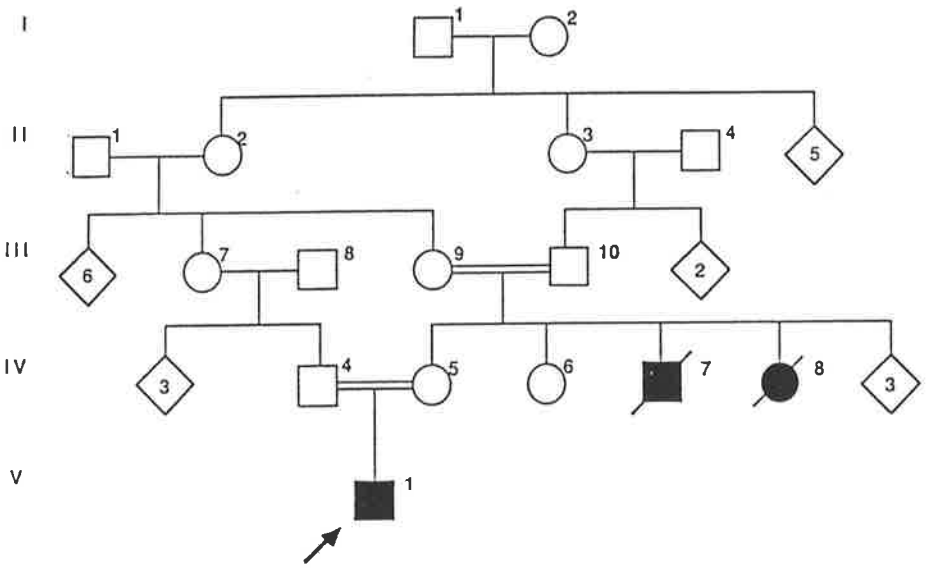


Figure 5.1

Pedigree of case 90 (arrowed) (the symbols are described in the notes to appendix 5.1).

in the 8 multiply affected sibships, arise as a result of autosomal recessive inheritance; they will be referred to as 'type III recessive' or simply 'type III' cases. (Parental gonadal mosaicism cannot be excluded however, particularly in the non-consanguineous families). The single cases (except case 90) will be referred to as 'sporadic type III/IV' or simply, type III/IV OI cases, since it is unknown whether they represent type III or IV OI.

The relationship of the time of birth of sibs to that of the proband is summarised in table 5.2. For the type IIA, type IIB and type IIC groups, the birth of an affected child had apparently not deterred the parents from further reproduction, since more or equal numbers of sibs were born after the proband than were born before. The same is probably also true for the type III/IV and unclassifiable perinatally lethal groups, since 41% of sibs were post-born in both.

5.2.3 Maternal miscarriages

The number of first trimester pregnancy losses experienced by the mothers is summarised in table 5.3. In addition, the mother of case 66 had a pregnancy loss at 5 months which is not included in the tables.

Mothers in the study group as a whole had lost about 10% of their pregnancies as early spontaneous miscarriages. This is not elevated above the estimate by Simpson et al. (1982), that about 10-15% of clinically recognisable pregnancies terminate in

Table 5.2

RELATIONSHIP OF TIME OF BIRTH OF SIBS TO THAT OF THE PROBAND

Type of OI	Total sibs	Pre-born sibs	Post-born sibs
IIA	30	19	19
IIB	13	4	9
IIC	3	1	2
Unclassifiable PNL	22	13	9
All III & III/IV	146	86	60
I new mutation	13	8	5

PNL - perinatally lethal

Table 5.3

MATERNAL MISCARRIAGES

a) Number of miscarriages per mother

	No. mothers					Total no. mothers
No. miscarriages	0	1	2	5	?*	
Type of OI						
IIA	17	4	2	1	6	30
IIB	13	-	-	-	1	14
IIC	3	-	-	-	-	3
Unclassifiable PNL	8	2	-	-	-	10
III/IV sporadic	70	15	2	1	1	89
III recessive	5	3	1	-	-	9
I new mutation	10	1	-	-	-	11
						166

PNL - perinatally lethal, ? unknown.

* See text.

b) Miscarriages as a percentage of total pregnancies

Type of OI	Total misc.	No. probands	No. sibs	Total pregnancies	% miscarriages
IIA	13	30	38	81	16.1
IIB	0	14	13	27	0
IIC	0	3	3	6	0
Unclassifiable PNL	2	10	22	34	5.9
III/IV sporadic	24	89	127	240	10.0
III recessive	5	9	19	33	15.2
I new mutation	1	11	13	25	4.0
	45	166	235	446	10.1

spontaneous abortion, usually during the first trimester. Table 5.3a shows that mothers mostly experienced only 1 or 2 losses; 2 mothers had had 5 miscarriages each. Table 5.3b shows the breakdown of miscarriages in the different types of OI. The reason for the notable difference in loss rates between the different groups is unknown. The maximum rate however (of 16.1% for mothers of type IIA probands) is only just outside normal limits. There is no definite evidence therefore of excessive fetal loss which could be attributable to fetuses affected with OI.

5.2.4 Delays in conceiving

Some mothers were asked if they had ever experienced delays in conceiving of greater than one year. A mother of a child with type IIB OI (case 163) had been unable to conceive until her ovarian cyst was removed surgically. The parents of 9 of the single cases of type III/IV OI reported a delay in conception of their first child, ranging from 15 months to 5 years. One couple (parents of case 64) had attended an infertility clinic. The parents of an affected sib pair had involuntarily waited 5.5 years before their first child was conceived. The information is summarised in table 5.4.

In total, 88 of 99 women (89%) reported that they had conceived within one year which is similar to the situation in the general population, in which 82% of women have achieved pregnancy after 12 months exposure (Shearman, 1986). It appears, therefore, that

Table 5.4

DELAYS IN CONCEIVING (No. cases)

Type of OI	Delay	No delay*	Unknown	Total
IIA	-	4	26	30
IIB	1	6	7	14
IIC	-	1	2	3
Unclassifiable PNL	-	1	9	10
III/IV sporadic	9 (12%)	65	15	89
III recessive	1	6	2	9
I new mutation	-	5	6	11

* Conception occurred within a year.

excessive early unrecognised loss of fetuses was not occurring, at least in the mothers who were asked.

5.2.5 Parental consanguinity and ethnic origins

Table 5.5 summarises the number of families in which parents were related, and the ethnic origins of the parents. Various points require clarification:

Type IIA OI

Two couples were definitely related, namely, the parents of case 120 who were first cousins and those of case 124 who were 'distant cousins'. In one other couple (case 121) remote consanguinity was suspected. All 3 couples originated from the Indian subcontinent. Three other couples were non-Caucasian, consisting of 2 couples in whom one parent was from India and the other from Tanzania and one couple were Indian Sikhs. One other couple comprised an English mother and a father who described himself as an Indian Arab. The parents of the remaining 23 cases were unrelated Caucasians.

Type IIB OI

In three families, the parents were related. These include first-cousin Pakistanis, who had 2 affected babies (cases 150 and 151) and first-cousin Sri Lankan Christians who were parents of 163. In the other family, the proband was the product of an

Table 5.5

PARENTAL CONSANGUINITY AND ETHNIC ORIGINS

Type of OI	Total no. of parent pairs	No. consanguineous couples	Ethnic origins of couples	No. non-consanguineous couples	Ethnic origins of couples
IIA	30	3*	Indian/ Pakistani 3	27	Caucasian** 24 Indian 3
IIB	14	3	Pakistani 1 West Indian 1 Sri Lankan Christian 1	11	Caucasian 9 Negro 1 West Indian/ Zimbabwe 1
IIC	3	0	-	3	Caucasian 2 African 1
Unclassifiable PNL	10	0	-	10	Caucasian 9 Indian sub-continant 1
III/IV sporadic	89	-		89	Caucasian 87 Jamaican 1 English mother Ethiopian father 1
III recessive	9	4	Muslim Indian 1 Jordanian 1 Irish gypsy 1 Pakistani 1≠	5	Caucasian 5
I	11	0	-	11	Caucasian 11

* Remote consanguinity suspected in one.
** In one case the mother was English and the father was an Indian Arab.
≠ Case 90.

incestuous union between a West Indian man and his daughter. Whereas consanguinity is common in Pakistan, it is unusual amongst Sri Lankan Christians and West Indians. In the remaining 11 families, the parents were unrelated and were Caucasian in 9, in one the mother was of mixed Caucasian and Negro origin and the father was Negro, and in another the mother was West Indian and the father was from Zimbabwe.

Type IIC OI and unclassifiable PNL OI

All parents were unrelated and Caucasian except in 2 cases in which the parents were African (type IIC) or from the Indian subcontinent (unclassifiable PNL).

Type III/IV OI

Eighty-nine of the single cases were born to unrelated parents of whom all but two were Caucasion; one (case 90) was born to first-cousin Pakistanis as described in section 5.2.2. Parents of 3 of the 8 sibships with 1 or more affected sibs were related and originated from racial groups in which consanguinity is common, namely Muslim Indian, Jordanian and Irish gypsy. The other 5 pairs of parents of type III cases were Caucasion.

Type I OI

All 11 pairs of parents were unrelated Caucasians.

5.2.6 Parental ages

Mean parental ages at the birth of the (first) affected child were compared to general population means for England and Wales, as shown in table 5.6. Babies with type II OI tended to have been born over a 10 year period, so population means for the modal year of birth of study cases were taken (Emery, 1986).

The patients with type III/IV sporadic, type III and type I OI tended to have been born over a greater time span (1930-1985; 1953-1985; 1944-1983 respectively) so that it was thought to be more accurate to take an average of population means (when available) and to weight these according to the number of study cases born in each year. Data was obtained from the Office of Population Censuses and Statistics Series FMI (HV), 1986. Figures for 'legitimate births for all birth orders' were used since all but one case were legitimate births.

Type IIA OI

There was a significant paternal and maternal age effect, more so for paternal age, when compared with the general population values for England and Wales in 1982 (Emery, 1986), the modal year of birth of the study cases. Fourteen fathers and 6 mothers were aged over 35 years at the time of birth of their affected child. (Note that the values are slightly different for paternal age from those in Young et al., 1987 because the age of one other father came to light after the paper was published).

Table 5.6

PARENTAL AGES AT THE BIRTH OF THE AFFECTED CHILD (MEAN ± 1 SD)

Type of OI	Mean paternal age (years)		t	p	Mean maternal age (years)		t	p
	Study cases	General population*			Study cases	General population*		
IIA	33.93 ± 7.18 (n = 29)	29.63	3.233	<0.005	28.87 ± 6.23 (n = 30)	26.37	2.198	<0.05
IIB**	25.44 ± 5.83 (n = 11)	29.80	-	-	22.28 ± 4.20 (n = 14)	26.45	-	-
IIC	27.77 ± 1.72 (n = 3)	29.49	-	-	26.62 ± 1.28 (n = 3)	26.33	-	-
Unclassifiable perinatally lethal	32.72 ± 9.21 (n = 9)	29.28	1.121	>0.2	27.98 ± 6.46 (n = 10)	26.24	0.853	p>0.4
III/IV sporadic	30.51 ± 6.20 (n = 89)	29.43	1.643	>0.1	27.82 ± 4.74 (n = 89)	27.16	1.313	p>0.1
III recessive**	29.05 ± 5.58 (n = 9)	29.70	-	-	24.49 ± 3.95 (n = 9)	27.22	-	-
I	31.87 ± 5.94 (n = 10)	29.43	1.299	>0.2	28.56 ± 5.58 (n = 10)	27.36	0.680	>0.5

t is t value using student's t test; p is probability. * Calculated as described in the text.
** Age of parents at the birth of the first affected child.

Type IIB OI and type IIC OI

Mean parental ages at the birth of the (first) affected child with type IIB and with type IIC OI were less than the general population values for England and Wales in 1983 and 1981 (Emery, 1986), the modal years of birth of the study cases, respectively.

Unclassifiable perinatally lethal OI

Although parental ages were above population means for 1979 (Emery, 1986), the modal year of birth of study cases, this was not statistically signficant.

Type III/IV OI

Mean parental ages at the birth of the single cases (excluding case 90, the presumed recessive single case) were elevated above the control population, but this was not statistically significant. The study group were born between 1930 and 1985 and the method to calculate general population means was described above.

Type III recessive OI

The mean parental age at birth was less than population means.

Type I OI

Although parents were older than population means this was not statistically significant. One mother and 4 fathers were aged over 35 years at the birth of the affected child.

5.2.7 Clinical assessment of parents

None of the parents was known to be affected with OI. Case 117 (type I OI) was adopted, but had been told that her natural parents were healthy. Appendix 5.2 summarises the various clinical features of the parents. Some points require clarification:

Number examined

The author personally examined 67 mothers and 58 fathers of 89 type III/IV single cases and 6 mothers and 5 fathers of type III recessive cases. One other couple with children with recessive OI was examined by a paediatrician. Twelve couples who had had a child with type IIA OI were examined by Dr Ian Young or the author and 2 by consultant geneticists. Of the 14 pairs of parents with a child with type IIB OI, 7 were seen by Dr Young or the author and 3 by a consultant geneticist. It was not possible to examine the parents of the affected sib pair with type IIB OI but hospital records stated that they were unaffected. The parents of 1 case of type IIC OI were seen by Dr Young; the

parents of the other type IIC probands were seen by consultant geneticists who noted no abnormalities. Six of the parents of the unclassifiable perinatally lethal OI cases were examined by Dr Young or the author. The author also examined 7 mothers and 4 fathers of type I OI cases. The findings tabulated in appendix 5.2 refer to parents personally examined by the author, Dr Young or another specialist, except where indicated. The small numbers in all the groups except type III/IV, do not allow comparisons between the groups to be made.

Fractures

A history of fractures was generally obtained from the parents themselves or if this was not possible, from the family. Major fractures (for example of long bones) and minor fractures (for example of fingers) were counted. The majority of parents in each group had never had a fracture. A maximum of 3 fractures was reported by three others. (The latter include the fathers of case 65 and 130 (types III/IV and IIA OI respectively) with no other signs of OI, and the mother of case 71 (type III/IV OI). She (the mother) had a history of unilateral congenital dislocated hip, with mild skin and joint laxity and pale blue sclerae. Her height was 5'7"). One parent had more than 3 fractures, namely the father of case 17 (a single case of type III/IV OI). He (the father) had had 7 fractures; he had pale blue sclerae, was 5'10" tall and had no other stigmata of OI.

In all parents, fractures were the result of some substantial

trauma and are thought not to signify bone fragility. Radiographs were taken of 2 sets of parents (both with children with type III OI, i.e. cases 104 and 105, and 106-108). None had Wormian bones of the skull or osteoporosis of long bones.

Scleral colour

The majority of parents had normal coloured sclerae, that is, white or very pale blue.

Pale blue sclerae were noted in about one-third of mothers and one-sixth of fathers overall. Although there was no formal control group, 16 Caucasian medical students were examined to obtain a feel for scleral colour. Ten of 11 female and all of 5 males had white or very pale blue (normal) sclerae. One female had pale blue sclerae. It is the author's feeling from this and general observations that pale blue sclerae may occur in the general population.

Seven parents, all mothers, had moderately blue sclerae; 6 were mothers of sporadic type III/IV cases (nos. 4, 11, 16, 19, 52 and 72) and one was a mother of a baby with type IIB OI (case 156). These mothers had never had a fracture (except the mother of case 4 who had had one) and they had no other significant clinical features of OI. The mother of case 4 also claimed to bruise easily and she had lax joints of the thumbs; the mother of case 11 had lax metacarpophalangeal joints and the mother of case 156 could hyperextend her elbows.

No parent had deep blue sclerae. In general, fathers were more likely to have normal coloured sclerae than mothers.

Joint laxity

The majority of parents did not have any joint laxity but those who did were more likely to be mothers (24%) than fathers (8%). However, any joint laxity noted in parents tended to be mild and was mostly confined to the small joints of the hands. Only 2 parents, both fathers, had significant generalised joint laxity. They were first, the father of case 36 (a single case of type III/IV OI), aged 44 years with marked generalised joint laxity. He had suffered 2 fractures in his life, had white sclerae, normal skin and hearing, and he was 5'11" tall. Case 36 herself had died at age 9 years but was not thought by the parents to be lax-jointed. She had no sibs. Secondly, the father of a sporadic case of type III/IV OI (case 26) had marked joint laxity as a child, but this had decreased with age, and at his present age of 35 years he was only mildly lax-jointed. He was otherwise normal. Case 26 himself had marked joint laxity; neither of his 2 sisters were examined, but the parents thought that their 8 year old daughter had joint laxity. Neither of the mothers of cases 26 or 36 had joint laxity. The only findings in them were very pale blue sclerae in the mother of case 26 and a report of easy bruising in the mother of case 36.

Easy bruising

A tendency to form bruises on the skin was reported by only 4 fathers (of cases 31, 34, 45 and 61), but was common amongst mothers, of whom 42 (39%) noted the tendency. Some women found that the bruising varied with their menstrual cycle, being more common around the time of menstruation.

Skin hyperextensibility

Skin hyperextensibility or 'stretchy' skin was noted in 3 mothers and 3 fathers of type III/IV sporadic cases (nos. 44, 47, 71 and 24, 45 and 58, respectively), in one mother and one father of type IIA cases (nos. 132 and 148, respectively) and in 1 father of a type IIB case (no. 163). It was mostly mild, except in case 44's mother whose skin stretchiness was of such a degree as to have become a family joke (figure 5.2). She had never suffered a fracture, and had pale blue sclerae, mildly lax hand joints, claimed to form tender bruises readily but thought that scar formation was normal. She reported that her sister, who had normal skin and pale blue sclerae, had a son with stretchy skin and pale blue sclerae who was late in walking. Case 44 herself showed mild skin stretchiness (but not as marked as her mother's) and generalised joint laxity and the father showed no abnormalities. Five of the other 8 parents with 'stretchy' skin also had mild joint laxity.

Figure 5.2

Hand of the mother of case 44. Note stretchy skin.

Cases 45 and 47 (both type III/IV sporadic cases) demonstrated a remarkable degree of joint hyperextensibility and soft, loose, stretchy skin; it is interesting to note that they both had a parent (a father and a mother respectively) with a mild degree of skin stretchiness, but no joint laxity. The father of case 45 stated that his mother had also had 'slack skin'. Both of these cases also had sibs with stretchy skin (see section 5.2.8). The corresponding parent, i.e. the mother of case 45 and the father of case 47 had mild joint laxity and no abnormalities, respectively.

History of hearing loss

One mother (of case 12) and 10 fathers (of cases 3, 21, 31, 35, 42, 47, 48, 60, 112 and 172) reported some degree of hearing loss. In 5 of the fathers it was thought to be due to local causes such as infections or to environmental noise. In the other parents, the cause of the hearing loss was not known.

Poor teeth

Three mothers (of cases 12, 47 and 60) and 2 fathers (of cases 50 and 60) were edentulous and gave a history of gingivitis or multiple caries as the cause of their poor teeth. None were aware of having dentinogenesis imperfecta.

Other clinical features

No parent was below the 3rd centile for height; 3 fathers of single cases of type III/IV OI (cases 12, 28 and 56) and 1 father of 3 presumed recessive type III cases (nos. 106-108) were on the 3rd centile for height which is probably not significant given that 2% of the population are on the 3rd centile. Other abnormalities in parents of single cases of type III/IV were macrocephaly in 1 mother (of case 7) and 2 fathers (of cases 68 and 69) (head circumferences greater than the 98th centile) and a spinal malformation in 1 mother (of case 48). One of the fathers of a case of IIA OI (no. 137) had ankylosing spondylitis.

5.2.8 Clinical assessment of unaffected sibs

Features pertaining to OI, other anomalies and any stillbirths were noted. Other anomalies and stillbirths are listed in table 5.7.

Type IIA OI

Of the 38 unaffected sibs, 11 were examined by Dr Ian Young or the author. In general, sibs showed no stigmata of OI apart from 2 sibs from separate families (a sister of case 132 and a brother of case 139) who had pale blue sclerae. Other anomalies in sibs included cleft palate, bilateral talipes and Kugelberg-Welander disease. One sib was stillborn at 24 weeks gestation, following onset of premature labour which was said to be related

Table 5.7

ABNORMALITIES AND STILLBIRTHS IN UNAFFECTED SIBS

Type of CI	Abnormalities and stillbirths	No. sibs	Sex of sib	Case no. of proband
IIA (n = 38)	Cleft palate	1	F	148
	Bilateral talipes	1	M	136
	Kugelberg-Welander disease	1	M	121
	Stillbirth at 24 weeks (maternal bicornuate uterus, fetus normal)	1	F	128
IIB (n = 12)	Small subaortic ventricular septal defect	1	F	156
	Cup-shaped ears	2	F,F	158
	Stillbirths - none			
IIC (n = 3)	No abnormalities or stillbirths	—	-	—
Unclassifiable PNL (n = 22)	Bilateral short 4th metacarpals	1	F	174
	Stillbirths - none			
III/IV sporadic (n = 127)	Bilateral inguinal herniae	3	M,M,M	40
	Death from congenital heart disease	1	F	52
	Death from undiagnosed severe hypotonia	1	M	52
	Early post-natal death from prematurity	1	M	23
	Early post-natal death - cause unknown	2	F,M	41,67
	Stillbirths - neural tube defect	1	F	16
	Prematurity due to - maternal cervical incompetance	2	M,M	39
	- cause unknown	1	?	77
III recessive (n = 9)	No abnormalities or stillbirths			
I (n = 13)	Stillbirth with neural tube defect	1	F	114

to the mother's bicornuate uterus; the fetus was normal.

Type IIB OI

Of the 12 unaffected sibs, 7 were examined by Dr Ian Young or the author. None had significant stigmata of OI. Case 158 had 2 sisters; the older had pale blue sclerae whereas the younger had normal sclerae and both had cup-shaped ears. Other anomalies in sibs included a small spontaneously-resolving sub-aortic ventricular septal defect. No stillbirths had occurred amongst the sibs.

Type IIC OI

None of the 3 sibs (of which one was examined by Dr Young) were known to have stigmata of OI, other anomalies, nor were any stillborn.

Unclassifiable perinatally lethal OI

Eleven of the 22 unaffected sibs were seen by Dr Young or the author. Apart from pale blue sclerae in 4 sibs (a brother and sister of case 172 and 2 brothers of case 176), they had no stigmata of OI. One sister had bilaterally short 4th metacarpals. No sib was stillborn.

Type III OI

Of the 9 unaffected sibs from the 9 sibships of presumed recessive type III cases, 4 were examined by the author and were found to have no significant stigmata of OI. The sister of cases 100 and 101 had mild calcaneus valgus and reduced hip movement at birth but these resolved spontaneously and she remained healthy. No sibs had any other anomalies or were stillborn.

Type III/IV OI

There were 127 sibs of the 89 sporadic cases of whom 55 were examined by the author, and are described as follows:

a) Fractures

Several sibs had suffered a single fracture following significant trauma. More notable was the brother of case 17, who had 7 fractures in his life, as had his father (see section 5.2.7), but these all resulted from substantial trauma. He had pale blue sclerae, was 5'11" tall at the age of 13 years and was otherwise normal. He was not considered to have OI.

b) Scleral colour

Twelve sisters (of cases 6, 12, 19, 49, 51, 52 (2 sisters), 61, 64, 65, 71, 73) and 8 brothers (of cases 10, 39, 46, 52, 58, 69, 71, 73) had pale blue sclerae which were not thought to be

significant. No sib had moderately or deep blue sclerae.

c) Joint laxity

Mild joint laxity was noted in 8 sisters (of cases 2, 15, 35, 48, 65, 68, 71, 73) and 5 brothers (of cases 14, 15, 47 (2 brothers), 63).

d) Easy bruising

A tendency towards ready formation of skin bruises was reported for 8 sisters (of cases 15, 29, 34, 35, 51, 55, 60, 61) and 3 brothers (of cases 3, 15, 52).

e) Skin hyperextensibility

Unusual skin hyperextensibility was noted in 4 sibs. These include the sibs of cases 45 and 47 who had marked joint and skin laxity, as described in section 5.2.7. The 25 year old sister of case 45 had 'slack' skin. She had suffered one fracture, was 5'5" tall and had white sclerae. Both brothers of case 47 had stretchy skin and mild joint laxity but no other stigmata of OI. The brother of case 52 was reported by the mother to have had lax skin; he had died at 4 years of an undiagnosed disorder (not OI) characterised by severe hypotonia.

f) Poor teeth

Two brothers, from separate families (of cases 39 and 61), had

carious, discoloured teeth but were otherwise normal.

g) Other anomalies

All 3 brothers of case 40 had bilateral inguinal herniae in infancy. These brothers and their 3 sisters had between them 13 children, of whom 12 (8 boys and 4 girls) also had bilateral inguinal herniae. They reportedly had no stigmata of OI.

The sister of case 52 died in infancy from congenital heart disease and their brother died from an undiagnosed disorder characterised by severe hypotonia, as described above.

Two sibs from different families had died in early infancy from unknown causes, and one had died at 3 days from causes related to prematurity.

h) Stillbirths

Four sibs were stillborn. One, the sister of case 16 had a neural tube defect. Two brothers of case 39 were stillborn preterm because of maternal cervical incompetence. The cause for the stillbirth of case 77's brother was unknown.

Type I OI

Of the 13 sibs, 6 were examined by the author. Most sibs had either no fractures or one; a brother (of case 115) had suffered

multiple leg fractures in a fall from a height of 14 feet. Sclerae were white or very pale blue in 5 and were pale blue in one (the sister of case 111). A sister of case 110, aged 20 years, had mild generalised joint laxity, bruised easily and had once dislocated a shoulder. Two other females aged 7 and 3 years, from the same sibship (of case 111) had mild laxity of the hand joints. These sibs had no other stigmata of OI. One sib (of case 114) was stillborn and had spina bifida.

5.2.9 The extended family (beyond first degree relatives)

Type IIA OI

There was no history of OI in the relatives of any proband. One mother's brother (case 123) had a neural tube defect, and her daughter by another marriage had 'clicky hips'. One mother (of case 134) had a sister with 'double-jointedness'.

Type IIB OI

No family gave a history of OI in the extended family. One mother (of case 152) reported that many of her immediate family members had poor teeth, and another mother with pale blue sclerae stated that her sister's sclerae were of the same colour.

Type IIC OI & unclassifiable perinatally lethal OI

There was no family history of OI other than in the probands.

Type III OI

In two families there were individuals with OI in the extended family. The first (family of case 90) is described in section 5.2.2 and is shown in figure 5.1. In the second (family of cases 95-97), the parents of 3 affected sibs were first-cousin Irish gypsies living in Wales. The mother was said to have had several affected sibs who had died in infancy of severe OI and it was thought that her parents were also related.

Type III/IV OI

Case 61, who himself had normal hearing, had a maternal aunt who had congenital deafness, as did 3 of her 5 offspring (by 2 spouses). One of these 5 children was mentally retarded. Case 72, who also had apparently normal hearing had a family history of late-onset deafness in her paternal grandmother and in that individual's father.

In 2 families (of cases 52 and 86) there were relatives in the extended family with probable OI. Attempts to confirm the histories by communication with appropriate doctors were unsuccessful. In the family of case 52, mother's brother's daughter, aged 11 years, was said to have had multiple fractures,

very blue sclerae and short stature. Her father was said to be unaffected; he had had no fractures, white sclerae and was 6 feet tall. Likewise, his sister, the mother of case 52, had no stigmata of OI apart from moderately blue sclerae. Case 52 himself had only pale blue sclerae. In the family of case 86, mother's maternal aunt aged 40 years was said to have OI, as was mother's brother's daughter. The author was not able to personally examine any members of this family but all other relatives were said to be normal.

Type I OI

No member of the extended families was known to have OI.

5.3 Discussion

5.3.1 Classification

The primary aim of the study was to determine empirical recurrence risk data for the severe forms of 'sporadic' OI. It was recognised that in order to provide data useful for genetic counselling, it was necessary to define precise and readily identifiable criteria for classifying groups to which the risk figures apply. It was found that the radiological appearances at birth allowed assignment of cases to one of the types of OI, as defined by Sillence and his colleagues (1979a and b, 1984) with one modification. In order to circumvent the difficulty in classifying a sporadic case of severe deforming OI as type III or

IV inherent in the Sillence classification, such cases were assigned to a combined III/IV group. Since OI is likely to be a group of monogenic disorders of type I collagen production, sporadic cases are likely to result from recessive inheritance or represent new dominant mutations themselves (or rarely dominant new somatic mutations in a parent lead to mosaicism involving the gonads) but unless they 'declare' themselves by the subsequent birth of an affected sib or offspring, the precise recurrence risk remains in doubt. For these cases, empirical recurrence risk data are particularly useful. The author wishes to stress that she is not advocating an alternative to the Sillence classification of OI, since clear-cut pedigrees of severe recessive (type III) and dominant (type IV) OI exist, but is suggesting that unclassifiable sporadic cases be grouped together for genetic counselling on recurrence risks.

It should be noted that the radiological appearances at birth had been described previously (Sillence 1979a&b, 1984) but the present study emphasises their importance, especially for the type III/IV group and clarifies some issues. First, the use of the term 'perinatally lethal' to describe type II OI (Sillence et al., 1984) is a potential source of confusion, implying that the time of death determines type. This has led to suggestions that type IIB and type III OI overlap (Spranger, 1984), since sib pairs were described in which one child died in infancy but the other survived (Glanzmann, 1944). The present study shows that babies with type IIB OI may survive the perinatal period, and babies with type III/IV may die at or soon after birth. Clearly

it is more reliable to separate radiological groups at birth where possible and indeed Sillence et al. have recently noted this (Sillence et al., 1986). Emphasis was placed in the present study on examining radiographs as soon as possible after birth. As was described in section 4.1.2.3, the modelled femur of type III/IV OI may become thick, rectangular and unmodelled (like the femur in type IIB OI) as early as one month after birth. Therefore, to avoid misclassifying a case of type III/IV as IIB, radiographs should be examined soon after birth, preferably during the first week of life. Failure to examine first-week radiographs in sibs probably led to suggestions that type IIB and III can occur in sibs. As mentioned above, Spranger 1984 suggested that the two sibs described by Glanzmann (1944) demonstrate type III (the girl) and type IIB (the boy). The girl was alive at 9 years and her first week radiographs were not presented; her brother died at 5 months. His radiograph revealed thickened long bones, but was taken at 4 weeks of age, so that it is not possible to classify him as having IIB or III OI.

Although it is possible that the type IIB appearance at birth is only a more severe form of type III, support for the non-identity of the radiologic appearance at birth of the two types is the fact that it was shown to 'breed true' in three sets of sibs, namely one set with type of IIB OI (cases 150 and 151) and two sets of type III OI (cases 95 and 96, and cases 106-108) in the present study. Difficulties in classifying reported cases as type III or IIB arise because of inadequate early radiological data, as indicated in tables 1.3 and 1.4.

Advantages of classifying cases on radiological appearance at birth are that the majority of cases do fit readily into one group, and the parents can be given genetic counselling as soon as is necessary. It should be noted that a few cases were difficult to classify. These included 3 cases with radiological manifestations generally consistent with type IIA OI but with thinner ribs than usual; two cases generally consistent with type IIB OI but with ribs of intermediate thickness and one baby with very poor modelling (type IIB) who had surprisingly good femoral length, as in type III/IV OI.

The evidence from the present study is that the radiological groups can be also differentiated in radiographs of terminated second trimester fetuses.

5.3.2 Recurrence risks and comments upon modes of inheritance

5.3.2.1 Type IIA OI

Given the basic assumption that OI is likely to result from monogenic disorders affecting type I collagen production, sporadic cases could result from autosomal recessive inheritance or represent new dominant mutations. The findings of this study strongly support the latter for type IIA OI. First, no affected sib pairs were ascertained, even though clinical genetic centres would be likely to know of affected sib pairs. In addition, it is unlikely that any bias was introduced due to unrecognised loss

in early pregnancy of affected fetuses and this applies to all the forms of OI in this study. Secondly, consanguinity was not observed amongst Caucasian parents. The consanguinity noted amongst Asian parents is of dubious significance, since consanguinity is common in some Asian populations. Thirdly, a significantly increased paternal age effect was noted, which is suggestive of new dominant mutations.

To try to ensure as far as possible that affected sib pairs are indeed uncommon in Great Britain, the author and her collaborators wrote to all members of the British Paediatric Association for Perinatal Paediatrics asking if members knew of any families in which there had been more than one affected child with lethal OI. No positive responses for definite type IIA OI were received.

Other authors have reported a deficiency of sibs of probands with lethal OI (Young and Harper, 1980; Spranger et al., 1982; Cohen et al., 1984). More recently, Beighton et al. (1988b) reported 9 type IIA probands, all of whom were sporadic cases.

Recent biochemical evidence also strongly supports the concept that type IIA OI mainly results from new dominant mutations, as was discussed in section 1.9.6.2.

The clinical observations from the present and other studies and the biochemical data tend to conflict with the conclusions of Sillence et al. (1984) that all cases of type IIA OI are likely

to be autosomal recessive. Their method of segregation analysis was the proband method which is suitable for multiple incomplete ascertainment (Emery, 1986). If, instead, the sib method had been applied, a segregation ratio of 0.128 with standard error of 0.05 would have been obtained. The authors reported on 28 sibships with type IIA OI, of which only three contained more than one affected child. Two of these sibships were being re-reported having been published previously.

Given the nature of the collagen molecule and its complex post-translational modification, it would not be too surprising if type IIA is indeed genetically heterogeneous. It is certainly possible that different mutations at the same locus could lead to either dominant or recessive inheritance. The present study does not rule out a small proportion of type IIA cases being autosomal recessive and there are a few reports of sib pairs with definite type IIA OI (Dinno et al., 1982; Shapiro JE et al., 1982). The molecular work, however, suggests that the occasional recurrence in a sibship is largely attributable to gonadal mosaicism in a parent (see section 1.9.6.2.8).

In summary, it is now becoming generally accepted that type IIA OI is mainly attributable to fresh dominant mutations (Beighton et al., 1988a). For purposes of genetic counselling, in the absence of biochemical or molecular investigation of an individual family, the author is currently quoting 2% as a reasonable estimate of the risk of recurrence, and mothers at risk can be offered detailed serial ultrasonography in the second

trimester of subsequent pregnancies for this and other forms of type II OI (see section 6.3.2).

5.3.2.2 Type IIB OI

The recurrence risk of 7.7% (1 in 13) is similar to that in the III/IV cases, but the numbers are considerably smaller. In addition, consanguinity was present in three families, two of whom come from racial groups where consanguinity is uncommon. At least 11 other examples of probable type IIB OI in sibs have been reported (see table 1.4), with parental consanguinity in two families. The parents of the single case of probable type IIB OI reported by Hein (1928) were also related. Parental ages in the present study were not elevated above population means.

The frequency of type II OI in Victoria, Australia was estimated at 1 in 62,000 live births (Sillence et al., 1984). Given that type IIB accounted for one-eighth of Sillence et al.'s cases and for about one-fifth of perinatally lethal cases in the present study, it is obviously a rare disease. The findings of recurrences in sibs and of parental consanguinity in this and other reports, suggests that autosomal inheritance may account for the majority of cases. There is as yet, however, no convincing evidence that this is so from biochemical studies. In Byers et al.'s study (1988c), there were 2 sibships with recurrent 'group V' perinatally lethal OI, which probably corresponds to Sillence type IIB. The parents were consanguineous in one family. These 2 families have not yet been

investigated for molecular defects.

Again, the possibility of parental germinal mosaicism to explain sib recurrences exists. In the family described by Horwitz et al. (1985), a mother of two babies with perinatally lethal OI had a third affected child by another unrelated husband. The babies had thin beaded ribs and so may have had type IIB OI. Biochemical studies were consistent with heterozygosity for an abnormal allele of one of the type I collagen genes (Byers et al., 1988c, family B).

At the present time, the author quotes a 25% recurrence risk for genetic counselling, because there is not enough evidence to exclude recessive inheritance for the majority of cases.

5.3.2.3 Type IIC OI

There were no recurrences in sibs in the present study, and there was neither consanguinity nor advanced age in the parents. The numbers, however, are too few to draw conclusions regarding mode of inheritance.

This is the rarest form of OI; only 2 other reports exist, both of which suggest autosomal recessive inheritance. The first is by Sillence et al. (1984) who described four cases in two families, three of whom were from one sibship; the second is by Danks (1975) who described two affected sibs, the children of first-cousin parents. No biochemical data for this type of OI is

available yet. For genetic counselling, the author quotes a 25% recurrence risk.

5.3.2.4 Unclassifiable perinatally lethal OI

If genetic counselling were requested following the birth of a child with radiologically proven perinatally lethal OI, but the radiograph had been lost so that classification was not possible, empiric recurrence risk figures of 3.8% could be given (table 5.1), derived from the number of affected sibs (N=3) out of total sibs (N=80) of all cases of perinatally lethal OI (N=60).

5.3.2.5 Type III/IV OI

The empirical recurrence risk of 6.9% for sporadic cases in this study is likely to be a maximum because if any bias in ascertainment is present, it would be towards finding affected sib pairs whose families are more likely to join the BBS or to be known to clinical geneticists. The recurrence risk could have been underestimated if parents had been deterred from further reproduction following the birth of one affected child. This does not appear to have been the case, as 41% of the 146 sibs were born after the proband (table 5.2).

The recurrence risk of 6.9% is consistent with the disease arising as a new autosomal dominant mutation in 72.4% of families and as an autosomal recessive in only 27.6%, assuming that these are the two genetic mechanisms operating. Indeed, biochemical

evidence for both dominant and recessive forms of moderately severe OI exists. In view of this heterogeneity, it is not surprising that parental age at the birth of sporadic type III/IV cases was not significantly greater than population means.

As might be expected, the presumed autosomal recessive cases appear to be over-represented in families in which the parents are consanguineous. In other words, the four families with related parents had other evidence of the disease being inherited in a recessive manner. These are two families with two affected sibs (cases 91 and 92, and cases 102 and 103), and the families of case 90 and of cases 95, 96 and 97 (three affected sibs) in which other individuals in the consanguineous pedigrees were affected. If these four families are excluded, then the empirical recurrence risk in families with unrelated parents is 4.4% (six affected sibs out of 135 sibs of 104 cases).

The current study has specifically addressed the problem of recurrence risk in sporadic cases of OI born with fractures. The author recognises that there are autosomal recessive forms of OI in which the first fracture occurs at some time after birth, as reported by Sillence et al., 1986; Lievre, 1959; Awwaad and Reda, 1960 (family 2) and Horan and Beighton, 1975; but these are probably rare.

The cases in the present study were mainly of Caucasian origin (see table 5.5). It is important to note that the recurrence risk of 6.9% may not apply to other ethnic groups. In

particular, Beighton and Versfeld (1985) found a relatively high incidence of type III OI in a survey of Black patients of various tribal origins attending a hospital in Johannesburg. Of the 23 patients classified as type III OI, 12 were familial and 11 were sporadic. For the latter, recessive inheritance was not proven. Nevertheless, it should be born in mind that the recurrence risk of 6.9% quoted herein may not represent the risk in some ethnic groups.

In summary, sporadic cases with the moderate bone changes of 'type III' OI at birth cannot be reliably diagnosed as type III or IV OI. The overall empirical recurrence risk is 7%. In practice, the author gives a recurrence risk of 4.4% to parents of a sporadic case born to unrelated Caucasian parents and a 25% risk if the parents are consanguineous.

Prenatal diagnosis for future pregnancies, in the form of ultrasound scanning, can be offered but the reliability of normal scans is uncertain (see section 6.3.2). As mentioned previously, it would be useful to be able to characterise the mutation in individual families, so that autosomal recessive cases could be identified and the parents offered a precise prenatal test in subsequent pregnancies. For those who were demonstrated to have 'new' dominant mutations, the possibility of gonadal mosaicism in a parent would have to be considered.

5.3.2.6 Type I OI

There were no affected sibs in this small group in the present study, as might be expected, assuming that the condition arose as a fresh dominant mutation in each case. Parents were older than population means at the birth of their affected child, but the data were not significant statistically. Carothers et al. (1986), however, studied 80 presumed new mutation cases of dominant OI (48 with type I and 32 with type IV) and found a significant increase in paternal age.

5.3.3 Discussion of clinical manifestations in family members

5.3.3.1 Parents

Although none of the parents was thought to be affected with OI, it is important to consider whether any demonstrated clinical manifestations which might be attributable to heterozygosity for a recessive form of OI. If this were the case, then it would be possible to predict clinically families with a 25% recurrence risk after the birth of one affected child.

As a starting point, it is logical to look at families with more than one affected sib (type III OI) in whom the disease is likely to be due to a recessive gene (or possibly to a dominant gene present in a parent with somatic and gonadal mosaicism), to see if parents who are expected to be heterozygotes for a recessive

gene (or somatic mosaics for a dominant gene) showed more manifestations than parents in whose affected child the disease presumably arose as a fresh mutation (type IIA and type I OI). Reference to appendix 5.2 shows that this is not the case in general. Clearly, the small numbers in most groups make statistical comparisons impossible. It is likely, but not proven, that these generally mild clinical findings do not denote carrier status of a recessive gene for OI, or somatic mosaicism for a dominant gene.

Autosomal recessive inheritance is more likely when parents are consanguineous. Unfortunately, the author was able to examine consanguineous parents in only one case of type IIB OI (case 163) and of only one sib pair with type III OI (cases 91&92); their findings are summarised in table 5.8. It has been the experience of the author in general medical practice that in individuals of Asian origin, as these 4 parents were, pale blue sclerae, joint laxity in the hands and short stature are common, so that no significance can be attached to these findings.

Another approach is to look for any parent with significant clinical findings which might suggest a defect of collagen. As noted in section 5.2.7, two parents, the fathers of cases 26 and 36, both sporadic cases of type III/IV OI, had significant joint laxity. However, neither of the mothers of these cases had any significant abnormalities. Only one of the probands themselves had joint laxity (case 26). It is impossible to determine clinically whether it can be inferred that these fathers carried

Table 5.8

MANIFESTATIONS IN CONSANGUINEOUS PARENTS OF PATIENTS WITH TYPE IIB AND III OI WHO WERE EXAMINED BY THE AUTHOR

Manifestation	Type IIB OI (Case 163)		Type III OI (Cases 91 & 92)	
Ethnic origin	Sri Lankan Christian		Muslim Indian	
	Mother	Father	Mother	Father
No. fractures	0	0	0	0
Scleral colour	pale blue	white	very pale blue	very pale blue
Joint laxity	hyperextensibility of interphalangeal and metacarpophalangeal joints in all 4 parents			
Easy brusing	yes	-	-	-
Skin hyperextensibility	-	mild	-	-
Height	-	-	-	5'4" (3rd centile)
Teeth and hearing normal in all 4 parents				

a defective collagen gene which might be important in the genesis of OI in the offspring. Likewise, the only parent with significant hyperextensibility of the skin had a normal husband and an affected daughter (with type III/IV OI - case 44) who had mild skin stretchiness, and it is not possible to determine clinically whether the mother's skin stretchiness is significant. The converse situation occurred in cases 45 and 47 (both with sporadic type III/IV OI). Both had marked skin and joint hyperextensibility, and one parent of each child had a mild degree of skin stretchiness. These families (of cases 26, 36, 44, 45 and 47) may well be good candidates for collagen protein and gene analysis.

In summary, there was no convincing clinical evidence for heterozygosity for a recessive OI gene (or for somatic mosaicism for a dominant OI gene) in the parents in the present study. Similarly, the consanguineous parents of a boy with severe deforming OI who was homozygous for an abnormality of the α2(I) chain of type I collagen were shown to be heterozygotes for the defect but they were clinically normal (Nicholls et al., 1979, 1984a).

5.3.3.2 Clinical manifestations in sibs

Amongst the 224 unaffected sibs none could be definitely pin-pointed as having any manifestations pertaining to heterozygosity for a recessive OI allele, or somatic mosaicism for a dominant mutation. The sibs had a variety of congenital anomalies and

other disorders including 2 with a neural tube defect. It is unlikely that these are of any significance.

5.3.3.3 The extended families

In two of the consanguineous families with type III OI there were affected individuals in two generations, which strongly suggests recessive inheritance in these families.

The two families (of cases 52 and 86 with severe type III/IV OI) in whom distant relatives of the proband reportedly had OI are more difficult to explain and unfortunately the information on them was somewhat anecdotal. The explanations include inheritance of a dominant gene with non-penetrance in some individuals, or separate fresh mutations within the families. Both of these seem unlikely. Another possibility is compound heterozygosity. One mild or silent dominant allele could have been inherited by affected individuals; the abnormality in the other allele could have arisen as a new mutation or could have been inherited. Again, this seems unlikley. These families would be good candidates for more detailed clinical examination and for biochemical analysis. Gillerot et al. (1983) described a baby with probable type II OI whose great-grandmother and other more distant relatives had mild dominant OI. As in our families, this tantilising (but rare) association of severe and mild OI in one family may be fortuitous.

In the other families in the present study, there were no

findings of particular note in the distant relatives, except for the ones with inguinal herniae. Familial bilateral inguinal hernia has been reported previously (no. 14235, McKusick, 1988) and may well be a fortuitous association with OI in the same family.

Addendum:

Recently, the author was informed that case 6 (type III/IV OI) had become pregnant, having been refused a sterilisation by her doctor on the grounds that she represented an 'anaesthetic risk'. She gave birth (by Caesarian section at 36 weeks gestation) to a daughter who unfortunately has OI. The baby has grey-blue sclerae, short bowed femora and tibiae, and Wormian bones of the skull. The radiographic features at birth were those of type III/IV OI, as described in section 4.1.2.1. Case 6 herself was 22 years when seen by the author; she had had 20 fractures and had moderately blue sclerae (grade 1-2), with short but only mildly bowed legs, straight arms and severe pectus carinatum and a marked kyphoscoliosis, with severe short stature (height - 9.7 SD). The defective gene in this family is most likely to be inherited as an autosomal dominant trait.