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Dominant mutations in familial lethal and severe osteogenesis imperfecta

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Summary. Four families presenting with familial osteogenesis imperfecta (OI) have been studied: 2 with the lethal type II and 2 with the severe type III form. Fibroblasts of the patients, all issue from non-consanguineous parents, produced normal and abnormal α(I) chains. These heterozygous mutations differentiate the recurrent forms from homozygous mutations characteristic of autosomal recessive forms. Although the identity of the mutations could not be determined, such recurrence of autosomal dominant OI is probably the result of germinal mosaicism in one of the parents. Biochemical results were consistent with a somatic mosaicism in the father's fibroblasts in one family. Moreover, our studies show that not only OI type II but also severe OI type III can arise from gonadal mosaicism. We discuss the importance of such a phenomenon for genetic counseling.

Introduction

Osteogenesis imperfecta (OI) is a heterogeneous group of inherited disorders in which bone fragility is the main feature. Controversy remains concerning the mode of inheritance of the lethal form OI type II (Maroteaux et al. 1986; Thompson et al. 1987), which was originally described as a recessive form (Sillence et al. 1979, 1984). However, biochemical and molecular studies (Bateman et al. 1986, 1987, 1988; Byers et al. 1988; Lamande et al. 1989; Baldwin et al. 1989; Cohn et al. 1986) together with other clinical investigations (Young and Harper 1980; Thompson et al. 1987) were compatible with new dominant mutations in one of the genes of type I collagen. Recently, recurrent dominant forms of lethal OI (type II) were described (Byers et al. 1988; Cohen-Solal et al. 1988) and the hypothesis of a parental germinal mosaicism for a mutation in one of the type I collagen genes was demonstrated (Wallis et al. 1990; Cohn et al. 1990).

We report here not only the study of 2 families presenting with recurrent OI type II but also 2 other families presenting with recurrent severe OI (type III) in which the biochemical data can be explained by parental germinal mosaicism. The relevance of such findings for genetic counseling is discussed.

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Materials and methods

Clinical reports

Family I. Parents came for genetic counseling in June 1986 after the termination of a first pregnancy at 22 weeks (I.2) on the basis of the shortening and bowing of limb bones. The radiographs showed beaded ribs, shortened broad and crumpled long bones and a markedly unmineralized skull (Fig. 1a), features typical of OI type IIA.

Six months later, a second pregnancy was terminated at 12 weeks on the basis of ultrasonography (I.3). The diagnosis of OI type II was subsequently confirmed by X-ray analysis. Finally, 18 months after the second termination, abortion was again achieved at 14 weeks (I.4). Radiographic and clinical features were again compatible with a diagnosis of OI type II.

Careful clinical examination of the parents was carried out. The father (I.F) was 28 years old and the mother (I.M) was 23 in 1986. They were both healthy and did not present any features of OI. Radiographs of the father's limbs confirmed the absence of fractures and osteopenia. They were not consanguineous. The mother had a non-affected daughter from her first marriage.

Family II. In this family a pregnancy (II.2) was terminated in 1987 at 30 weeks because of the shortness and bowing of the limbs associated with "hydramnios". In X-rays, the typical features of OI type II, including the beaded aspect of the ribs because of multiple fractures, and crumpling and angulation of the long bones, clearly confirmed the diagnosis of lethal OI (Fig. 1b). The unrelated parents were healthy and there was no history of skeletal problems in their close relatives.

They were respectively 29 years old (mother) and 25.5 years old (father) at that time. They already had a daughter, who was phenotypically normal, but who at the age of 3 began to suffer acute lymphoblastic leukemia.

In 1989, the ultrasound examination performed during the course of a third pregnancy again revealed symptoms of lethal OI, which was confirmed by X-ray analysis. Abortion was performed at 24 weeks of pregnancy (II.3); the karyotype demonstrated the presence of a triploidy. The meaning of this unexpected observation with respect to OI is unclear.

Family III. In this family, 2 sibs out of 3 presented a severe form of OI. The brother (III.2) was 30 years old and the sister (III.3) 33 years old. Their parents were not consanguineous and did not have any features of OI.

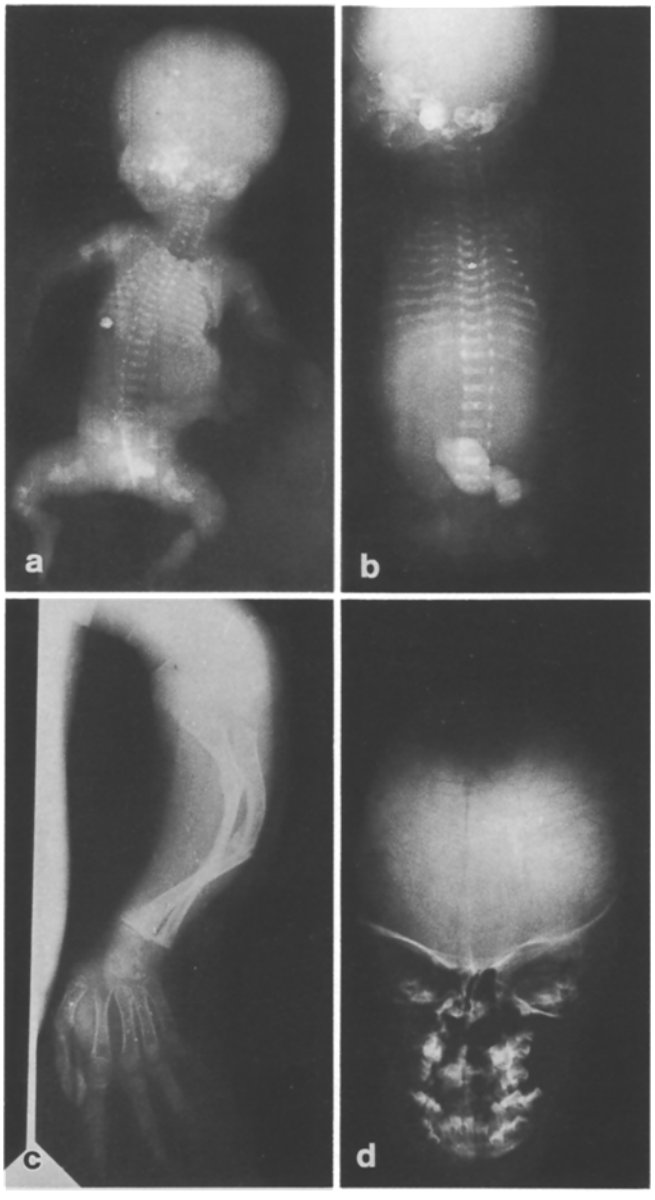

Fig. 1a–d. Radiographs of patients with lethal and severe osteogenesis imperfecta. **a** X-rays of fetus I.3 with typical features of OI type II. **b** X-rays of fetus II.3 with OI type II. **c** X-rays of the upper limb of patient IV.1 affected with severe OI. **d** X-rays of the skull of patient IV.1 showing Wormian bones

On clinical examination, these patients presented with very severe growth retardation. The brother is bed-ridden, whereas his sister can sit but not walk. The skull of each patient was enlarged transversally and their limbs excessively bowed. The presence of kyphoscoliosis was more obvious in the sister. She suffered from hearing impairment, although her brother did not. Their sclerae were normal and dentinogenesis imperfecta was not detectable.

Family IV. In this family, two girls (IV.1 and IV.2) were born of normal, non-related parents. They both suffered a severe form of OI that following the multiple deformations of the limbs because of fractures, was diagnosed at birth. Their growth had been severely delayed: at 31 months, the elder measured 73 cm. The skull of each child was enlarged transversally. We noted excessive joint laxity. Their sclerae were blue and dentinogenesis imperfecta of the deciduous teeth was present.

Radiographs confirmed the slenderness and the incurvation of long bones (Fig. 1c). The skull showed multiple Wormian bones (Fig. 1d). Reduced height of vertebrae was observed. The mental development of the elder was retarded.

Tissue sampling

Bone and skin were obtained with the appropriate consent of the families. Bone and skin of I.3 and II.3 allowed biochemical studies of tissue collagen. Fibroblast cultures were initiated from dermal biopsies of affected infants and parents of family I.

Solubility of dermal and bone collagens

Dermal samples were ground in liquid nitrogen and lyophilized. Samples were then extracted in acetic acid followed by pepsin digestion. After acid hydrolysis, hydroxyproline was measured (Stegeman 1958) in the whole skin, in the different solubilized fractions, and in the final insoluble residue. A similar protocol was followed with powdered bone samples after demineralization in 0.5 *M* EDTA (Bonaventure et al. 1989).

Analysis of collagens synthesized by cultured dermal fibroblasts

Fibroblast cultures and collagen labeling with L [4–5^{3}H]-proline were performed as described previously (Bonaventure et al. 1986). Collagen synthesis was measured and evaluated according to Wiestner et al. (1979).

Procollagen- and pepsin-treated collagen chains were analyzed by 5% SDS-PAGE (Neville and Glossman 1974). Unmodified α chains were obtained after incubation of cells in the presence of αα′dipyridyl (Steinmann et al. 1984).

Temperature of denaturation

Melting temperatures of the triple helix of normal and abnormal collagens were determinated as described by Bruckner and Prockop (1981). Samples warmed from 30°C to 43°C were treated with trypsin (100 μg/ml final concentration) and analyzed by 5% SDS-PAGE.

Identification of overmodified peptides

Collagen α chains or vertebrate collagenase-treated α chains were separated by 5% SDS-PAGE. The gel strips were treated with CNBr (50 mg/ml in 70% formic acid) and the peptides obtained in situ separated by 12.5% SDS-PAGE (Barsh et al. 1981).

Results

Tissue collagens

Only the tissues of lethal forms I.3 and II.3 were available for analysis. The collagen content of the dermis and bone of these patients was dramatically decreased by more than 75%. Collagen solubility was modified but differences were noticed between the two patients. The collagen extracted from the dermis of I.3 was mostly solubilized by pepsin treatment, whereas an important proportion of the collagen from the dermis of II.3 was soluble in acid. The bone collagen of I.3 had the same solubility as the dermal collagen; in II.3, the solubility was normal compared with a 17-week-old control fetus (Table 1).

Table 1. Collagen composition of dermis and bone of OI type II

	Dermis			Bone[a]		
	Controls[b]	I.3	II.3	Controls[b]	I.3	II.3
Fetal age	17–29 weeks	12 weeks	20 weeks	17–29 weeks	12 weeks	20 weeks
Hydroxyproline μg/mg dry weight	58.1 ± 8.7	14.0[c]	12.8[c]	48.5 ± 10.4	15.4[c]	9.7[c]
Collagen solubility % hydroxyproline						
Acid soluble	32.5 ± 5.3	4.7[c]	64.4[c]	3.3 ± 0.1	~0	2.0[d]
Pepsin soluble	65.2 ± 8.7	94.1[c]	18.0[c]	28.8 ± 3.0	90.1[c]	25.7[d]
Residue	2.3 ± 0.8	0.1	12.7[c]	67.7 ± 3.2	9.9[c]	72.3[d]

[a] Result expressed per mg/dry weight of undemineralized bone
[b] Values for controls: mean ± SD of two different fetuses
[c] Significantly different: values outside the control mean ± 2 SD
[d] Not significantly different

Table 2. Collagen synthesized by fibroblasts in culture medium and cell layer (% total protein)

Family	Medium	Cells
Family I		
I.3, fetus, 12 weeks	8.2	1.7
I.4, fetus, 15 weeks	15.3	2.2
I.F, 30 years	9.5 ± 1.1[a] ($n = 2$)	1.1 ± 0.02[a]
	3.1 ± 0.01[b] ($n = 2$)	1.0 ± 0.01[b]
I.M., 25 years	6.9[c] ± 0.3 ($n = 2$)	3.0 ± 0.1[c]
	8.6[c] ± 0.5 ($n = 2$)	2.0 ± 0.05[c]
Family II		
II.3, fetus, 20 weeks	12.2	1.1
Family III		
III.2, 30 years	11.9	1.3
III.2, 33 years	14.5	1.6
Family IV		
IV.1, 6 years	3.9	1.1
Controls		
Fetus ($n = 2$), 11–17 weeks	23.2 ± 3.8	2.4 ± 1.2
Infant ($n = 2$), 3–6 years	13.4 ± 1.8	1.3 ± 0.6
Adult ($n = 3$), 30 years	11.5 ± 2.5	1.1 ± 0.4

[a] Mean of values ± SD obtained with fibroblasts from two different skin biopsies that synthesized normal type I collagen
[b] Mean of value ± SD obtained with fibroblasts from two different skin biopsies that synthesized normal and abnormal type I collagen
[c] Mean of values ± SD obtained with fibroblasts from two different skin biopsies

Collagen synthesis

The collagen synthesized by cultured fibroblasts from the probands was measured. Parental cells were also studied when available. In the lethal forms, collagen synthesis was significantly decreased after 20 h labeling, confirming the low collagen content of the tissues. Parents in family I had normal or lightly decreased synthesis when compared with an age-matched control. Repeated determinations on different cell batches from the parents showed some variability in the collagen synthesis of the father's fibroblasts from the same biopsy or from two different biopsies (Table 2).

In severe forms, the fibroblasts of the two probands of family III showed normal collagen synthesis, whereas the proband of family IV exhibited a dramatic decrease of collagen synthesis (we failed to culture the fibroblasts of IV-2). Results are summarized in Table 2.

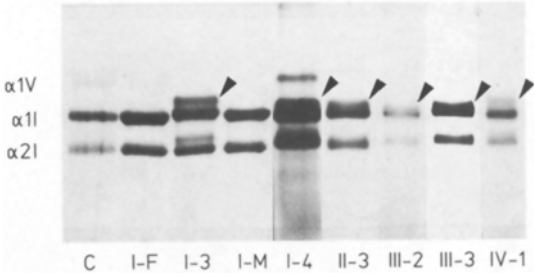

Fig. 2. Elecrophoretic migration of type I collagen chains secreted by fibroblasts in the culture medium. *C* Control, *I. F.* father from family I, *I.M.* mother from family I

Analysis of collagen chains and peptides from cultured fibroblasts

Examination of the electrophoretic migration of type I procollagen chains and α chains obtained after pepsin digestion revealed the same electrophoretic pattern in the lethal and severe forms. Two distinct populations of α1(I) and α2(I) chains were present (Fig. 2), one co-migrating with the control and the other showing a delayed migration, the sign of a heterozygous mutation. Intracellular collagen accumulation was noticed only in the lethal cases. Slower electrophoretic mobility of part of the α chains was related to post-translational overmodification of lysine residues because of the delay in the folding of the heterotrimer containing mutant chains. Indeed, overmodifications were abolished by αα′dipyridyl treatment, which blocks lysine hydroxylase (not shown). This result favored the hypothesis of a point mutation in one allele of COL1A1 or COL1A2. In family I, the collagen synthesized by the parents was carefully examined. A first study had shown a normal migration of α1(I) and α2(I)

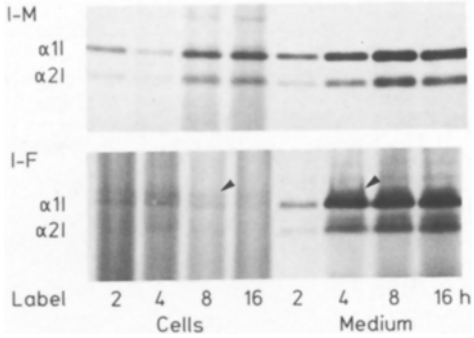

Fig. 3. Type I collagen synthesized by skin fibroblasts of the mother *(I. M.)* and father *(I. F.)* (family I) during increasing labeling periods

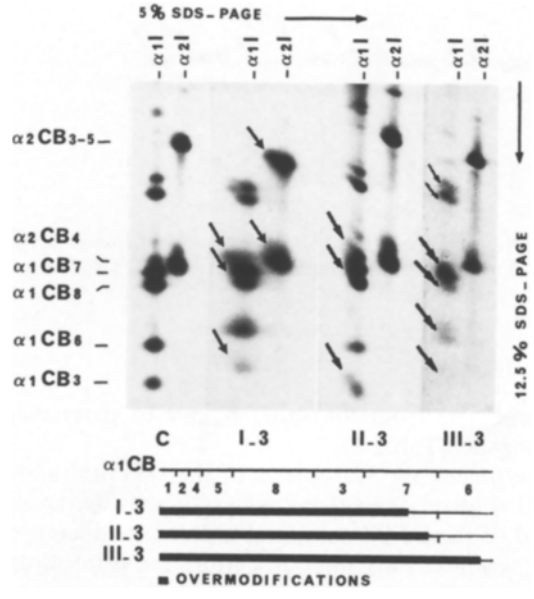

Fig. 4. Peptide mapping of collagen type I chains obtained by CNBr cleavage in affected subjects. The extent of overmodification is diagrammatically presented for each patient *(below)*

collagen chains from the mother and father. A second study with shorter labeling periods (2, 4, 8, 16 h) was undertaken in order to test if one of them or both synthesized an abnormal chain that was rapidly degraded. The father's fibroblasts synthesized a small amount of a population of slow migrating chains (Fig. 3). This result was confirmed with the fibroblasts from a second biopsy. Repeated experiments with different cell batches from the 2 biopsies gave either only normal or normal and abnormal chains. Moreover, the presence of abnormal chains was associated with a decrease in collagen synthesis. Some factors during the culture process may have allowed the selection of normal cells, which would grow faster than the mutant fibroblasts.

Cyanogen bromide peptide mapping showed that, in the probands of families I and II, overmodification was initiated in the α1CB7 peptide (Fig. 4), and more precisely in the α1CB7A or α2CB3–5A peptide as far as the

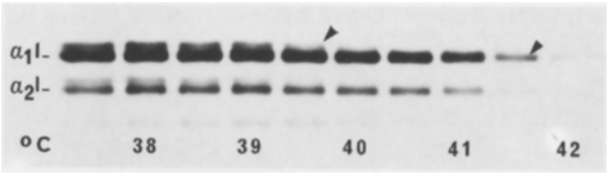

Fig. 5. Denaturation temperature of the triple helix of type I collagen synthesized by patient I.4. *Arrows* indicate the melting temperature of overmodified α chains

two probands I-3 and I-4 are concerned. In the severe forms, only peptide mapping of OI III.1 and OI III.2 could be performed showing that the mutation was located at the α1CB6 level.

As expected, the thermal stability of the triple helix of the mutant collagen synthesized by probands affected with lethal OI was decreased to 39.5°C for I.3 and I.4 (Fig. 5) and to 37.5°C for II.3.

Discussion

Our results clearly demonstrate that, in these four families, the affected infants present a heterozygous mutation in their type I collagen, thus excluding a recessive transmission of the disease. Consistent with recent results from Cohn et al. (1990), Wallis et al. (1990) and Constantinou et al. (1989, 1990), the clinical and biochemical data favor the hypothesis of a germinal mosaicism in one of the parents. In one case (family II), we have now identified the mutation as a single base substitution in a glycine codon of the COL1A1 gene (unpublished results). Such a point substitution is usually sufficient to produce a lethal phenotype but it does not preclude the occurrence of a second unrelated mutation; this however remains unlikely. DNA analysis of parental germ and somatic cells will be required in order to validate the hypothesis.

In family I, the poor secretion by the father's fibroblasts of normal and overmodified α chains was indicative of somatic cell mosaicism. Since somatic and germline mosaicism have been shown to be regularly associated in several recurrent cases (Sykes et al. 1990), we suggest that this association also occurs in the families reported here.

In addition to recurrent lethal OI (type II), we have some indications that severe OI (type III) can also arise from germinal mutations. This is not surprising since variability in the severity of the phenotype would depend on the position and nature of the substituting amino acid as demonstrated by Starman et al. (1989), Wenstrup et al. (1990), and Cole et al. (1990).

Two clinical implications emerge from our data. First, contrary to early assumptions by Sillence et al. (1979), 1984, 1986), it appears that recurrent lethal or severe OI in children from non-consanguineous parents is caused by parental mosaicism rather than by recessive inheritance, which seems to be restricted to related parents.

Secondly, genetic counseling must be reconsidered on the basis of this concept. Whereas the recurrence risk

in recessive disease is 25%, this value is much lower if the transmission is dominant. An estimated recurrent risk of 6%–7% was proposed by Byers et al. (1988), a value which, according to our own clinical experience, could be an overestimate. Although we have to be aware of possible parental mosaicism in sporadic new cases, we think that genetic counseling must remain reassuring. In families with a first affected infant, if one of the parents proves to be mosaic for the mutation, great care should be taken in counseling, since recurrence will depend on the extent of germinal mosaicism.

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