Original Paper

European Journal of Human Genetics

Eur J Hum Genet 1996;4:39–45

Allan M. Lund[a]
Marianne Schwartz[a]
Michael Raghunath[b]
Beat Steinmann[b]
Flemming Skovby[a]

[a] Juliane Marie Centre, Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark;
[b] Division of Metabolic and Molecular Diseases, University Children's Hospital, Zurich, Switzerland

Gly802Asp Substitution in the proα2(I) Collagen Chain in a Family with Recurrent Osteogenesis imperfecta due to Paternal Mosaicism

Key Words
Mosaicism
Germinal mosaicism
Somatic mosaicism
Non-lethal osteogenesis imperfecta
Aspartic acid
Genetic counselling
Sequencing

Abstract
A proband with osteogenesis imperfecta (OI) type III/IV was born to clinically normal parents, who subsequently had two pregnancies terminated because of OI in the fetuses. Cultured fibroblasts from the proband, one fetus and the father produced abnormal collagen I. Cyanogen bromide mapping localised the defect to the region of the α1(I)CB7 peptide. Sequencing revealed a G to A transition at nucleotide 2814 in COL1A2 in the proband, the fetus, and the father, which resulted in a Gly802Asp substitution in the proα2(I) collagen chain. About 25% of the paternal alleles from fibroblasts and leucocytes and 40% of paternal alleles from spermatocytes carried the mutation consistent with somatic and germinal mosaicism. For genetic counselling, parental mosaicism must be considered in all sporadic cases of OI.

Introduction

Osteogenesis imperfecta (OI) is an inherited generalised connective tissue disorder with a wide clinical spectrum ranging from perinatal death to life-long mild osseous fragility. On the basis of clinical, radiographic and genetic features, OI can be divided into four types [1]. At least 95% of cases are caused by mutations in COL1A1 or COL1A2, the genes encoding the α1(I) or the α2(I) subunits of collagen I [2], and molecular and protein-chemical studies have shown most patients with OI to be heterozygous for a dominant mutation in COL1A1 or COL1A2 [3].

Genetic counselling is difficult in families with more than one affected child born to unaffected parents. Autosomal recessive inheritance has been shown in very few families with OI [4–6] and only in a single case with abnormal collagen I [7–9], but it cannot be excluded. The electrophoretic finding of both normal and abnormal collagen I may, however, make autosomal recessive inheritance unlikely. Instead germinal mosaicism for a dominant mutation in COL1A1 or COL1A2 in one of the parents may be a more plausible hypothesis, which has been confirmed in several families with OI type II [10–17]. In a smaller number of cases, parental mosaicism has also been shown to account for recurrence of non-lethal OI (OI types III and IV) [18–20].

We describe a family with one affected child and two fetuses with OI and show that they inherited a mutant COL1A2 allele from their clinically unaffected father.

KARGER
E-Mail karger@karger.ch
Fax + 41 61 306 12 34

1018–4813/96/0041–0039$10 00/0

Allan M. Lund
Juliane Marie Centre
Department of Clinical Genetics
Rigshospitalet, Blegdamsvej 9
DK–2100 Copenhagen (Denmark)

Received: June 28, 1995
Revision received: October 18, 1995
Accepted: October 19, 1995

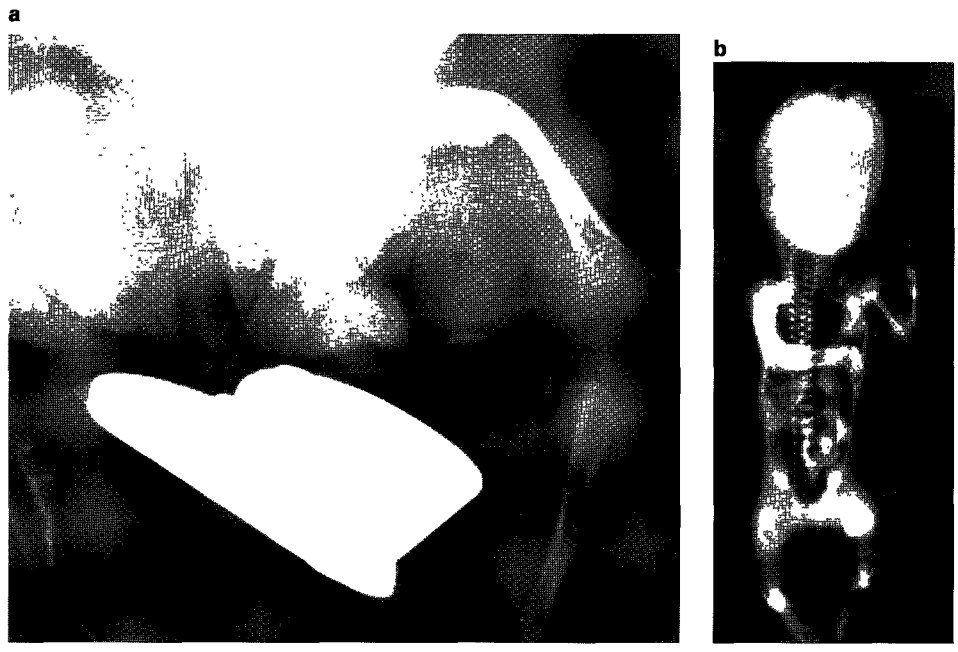

Fig. 1. a X-ray of the proband's lower limbs at 1 year. **b** X-ray of the second fetus aborted after 22 weeks of gestation.

Clinical Summary

As a newborn, the proband (L.P., born 1984, fig. 1) was noted to have blue sclerae, a small thorax, short limbs and a big head with soft calvaria. The birth weight was 2,650 g, length 45 cm. X-rays at birth revealed wormian bones, shortened long bones and fractures of varying ages of the ribs and lower extremities. His subsequent growth has been severely retarded: at 5 and 9 years his height was 75 and 97 cm, respectively. He has had 5 fractures of the lower extremities, but is able to walk independently, even though he prefers to use a wheelchair. Both femora are bowed in an anterolateral direction. He is loose-jointed in hands and feet, whereas hips, knees and elbows show a normal mobility, except for the right knee, which has a flexion contracture of about 15°. The skin is normal. The teeth are affected by dentinogenesis imperfecta: they are brownish and slightly transparent, but there are no fractures. No Panoramix pictures are available. The color of his sclerae has gradually faded into light grey-blue.

The parents are not related and both appear healthy. At initial genetic counselling they were told that the child most probably had a de novo mutation and that the recurrence risk was small. Biochemical studies of collagen from the proband were not done at that time, and second trimester ultrasound examination in future pregnancies was recommended. Two subsequent pregnancies (in 1988 and 1989) were terminated in the 21st and 22nd week of gestation, respectively, because of abnormal ultrasound findings: in the first, there were bilateral fractures of the crus and shortened long bones, especially the femora, and in the second, shortened long bones and severe bowing of the femora. In both fetuses, the biparietal diameter was normal. Autopsy and X-rays confirmed the ultrasound findings in the first fetus. In the second fetus, X-rays confirmed the ultrasound findings (fig. 1). X-rays at birth from the proband and X-rays from the two fetuses were roughly identical and compatible with the same severity of OI. Unfortunately, tissue was obtained only from the second fetus.

The father has had no fractures, and he has no bony deformities. The scleral hue is normal. The teeth are normal with no discoloration or any abnormal tendency to caries or fractures. His skin is normal in terms of thickness, elasticity and strength, and heals without abnormal scarring. His joints are not hypermobile, and he has no hearing impairment. His height is 166 cm, which is just below the 3rd percentile of the height of Danish men. His mother is 158 cm and his father 165 cm. His only sib, a sister, is 162 cm. To evaluate bone mineralisation we did a whole-body densitometric measurement of total mineral content (DXA), which showed values at the 7th percentile for normal Danish men. An X-ray survey is not available. He has been employed as a plumber and as a smith without physical complaints.

Table 1. Sequence and position of primers in COL1A2

Primer	Position	Sequence	Annealing temperature, °C
A	exon 41	5′-TCTTCTTGGTGCTCCTGGTA-3′ (forward)	62
B	exon 42	5′-ATCACGACCAGCTTCACCAG-3′ (reverse)	62
C	intron 41	5′-CCATTGTGTGACCCATTCAC-3′ (forward, biotinylated)	62
D	exon 42	5′-CACCGTTGACTCCAGGACTA-3′ (reverse)	62
E	exon 40	5′-TCCCTCTGGAGAGGCTGGTACT-3′ (forward)	68
F	exons 49/50	5′-TAGTAACCACTGCTCCACTC-3′ (reverse, biotinylated)	68

The mother of the proband has had no fractures, and her scleral hue, teeth, and skin are all normal. Her height is 170 cm. DXA scanning was not done.

Material and Methods

Collagen Extraction from Fetal Tissues

Following cleaning and removal of subcutaneous fat, skin was homogenised and lyophilised. Chorionic villi were carefully separated from maternal decidua under a dissection microscope, homogenised and lyophilised. Substantia compacta was separated from the spongiosa and periosteal bone and cleaned in 70% ethanol. After grinding in liquid nitrogen and lyophilisation, it was decalcified by dialysis at 4 °C against 0.1 *M* EDTA (pH 7.4) with repeated changes over 3 weeks and dried by lyophilisation. Samples of skin, placenta and bone were digested with pepsin (Sigma, 3 U/mg protein) in 0.5 *M* acetic acid (0.1 mg pepsin/ml acetic acid) at 4 °C for 48 h. Collagens were precipitated with 30% ethanol and collected by centrifugation. The pellet was solubilised in 0.5 *M* acetic acid and analysed by SDS-PAGE as described below. After electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250.

Analysis of Radioactively Labelled Procollagen

Fibroblast cultures, established from skin biopsies from the proband, the parents and the second fetus, were grown under standard conditions. Radiolabelled procollagen was obtained from cell cultures incubated at 37 °C in medium containing ascorbic acid (18 µg/ml), catalase (50 µg/ml), 2,3-[^{3}H]proline (10 µCi/ml, specific activity 38.1 Ci/mmol) and [^{3}H]glycine (10 µCi/ml, 38.8 Ci/mmol) as described elsewhere [21]. Medium and cell layer proteins were harvested and analysed separately. Collagen was obtained by digestion of procollagen with pepsin [21], and both procollagen and collagen were ethanol-precipitated, redissolved in sample buffer and analysed by SDS-PAGE using a discontinuous buffer system with 0.5 *M* urea [22]. Two-dimensional mapping of cyanogen bromide peptides from collagen was performed as described by Steinmann et al. [21]. The thermal stability of collagen was determined using the heat-trypsin assay as described previously [23, 24]. Briefly, pepsinised collagen was heated from 30 to 45 °C and submitted to digestion with trypsin (Sigma, 12.7 U/mg protein). After digestion the collagens were analysed by SDS-PAGE.

Isolation of Total RNA and Reverse Transcription

Total RNA was prepared from cultured fibroblasts by lysis of cells in a 0.65% NP40 solution containing 10 m*M* Tris (pH 7.5), 150 m*M* NaCl and 1.5 m*M* $MgCl_2$. After lysis, the cell suspension was centrifuged and the supernatant containing total cytoplasmic RNA was incubated in a 7 *M* urea buffer containing 10 m*M* Tris (pH 7.5), 10 m*M* EDTA, 350 m*M* NaCl and 1% SDS. Subsequently, RNA was extracted with phenol and chloroform/isoamylalcohol and precipitated with ethanol.

cDNA was generated with the GeneAmp RNA-PCR kit from Perkin Elmer using MuLV reverse transcriptase and random hexamers according to the guidelines from the manufacturer.

Amplification of cDNA and DNA Sequences

All PCR reactions were done in a Cetus Thermal Cycler. An initial denaturation of 2 min at 95 °C was followed by 30 cycles of 1 min denaturation at 95 °C, 1 min annealing at temperatures indicated in table 1, 1 min elongation at 72 °C and a final extension at 72 °C for 10 min. PCR was carried out in a total volume of 100 µl containing 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 10 m*M* Tris-HCl (pH 9.0), 0.1% Triton X-100, 0.2 m*M* of each dNTP, 0.5 µ*M* of each primer and 2 U Taq polymerase (Perkin Elmer). For amplification of cDNA, 10 µl of the reverse transcription mixture was used in each PCR reaction with the primers E and F (table 1).

Genomic DNA was isolated from blood and cultured fibroblasts by standard methods, and 1 µg was used in each PCR reaction with the primer pairs A + B and C + D (table 1).

Sequence Analysis

PCR was performed as described above and the products were purified using the QIAquick PCR purification kit from Qiagen. Single-stranded DNA was obtained by the use of Dynabeads M-250 streptavidin and separation in a Dynal magnetic separator. Sequencing was done using the AutoRead sequencing kit from Pharmacia. Only nested sequencing primers were used and 150 pmol were used per reaction. Fluorescent dATP was included in the reaction. The products were analysed on an ALF DNA sequencer from Pharmacia.

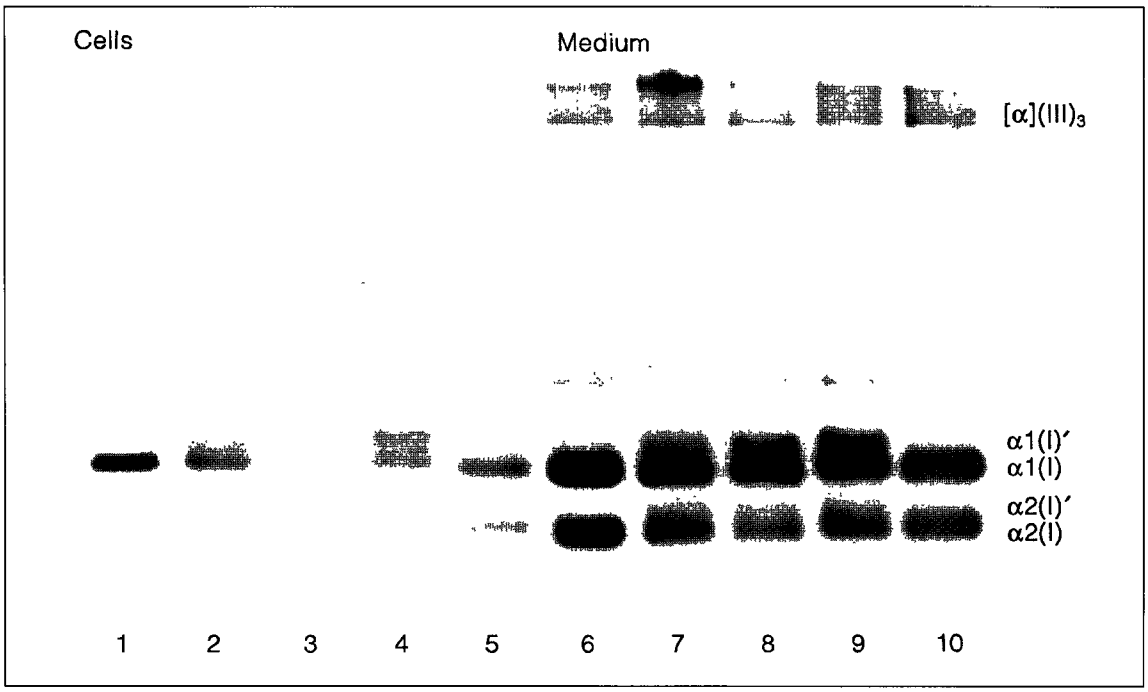

Fig. 2. Gel electrophoresis of pepsin-treated radiolabelled procollagen I produced by cultured fibroblasts. Cells (lanes 1–5) and medium (lanes 6–10) were harvested separately, treated with pepsin and the collagens electrophoresed on a 5% SDS-polyacrylamide gel. Lanes 1, 6 = control; lanes 2, 7 = father; lanes 3, 8 = proband; lanes 4, 9 = fetus; lanes 5, 10 = mother. α1(I)′ and α2(I)′ mark the overmodified chains of collagen I.

The mutation introduced a *Fok*I and removed an *Hph*I restriction site. The PCR products were cut with 10 U each of these enzymes in a 25-μl reaction and run on a 3% agarose gel.

Quantification of the Mutant Allele

Estimation of the proportion of the mutant to the normal allele in leukocytes, fibroblasts and spermatocytes from the father was done by densitometry. PCR products containing the mutation were generated with primers C and D in intron 41 and exon 42, respectively. Primer D was kinase-labelled with [^{32}P]ATP. To ensure a linear relationship between the amount of PCR product and alleles present in the patient sample, the PCR reaction was run for only 20 cycles. The PCR products were digested with *Fok*I (and in other experiments *Hph*I) and run on a 12% polyacrylamide gel. The density of the bands of the uncleaved and cleaved reaction products was measured by scanning the autoradiogram followed by quantification using the ImageQuant software. At least 10 different digestions and measurements were done for each tissue and complete digestion was checked by codigestion and electrophoresis of PCR products from the proband and the mother on the same gel.

Numbering

The amino acids of collagen I are numbered from the beginning (N terminus) of the triple-helical domain of procollagen type I, and nucleotides are numbered according to the sequence data from the Osteogenesis Imperfecta Genetic Analysis Consortium (OIAC Newsletter, April 1, 1994).

Results

Biochemical Analysis of Collagen

Analysis by SDS-PAGE of ^{3}H-labelled procollagen and collagen from cultured skin fibroblast showed, in addition to normal α(I) chains, the presence of slower-migrating, overmodified α1(I) and α2(I) chains of collagen I in the proband, the father and the second fetus (fig. 2). Intracellular retention of abnormal collagen I was evident. To determine those parts of the collagen triple helix that were overmodified, pepsinised collagen was cleaved with cyanogen bromide. All α1(I)- and α2(I)-derived peptides were overmodified except for α1(I)CB6 in the proband, the father and the fetus (not shown).

Thermal stability experiments revealed that the overmodified population of collagen I had a T_m value of 38 °C, compared with 41 °C for normal collagen I (not shown). Collagen extracted from skin, bone, and placenta from the fetus was also analysed by SDS-PAGE; the delayed migration of both the α1(I) and α2(I) chains of collagen I from these tissues confirmed that overmodified collagen was also produced in vivo (not shown).

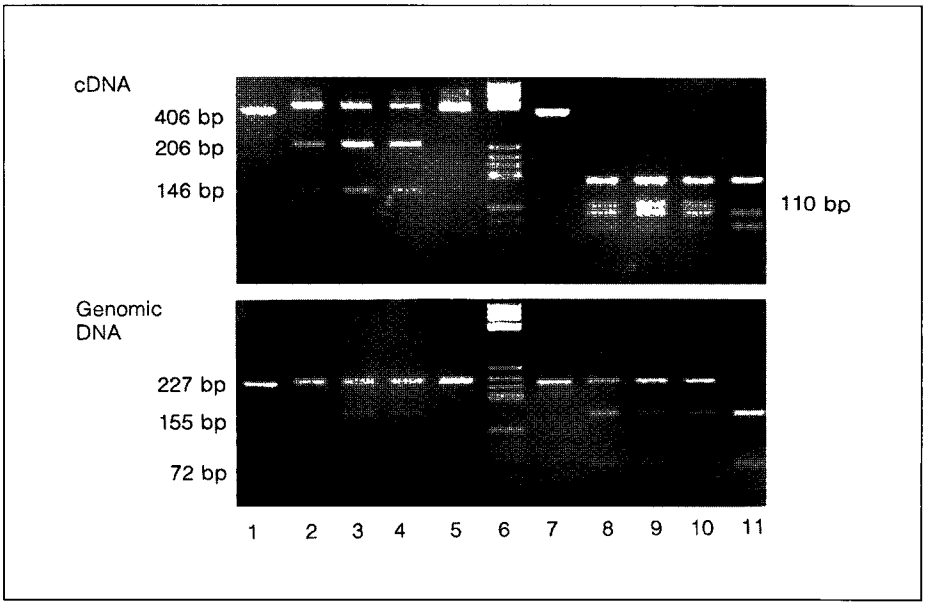

Fig. 3. The mutation at nucleotide 2814 introduced a *Fok*I restriction site and destroyed an *Hph*I restriction site. The figure shows the digests of the PCR products from cDNA and from leucocytic genomic DNA (see text) with *Fok*I to the left of the molecular-weight marker (lane 6) and *Hph*I to the right. With *Fok*I, the PCR product generated from cells from the father (lane 2), the proband (lane 3) and the fetus (lane 4) is cut, whereas that from cells from the mother (lane 5) remains uncut. With *Hph*I only the PCR product from the mother (lane 11) is cut completely, whereas the digests of the PCR products from the father (lane 8), the proband (lane 9) and the fetus (lane 10) show a heterozygous pattern. Thus, the mother is homozygous at nucleotide 2814, whereas the father, the proband and the fetus are heterozygous. Lanes 1, 7 = uncleaved PCR product from a control.

Molecular Studies

The biochemical analysis of collagen suggested that a mutation in COL1A1 or COL1A2 would most likely be localised in the region comprising the α1(I)CB7 peptide, corresponding to amino acids 552–822 of the collagen triple helix. After reverse transcription of mRNA and preparation of cDNA, a region corresponding to the α1(I)CB7 peptide was amplified by PCR. Sequencing of the PCR products from the proband revealed a G to A transition in the second base of the first codon in exon 42 of COL1A2 (nucleotide 2814; not shown). This mutation resulted in a Gly802Asp substitution in the proα2(I) chain of procollagen I. The mutation introduced a *Fok*I and removed an *Hph*I restriction site. PCR fragments of 406 bp containing the mutation were cleaved with these enzymes and confirmed the presence of the mutation in both the proband, the fetus, and the father (fig. 3).

The location of the mutation in the first codon of exon 42 made its confirmation difficult at the genomic DNA level. However, using a forward primer in exon 41 and a reverse primer in exon 42, PCR was able to span the whole intron. We sequenced the 3′ end of the intron and designed a forward primer (primer C, table 1) from the sequence obtained. This primer was positioned 142 bp from the 3′ end of intron 41. Using this primer and a downstream primer in exon 42, we amplified a 227-bp fragment from genomic DNA (fig. 3). Cleavage with *Fok*I and *Hph*I confirmed the presence of the mutation in genomic DNA from both leucocytes and cultured fibroblasts from the proband, the fetus and the father. The mutation was absent in leucocytes from the father's parents (not shown).

Estimation of the proportion of the mutant to the normal allele in leucocytes and cultured fibroblast from the father and the proband and in spermatocytes from the father was done by densitometry. The reverse primer D was kinase-labelled with [^{32}P]ATP, and the 227-bp PCR product containing the mutation was generated as above and subsequently cleaved with *Fok*I (fig. 4) and in other experiments with *Hph*I. The proportion between the uncleaved 227 bp band and the cleaved 72 bp band was calculated from the densities of the bands. The ratio between the 227- and 72-bp bands was 1:1 in both leucocytes and cultured fibroblasts from the proband, whereas it was 2.4, 2.9 and 1.7 in leucocytes, fibroblasts and spermatocytes, respectively, from the father, suggesting that 29, 25 and 40%, respectively, of his alleles carried the mutation (assuming complete cleavage of the PCR products as mentioned in Methods).

Discussion

We have described a family with recurrent OI caused by germinal mosaicism in the father. The molecular background was a nucleotide substitution in the COL1A2 gene, resulting in a Gly802Asp substitution in the proα2(I) chain of collagen I.

When genetic counselling was first given to the family, two possible modes of inheritance were considered: auto-

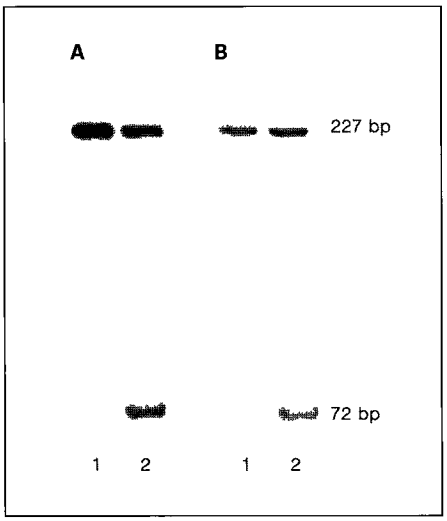

Fig. 4. Estimation of the proportion of the mutant to normal allele in leucocytes (**A**) and fibroblast (**B**) from the father (lane 1) and the proband (lane 2) was done by densitometric measurement of the 227-bp PCR product containing the mutation and the *Fok*I-cleaved 72-bp reaction products. The ratio between these products was 1 in leukocytes and cultured fibroblasts from the proband, whereas leucocytes and cultured fibroblasts from the father showed a ratio of 2.4 and 2.9, respectively. In spermatocytes, a ratio of 1.7 was found (not shown).

somal recessive inheritance and a de novo dominant mutation in the proband. The biochemical finding of a normal and an overmodified population of collagen I in the proband subsequently made heterozygosity for a dominant mutation more likely. Two pregnancies were terminated because of similarly affected fetuses detected by ultrasound, raising the possibility of germinal mosaicism in one of the parents. We found both normal and abnormal collagen I in cultured fibroblasts from the father, and the same mutation was found in the father, the proband, and the second fetus. This proves mosaicism for the mutation in the father. His short stature and borderline low bone mineral content may reflect somatic mosaicism. On the other hand, all his first-degree relatives have a similar short stature.

Mosaicism has now been reported in several families with both lethal and non-lethal OI [10–19, 25, 26], and our study underscores the importance of considering mosaicism in all sporadic cases of OI.

With some exceptions [12, 16, 18, 27], most parents with germinal and somatic mosaicism for a mutation in COL1A1 or COL1A2 do not express the mutation in fibroblasts and do not produce abnormal collagen I, even when the mutation is found in up to 50% of alleles in leucocytes, sperm and hair bulb roots [10, 12, 13, 19]. Cultured fibroblasts from our proband's father produced a substantial amount of slower migrating collagen I, which had the same reduced thermal stability as did abnormal collagen I from the proband and the fetus. The normal phenotype of the father as opposed to the phenotype of the proband may be explained by a smaller amount of the mutated allele in the former, which may be influenced also by differences in the tissue distribution of the mutated allele and by a different genetic background. We found that the relative abundance of the mutant allele in leucocytes and fibroblasts from the clinically unaffected father was about 25%, a rather high frequency, which, however, still leaves 50% of his cells functioning normally, assuming that the fibroblasts from the skin biopsy are representative. Regarding glycine for aspartic acid substitutions, 50% normal cells may be enough to produce a normal phenotype. Bone from the father was not available for study, but we speculate that the frequency of the mutant allele in osteogenic cells is lower than in fibroblasts and leucocytes; the normal bone mineral content gives some support to this.

Six other published aspartic acid for glycine substitutions in the proα2(I) chain have resulted in perinatally lethal OI [3, 27–31]. One triplet more C terminal than the substitution reported here, a Gly805Asp substitution [29] in a child with lethal OI has been reported, pointing to rather short segments of the proα2(I) chain with differing importance for the stability of the helix [32] or for the function of mature collagen in the extracellular matrix. On the other hand, a valine for glycine substitution in the corresponding position 802 in the proα1(I) chain has been reported in a family with recurrent, lethal OI [13]. This finding may argue against this segment of the triple helix having lesser importance for the integrity of the extracellular matrix. However, the difference in the clinical presentations (nonlethal as opposed to lethal) may be explained by the fact that a COL1A1 mutation results in production of collagen I, of which 75% is abnormal, whereas the COL1A2 mutation reported here results in production of collagen I, of which only half is abnormal.

Acknowledgments

This work was supported in part by grants from the Novo Nordisk Foundation, the Foundation of the Queen Louise's Children's Hospital, The Foundation of Henny and Helge Holgersen, Vanførefonden, the European Community Concerted Action on Heritable Connective Tissue Disorders 1990–1992, and the Swiss National Science Foundation (grant No. 32-42198.94 to B.S.). The skilful technical support from Bodil Mogensen is greatly appreciated.

References

1 Sillence DO, Seen A, Danks DM: Genetic heterogeneity in osteogenesis imperfecta. J Med Genet 1979;16:101–116.

2 Sykes B: Linkage analysis in dominantly inherited osteogenesis imperfecta. Am J Med Genet 1993;45:212–216.

3 Byers PH: Osteogenesis imperfecta; in Royce PM, Steinmann B (eds): Connective Tissue and Its Heritable Disorders: Molecular, Genetic and Medical Aspects. New York, Wiley-Liss, 1993, pp 317–350.

4 Beighton P, Spranger J, Versveld G: Skeletal complications in osteogenesis imperfecta: A review of 153 South African patients. S Afr Med J 1983;64:565–568.

5 Aitchison K, Ogilvie D, Honeyman M, Thompson E, Sykes B: Homozygous osteogenesis imperfecta unlinked to collagen I genes. Hum Genet 1988;78:233–236.

6 Wallis GA, Sykes B, Byers PH, Mathew CG, Viljoen D, Beighton P: Osteogenesis imperfecta type-III – Mutations in the type-I collagen structural genes, COL1A1 and COL1A2, are not necessarily responsible. J Med Genet 1993; 30:492–496.

7 Deak SB, Nicholls A, Pope FM, Prockop DJ: The molecular defect in a nonlethal variant of osteogenesis imperfecta: Synthesis of pro-α2(I) chains which are not incorporated into trimers of type I procollagen. J Biol Chem 1983;258: 15192–15197.

8 Pihlajaniemi T, Dickson LA, Pope FM, Korhonen VR, Nicholls A, Prockop DJ, Myers JC: Osteogenesis imperfecta: Cloning of a pro-α2(I) collagen gene with a frameshift mutation. J Biol Chem 1984;259:12941–12944.

9 Nicholls AC, Osse G, Schloon HG, Lenard HG, Deak S, Myers JC, Prockop DJ, Weigel WR, Fryer P, Pope FM: The clinical features of homozygous α2(I) collagen deficient osteogenesis imperfecta. J Med Genet 1984;21:257–262.

10 Cohn DH, Starman BJ, Blumberg B, Byers PH: Recurrence of lethal osteogenesis imperfecta due to parental mosaicism for a dominant mutation in a human type I collagen gene (COL1A1). Am J Hum Genet 1990;46:591–601.

11 Constantinou CD, Pack M, Young SB, Prockop DJ: Phenotypic heterogeneity in osteogenesis imperfecta: The mildly affected mother of a proband with a lethal variant has the same mutation substituting cysteine for α1-glycine 904 in a type I procollagen gene (COL1A1). Am J Hum Genet 1990;47:670–679.

12 Wallis GA, Starman BJ, Zin AB, Byers PH: Variable expression of osteogenesis imperfecta in a nuclear family is explained by somatic mosaicism for a lethal point mutation in the α1(I) gene (COL1A1) of type I collagen in a parent. Am J Hum Genet 1990;46:1034–1040.

13 Bonaventure J, Cohen-Solal L, Lasselin C, Maroteaux P: A dominant mutation in the COL1A1 gene that substitutes glycine for valine causes recurrent lethal osteogenesis imperfecta. Hum Genet 1992;89:640–646.

14 Edwards MJ, Wenstrup RJ, Byers PH, Cohn DH: Recurrence of lethal osteogenesis imperfecta due to parental mosaicism for a mutation in the COL1A2 gene of type I collagen: The mosaic parent exhibits phenotypic features of a mild form of the disease. Hum Mutat 1992;1: 47–54.

15 Mottes M, Lira MMG, Valli M, Scarano G, Lonardo F, Forlino A, Cetta G, Pignatti PF: Paternal mosaicism for a COL1A1 dominant mutation (α-1 Ser-415) causes recurrent osteogenesis imperfecta. Hum Mutat 1993;2:196–204.

16 Constantinou-Deltas CD, Ladda RL, Prockop DJ: Somatic cell mosaicism – Another source of phenotypic heterogeneity in nuclear families with osteogenesis imperfecta. Am J Med Genet 1993;45:246–251.

17 Raghunath M, Steinmann B, Delozier-Blanchet C, Extermann P, Superti-Furga A: Prenatal diagnosis of collagen disorders by direct biochemical analysis of chorionic villus biopsies. Pediatr Res 1994;36:441–448.

18 Cohen Solal L, Bonaventure J, Maroteaux P: Dominant mutations in familial lethal and severe osteogenesis imperfecta. Hum Genet 1991;87:297–301.

19 Raghunath M, Mackay K, Dalgleish R, Steinmann B: Genetic counselling on brittle grounds: Recurring osteogenesis imperfecta due to parental mosaicism for a dominant mutation. Eur J Pediatr 1995;154:123–129.

20 Namikawa C, Suzumori K, Fukushima Y, Sasaki M, Hata A: Recurrence of osteogenesis imperfecta because of paternal mosaicism: Gly(862) → Ser substitution in a type I collagen gene (COL1A1). Hum Genet 1995;95:666–670.

21 Steinmann B, Rao VH, Vogel A, Bruckner P, Gitzelmann R, Byers PH: Cysteine in the triple-helical domain of one allelic product of the α1(I) gene of type I collagen produces a lethal form of osteogenesis imperfecta. J Biol Chem 1984;259:11129–11138.

22 Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–685.

23 Bruckner P, Prockop DJ: Proteolytic enzymes as probes for the triple-helical conformation of procollagen. Anal Biochem 1981;110:360–368.

24 Steinmann B, Westerhausen A, Constantinou DC, Superti-Furga A, Prockop DJ: Substitution of cysteine for glycine-α1-691 in the pro α1(I) chain of type I procollagen in a proband with lethal osteogenesis imperfecta destabilizes the triple helix at a site C-terminal to the substitution. Biochem J 1991;279:747–752.

25 Marini JC, Lewis MB, Wang Q, Chen KJ, Orrison BM: Serine for glycine substitutions in type I collagen in 2 cases of type IV osteogenesis imperfecta (OI) – Additional evidence for a regional model of OI pathophysiology. J Biol Chem 1993;268:2667–2673.

26 Cohn DH, Wallis G, Zhang X, Byers PH: Serine for glycine substitutions in the α1(I) chain of type I collagen: Biological plasticity in the Gly-Pro-Hyp clamp at the carboxy-terminal end of the triple helical domain (abstract). Matrix 1990;10:236.

27 Byers PH, Starman BJ, Atkinson M, Willing MC, Cohn DH: Mosaicism in osteogenesis imperfecta (abstract). 5th Int Conf on Osteogenesis Imperfecta, 1993.

28 Baldwin CT, Constantinou CD, Dumars KW, Prockop DJ: A single base mutation that converts glycine 907 of the α2(I) chain of type I procollagen to aspartate in a lethal variant of osteogenesis imperfecta: The single amino acid substitution near the carboxyl terminus destabilizes the whole triple helix. J Biol Chem 1989;264:3002–3006.

29 Grange DK, Gottesman GS, Lewis MB, Marini JC: Detection of point mutations in type I collagen by RNase digestion of RNA/RNA hybrids. Nucleic Acids Res 1990;18:4227–4236.

30 Niyibizi C, Bonadio J, Byers PJ, Eyre DR: Incorporation of type I collagen molecules that contain a mutant α2(I) chain (Gly580 → Asp) into bone matrix in a lethal case of osteogenesis imperfecta. J Biol Chem 1992;267:23108–23112.

31 Cohen-Solal L, Zylberberg L, Sangalli A, Gomez Lira M, Mottes M: Substitution of an aspartic acid for glycine 700 in the α2(I) chain of type I collagen in a recurrent lethal type II osteogenesis imperfecta dramatically affects the mineralization of bone. J Biol Chem 1994; 269:14751–14758.

32 Wenstrup RJ, Shrago Howe AW, Lever LW, Phillips CL, Byers PH, Cohn DH: The effects of different cysteine for glycine substitutions within α2(I) chains: Evidence of distinct structural domains within the type I collagen triple helix. J Biol Chem 1991;266:2590–2594.