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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)

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Summary

A proband with a lethal variant of osteogenesis imperfecta (OI) has been shown to have, in one allele in a gene for type I procollagen (COL1A1), a single base mutation that converted the codon for α1-glycine 904 to a codon for cysteine. The mutation caused the synthesis of type I procollagen that was posttranslationally overmodified, secreted at a decreased rate, and had a decreased thermal stability. The results here demonstrate that the proband's mother had the same single base mutation as the proband. The mother had no fractures and no signs of OI except for short stature, slightly blue sclerae, and mild frontal bossing. As a child, however, she had the triangular facies frequently seen in many patients with OI. On repeated subculturing, the proband's fibroblasts grew more slowly than the mother's, but they continued to synthesize large amounts of the mutated procollagen in passages 7–14. In contrast, the mother's fibroblasts synthesized decreasing amounts of the mutated procollagen after passage 11. Also, the relative amount of the mutated allele in the mother's fibroblasts decreased with passage number. In addition, the ratio of the mutated allele to the normal allele in leukocyte DNA from the mother was half the value in fibroblast DNA from the proband. The simplest interpretation of the data is that the mother was mildly affected because she was a mosaic for the mutation that produced a lethal phenotype in one of her three children.

Introduction

More than 30 different mutations in the two structural genes of proα1(I) and proα2(I) chains of type I procollagen (COL1A1 and COL1A2, respectively) have been shown to cause osteogenesis imperfecta (OI), a genetic disease characterized by brittleness of bones (Prockop and Kivirikko 1984; Cheah 1985; Byers et al. 1988*a*; Constantinou et al. 1989; Prockop et al. 1989, 1990). Similar mutations in either of the two genes for type I procollagen have also been shown to cause a few variants of Ehlers-Danlos syndrome (EDS). With some exceptions, the mutations cause synthesis of structurally abnormal but partially functional proα1(I) or proα2(I) chains. The deleterious effects of the mutations are largely explained by the fact that the structurally abnormal proα chains are assembled into procollagen and that presence of one or two abnormal proα chains in the protomer interferes with folding of the protein into a stable triple-helical conformation, the processing of procollagen to collagen, or the self-assembly of collagen into normal fibrils (Prockop et al. 1989, 1990). The mutations defined to date include partial deletions of the genes and RNA splicing mutations. The largest category of mutations are, however, single base mutations that cause substitution of bulkier amino acids for glycine residues that are found as every third amino acid in the repeating -Gly-X-Y- sequences characteristic of the triple-helical domain of collagen (Prockop and Kivirikko 1984; Cheah 1985; Byers et al. 1988*a*; Constantinou et al. 1989; Prockop et al. 1989, 1990).

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We recently reported that one lethal variant of OI was caused by a single base mutation that substituted cysteine for glycine 904 in the α1(I) chain domain of type I procollagen (Constantinou et al. 1989). The allele containing the mutation was expressed at a high level and caused synthesis of type I procollagen molecules containing one or two mutated proα1(I) chains. Some of the molecules containing two mutated chains had disulfide bonds bridging the chains. Of special importance was that the presence of one or two mutated chains decreased the thermal stability of type I procollagen. Also, it caused posttranslational overmodification and decreased the rate of secretion of the protein. All the findings, therefore, were consistent with a series of previous single base mutations that substituted a bulkier amino acid for glycine, led to synthesis of structurally abnormal type I procollagen molecules, and caused severe or lethal OI phenotypes (see Byers et al. 1988*a;* Prockop et al. 1989, 1990). It is surprising, however, that fibroblasts from the proband's mildly affected mother were also found to synthesize a structurally abnormal type I procollagen. In addition, preliminary observations suggested that the mother had the same mutation as the proband (Constantinou et al. 1989, 1990). Here we demonstrate that the mother does have the same mutation but probably has somatic cell mosaicism for the mutated allele.

Methods

Cell Culturing and Labeling

Skin fibroblasts were prepared from the proband, two normal brothers of the proband, the proband's parents, the proband's maternal grandparents, and one maternal uncle. As reported elsewhere (Constantinou et al. 1989), the skin biopsy from the proband was obtained following stillbirth at 31 wk gestation. Skin biopsies from the proband's relatives were taken from the forearm by excision with a scalpel. The biopsies were used to prepare cultured fibroblasts under standard conditions (Constantinou et al. 1989). The cells were grown in either 25-cm^2 or 75-cm^2 plastic flasks (Falcon Labware, Oxnard, CA) in Dulbecco's modified Eagle's medium with 10% FCS. The cell cultures were amplified by allowing the cultures to grow to confluency and diluting the cells 1:4 before transferring to new flasks. For the experiments here, the mother's and proband's cells were examined at passages ranging from 7 to 14 or 15. The mother's fibroblasts grew more rapidly than the proband's. Therefore, the mother's fibroblasts were transferred after about 7 d, and the proband's were transferred after 10–12 d.

Analysis of Newly Synthesized Proteins

Newly synthesized proteins were analyzed by labeling the fibroblasts in culture with 50 μCi/ml L-[2,3,4,5-^{3}H]proline (102 Ci/mmol; Amersham, Arlington Heights, IL). The cell layer and medium proteins were digested with pepsin or with a mixture of trypsin and chymotrypsin according to a method described elsewhere (Constantinou et al. 1989). The protein digests were then analyzed by electrophoresis in SDS-polyacrylamide gels and by fluorography.

Polymerase Chain Reaction and Nucleotide Sequencing

To isolate genomic DNA from cultured skin fibroblasts, the cells were grown to confluency in either 75-cm^2 or 175-cm^2 flasks, and DNA was extracted using a procedure involving treatment with SDS and digestion with proteinase K (Maniatis et al. 1982). For experiments in which cells from successive passages were used for DNA isolation, two 75-cm^2 flasks were used. To extract genomic DNA from single hairs, the similar procedure of Higuchi et al. (1988) was used. To extract DNA from peripheral blood, 10-ml samples anticoagulated with EDTA were mixed with an equal volume of lysis buffer that consisted of 1 mM $MgCl_2$, 0.8% Nonidet P-40, and 0.4% sodium deoxycholate in 1 mM sodium phosphate buffer (pH 6.5). Nuclei were isolated by centrifugation and were suspended in 20 ml of 0.15 M NaCl and 5 mM EDTA in 10 mM Tris-HCl buffer (pH 8.3). The sample was adjusted to 1% SDS and 1 mg of proteinase K/ml and was incubated at 55°C–65°C for 1–2 h. Sodium perchlorate was added to a final concentration of 1 M, and the DNA was extracted with chloroform:isoamyl alcohol (24:1) twice before precipitation with cold ethanol.

The polymerase chain reaction (Saiki et al. 1985) was performed on heat-denatured genomic DNA. The primers were 28-mer oligonucleotides in which 20 bases were complementary to the proα1(I) gene and in which eight bases contained a recognition site for a restriction endonuclease. The 5′ primer pCDC22/*Eco*RI hybridized to exon 44 of the proα1(I) gene and had the sequence 5′-GGAATTCCTTGGCCCTGCTGGCAAGAGT-3′. The 3′ primer pCDC26/*Bam*HI hybridized to intron 45 of the proα1(I) gene and had the sequence 5′-AAGGATCCCAGGCGGAAGTTCCATTGGC-3′. The amplification was performed for 25 or 40 cycles under conditions suggested by the commercial supplier of the reaction kit and instrument (Perkin-Elmer). The

amplified product was 571 bp long. It was digested with *Eco*RI and *Bam*HI (New England Bio-Labs, Beverly, MA) and was cloned into M13mp18 and M13mp19 bacteriophage for nucleotide sequencing with the dideoxynucleotide method (Sanger et al. 1977).

Hybridization with Allele-specific Oligonucleotides

Aliquots of the products of the polymerase chain reaction were either dot blotted or slot blotted in duplicate on nitrocellulose membranes. The blots were prehybridized for 2 h in 6 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 6.8), 5 × Denhardt's solution, 0.5% SDS, and 0.25 mg denatured salmon sperm DNA/ml. Hybridization was performed at 58°C for 15 h after the addition of ^{32}P-end-labeled oligonucleotides to the same solution (Maniatis et al. 1982). Two 19-mers spanning amino acid position 904 of the α1(I) chain were used. One of them (ASO-Gly^{904}) had the normal sequence, and the other (ASO-Cys^{904}) was the same except for a G-to-T substitution, in the middle of the sequence, that converted the codon for α1-glycine 904 to a codon for cysteine. Washes were performed in 2 × SSC for 15 min at room temperature, in 2 × SSC/0.1% SDS for 1 h at room temperature, and then in 2 × SSC for 3 min at 67°C with the ASO-Gly^{904} probe and at 65°C with the ASO-Cys^{904} probe. The filters were exposed to X-ray film, and the slots were excised from the nitrocellulose membranes for assay by liquid scintillation counting in Ecoscint (National Diagnostics, Manville, NJ).

Southern Blot Analysis

About 20 μg of genomic DNA isolated from fibroblasts was digested with 100 units of *Taq*I for 5 h at 65°C in the buffer recommended by the manufacturer (New England Bio-Labs). Southern blotting and hybridization were carried out under standard conditions (Maniatis et al. 1982).

Results

Synthesis of Cysteine-substituted α1(I) Chains by the Mother's Fibroblasts

As reported elsewhere (Constantinou et al. 1989), the proband was stillborn after 31 wk gestation and had the typical findings of type II OI as defined by Sillence et al. (1979). The family resided on a remote peninsula of Denmark, but all the immediate members of the proband's family except for one reportedly normal maternal uncle were interviewed and examined by one of us (M.P.). Except for the mother, none had any evidence of either OI or any related disorder of connective tissue. The mother was 26 years old at the time of the proband's pregnancy. She subsequently had two normal sons. She had no history of fractures. Extensive evaluation of the bone status of the mother showed no striking abnormalities (Constantinou et al. 1989). At the time that the mother was examined at age 35 years, her only notable features were slightly blue sclerae and mild frontal bossing. Also, she was 153 cm (5 feet) tall and 7 cm shorter than her mother and 17 cm shorter than her father. However, as a child (fig. 1) she had the triangular facies frequently seen in many patients with OI (Smith et al. 1983).

Initial experiments (Constantinou et al. 1989) indicated that the mother's fibroblasts synthesized a small amount of type I procollagen that was overmodified

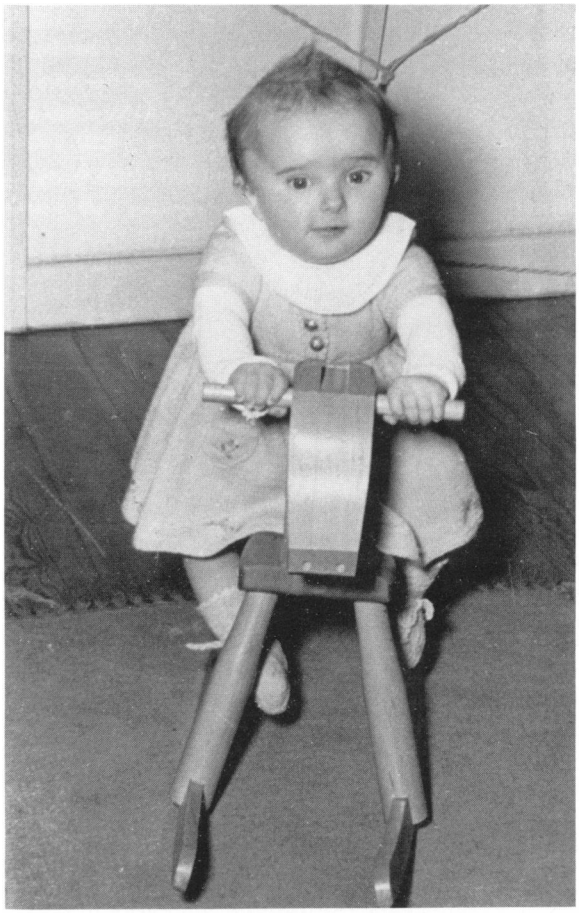

Figure 1 Picture of the proband's mother as a 2-year old child. When she was examined at the age of 35 years, the frontal bossing was less apparent, and her face was not unusually triangular.

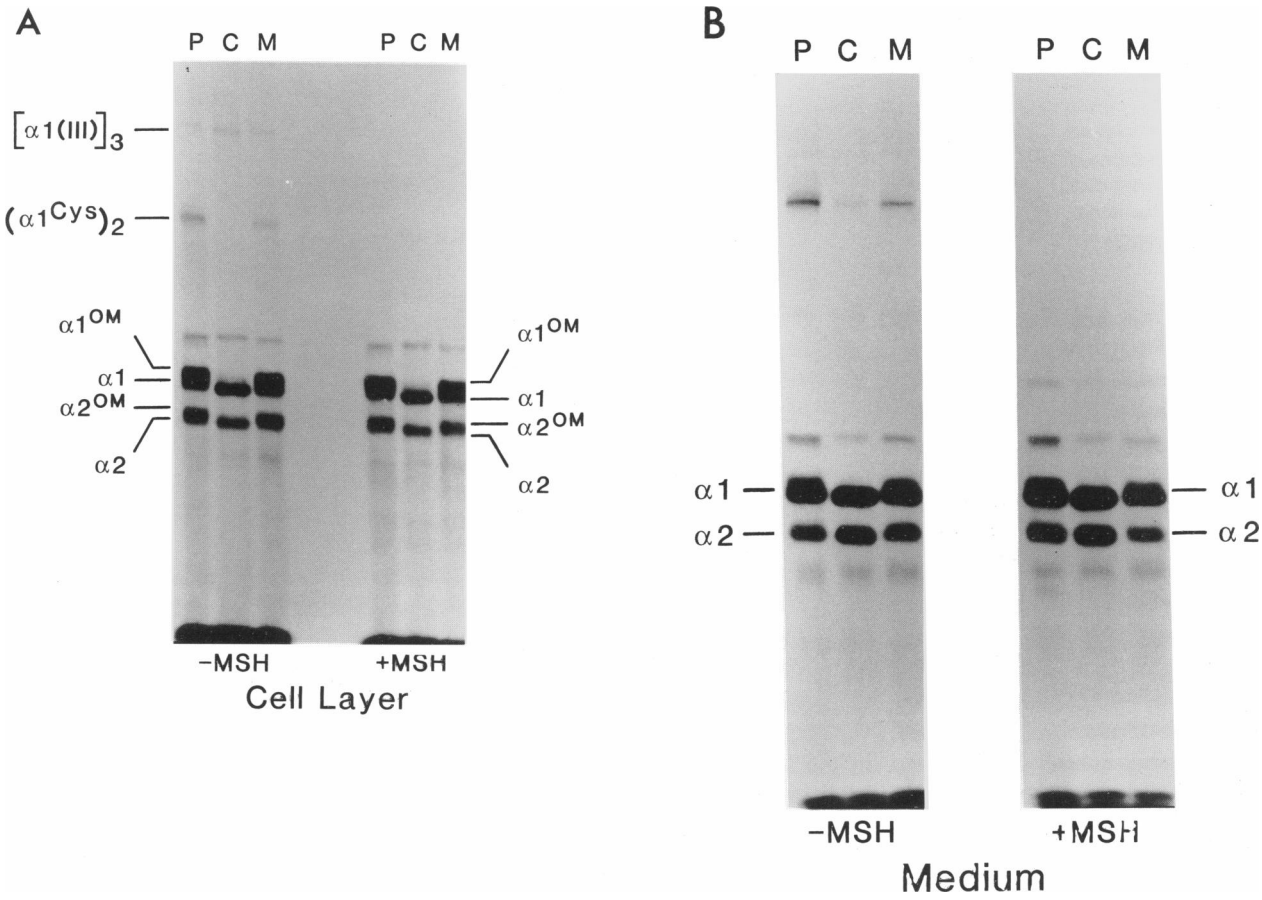

Figure 2 SDS-PAGE analysis of collagens from the fibroblasts cultures. The cell-layer (*A*) and medium (*B*) samples were digested with pepsin and then were examined by gel electrophoresis either with or without reduction with 2-mercaptoethanol. MSH = 2-mercaptoethanol; $[\alpha 1(III)]_3$ = trimers of unreduced α1(III) chain of type III collagen; $(\alpha 1^{cys})_2$ = disulfide-linked dimers of α1 chains containing the substitution of cysteine for glycine 904; $\alpha 1^{OM}$ = overmodified α1(I) chains; $\alpha 2^{OM}$ = overmodified α2(I) chains. The band of disulfide-linked dimers of α1 chains was elsewhere identified by two-dimensional analysis of cyanogen bromide peptides (Constantinou et al. 1989).

by posttranslational reactions and that had a decreased thermal stability. There was no evidence of disulfide-linked α1(I) chains seen by analysis of the proband's fibroblasts, and, therefore, it appeared that the mother had a type I procollagen mutation different than the α1-cysteine 904 found in the proband. Here we examined the mother's fibroblasts at earlier stages in culture, i.e., passages 7 and 8 instead of passages 10–14. Disulfide-linked α1(I) chains were present in the cell layer of passage 7 fibroblasts from the mother (fig. 2*A*). Also, posttranslationally overmodified α chains were found in digests of both the cell layer (fig. 2*A*) and the medium (fig. 2*B*). However, there were no disulfide-linked dimers in the medium. Also, the relative amount of overmodified α chains was less in the medium than in the cell layer. Therefore, the results suggested that the abnormal procollagen was secreted more slowly than normal type I procollagen synthesized by the same fibroblasts. Similar observations were made earlier with the proband's fibroblasts (Constantinou et al. 1989).

Presence of the α1-Cysteine 904 Mutation in the Mother's DNA

Because the mother appeared to have the same protein defect as was found in the proband, mRNA-derived cDNA and genomic DNA from the mother was examined for the presence of the single base substitution that converted the codon for α1-glycine 904 to a codon for cysteine (Constantinou et al. 1989). In the first series of experiments, the polymerase chain reaction was car-

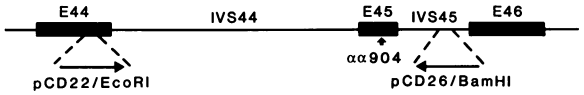

Figure 3 Scheme showing location of the α1-cysteine 904 mutation and the strategy for using oligonucleotide primers in the polymerase chain reaction. The amino acid positions are numbered by the standard convention, in which the first glycine of the triple-helical domain is number 1.

ried out by using genomic DNA from the mother's fibroblasts as template and by using a set of primers that spanned the gene from the 3′ end of exon 44 to the 3′ end of intron 45 (fig. 3). The products of polymerase chain reaction were cloned into M13 and sequenced. As indicated in figure 4, the α1-cysteine 904 mutation was found in clones from the mother's genomic DNA. The mutation was present in two of 18 independent M13 clones.

To confirm the presence of the mutation in the mother, a second series of polymerase chain reactions were carried out using RNA from the mother's fibroblasts to generate cDNA, and the cDNA was used as a template for the polymerase chain reaction. The products of the polymerase chain reaction were again cloned into M13 and sequenced. The single base mutation converting

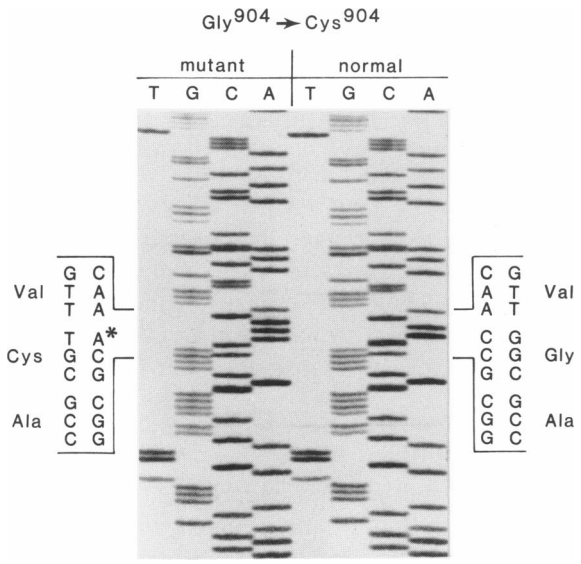

Figure 4 DNA sequence of polymerase chain reaction prepared using genomic DNA as a template. The asterisk marks the A-for-C substitution, in the antisense strand, that converted the codon for glycine to a codon for cysteine. In the normal sequence there is a compression in the two C's of the antisense codon for glycine 904.

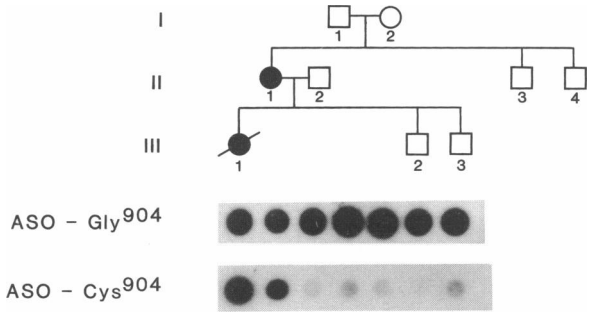

Figure 5 The pedigree of the family and the results of allele-specific dot-blot analyses. As indicated, only the proband and the proband's mother were positive for the α1-cysteine 904 mutation. The film is slightly overexposed but indicates that the mutant-to-normal allele ratio in the mother's fibroblasts (passage 7) is less than that in the proband's fibroblasts.

the codon for α1-glycine 904 to cysteine was found in 21 of 22 M13 clones (data not shown).

Inheritance of the Mutation in the Proband's Family

To examine inheritance of the α1-cysteine 904 mutation in the proband's family, genomic DNA from cultured fibroblasts was used as a template for the polymerase chain reaction. The products of the polymerase chain reaction were then dot blotted and hybridized with allele-specific oligonucleotides. As indicated in figure 5, DNA from the proband and from the proband's mother was positive for the α1-cysteine 904 mutation. DNA samples from other members of the family examined were negative.

Effect of Passage Number on the Synthesis of Mutated Procollagen by the Mother's Fibroblasts

Because synthesis of the disulfide-linked dimers of α1(I) chain was not detected in initial studies on late-passage fibroblasts from the mother (Constantinou et al. 1989), fibroblasts from both the proband and mother were examined at various stages of passage in culture (fig. 6). The presence of overmodified α1(I) and α2(I) chains was consistently seen in both late- and early-passage cells from the proband (fig. 6*B*). The relative amounts of the overmodified chain did not change with passage number. In contrast, overmodified α1(I) and α2(I) chains were present only in early-passage fibroblasts from the proband's mother (fig. 6*A*). There was a marked decrease in the amount of overmodified chains seen in fibroblasts from the mother after passage 11 or 12. Under the conditions of the experiment shown in figure 6, only small amounts of the disulfide-linked

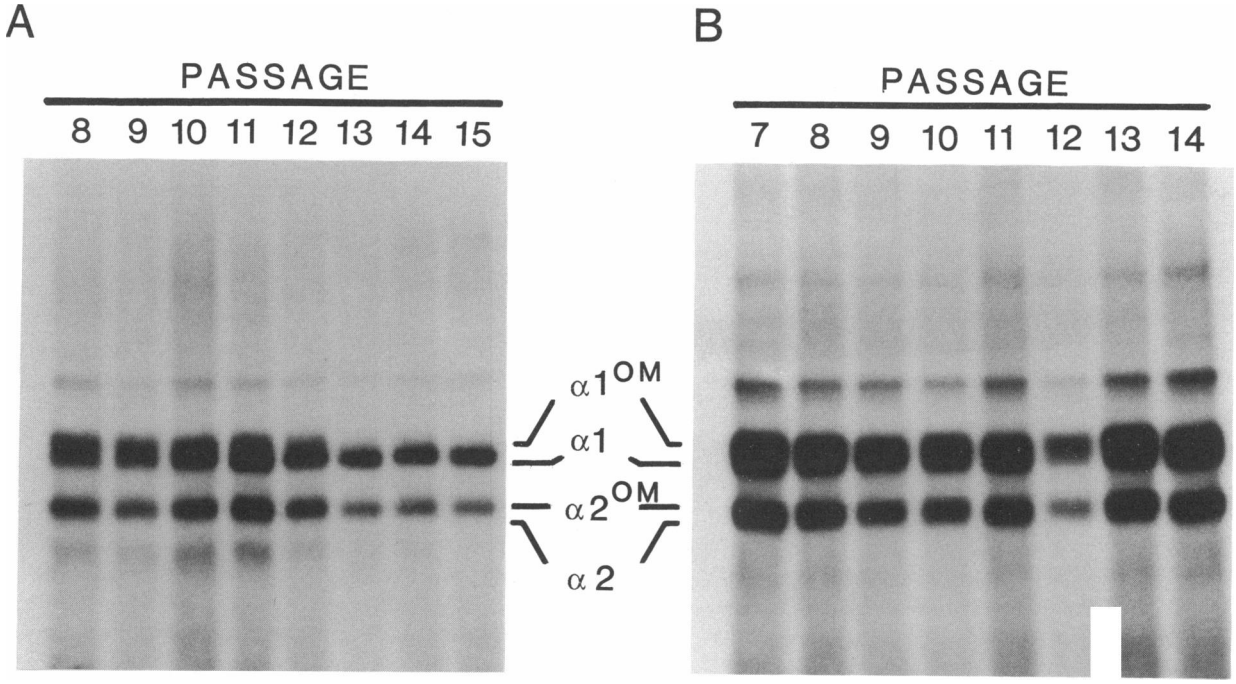

Figure 6 SDS-PAGE analysis of collagens from the cell layer, as a function of passage number. *A*, Collagens from passages 8–15 of the mother's fibroblasts. *B*, Collagens from passages 7–14 of the proband's fibroblasts.

α1(I) chains were seen in fibroblasts from the proband. In other experiments (see fig. 2*A*) small amounts of the disulfide-linked dimer were also seen in early-passage cells from the mother but not in fibroblasts from subsequent passages.

Effect of Passage Number on Presence of the Mutated Allele in the Mother's Fibroblasts

The decreased synthesis of abnormal procollagen with increasing passage number (fig. 6*A*) suggested the possibility that the mother had somatic cell mosaicism for the mutation and that, in vitro, the normal fibroblasts outgrew fibroblasts containing the mutation during replication. Therefore, we decided to test whether the mother's fibroblasts were mosaic for the mutation.

The polymerase chain reaction and allele-specific oligonucleotides were used to assay, as a function of passage number, the relative amounts of normal and mutated alleles in genomic DNA from fibroblasts from the mother and from the proband. As shown in figures 7 and 8, assay of the mother's fibroblasts demonstrated a progressive decrease in the ratio of the mutated allele to the normal allele. In contrast, assay of the proband's fibroblasts demonstrated that there was no effect of passage number. (Because the assays of the mother's and

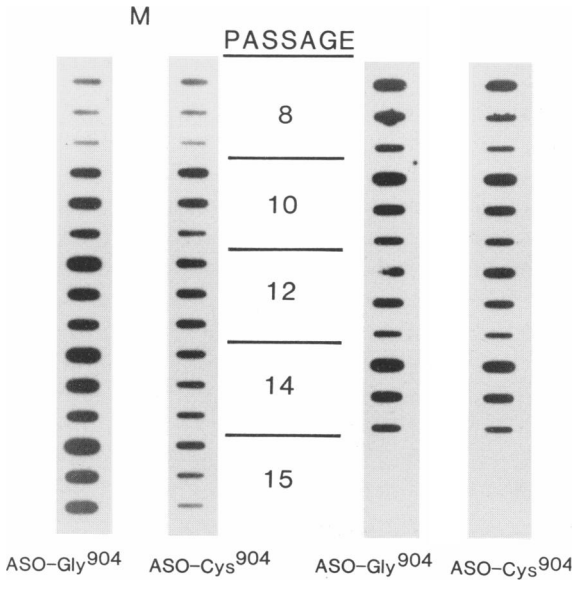

Figure 7 Slot-blot analysis of polymerase chain reaction products with allele-specific oligonucleotides. Unequal amounts of DNA were used in cells from different passages. However, it is apparent that the relative amount of DNA hybridizing with the ASO-Cys^{904} probe decreases with passage number in the case of the mother's cells but not in the case of the proband's cells.

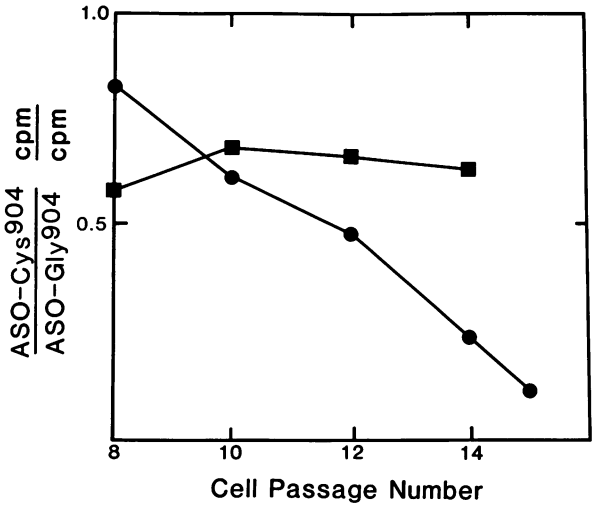

Figure 8 Analysis of the mutant-to-normal allele ratio in the proband's fibroblasts (■) and mother's fibroblasts (●), as a function of passage number. Slot blots shown in fig. 7 were excised and assayed by liquid scintillation counting. Values are the means of the three slots.

proband's fibroblasts were carried out in separate experiments with slight differences in hybridization and washing conditions, the absolute values of the ratios shown in fig. 8 are not comparable.)

To rule out the possibility that the decreasing ratio in the mother's fibroblasts (figs. 7 and 8) was explained by contamination of the mother's fibroblast cultures by another strain of normal fibroblasts, Southern blot analysis was performed using a DNA probe for a hypervariable locus on the X chromosome (Knowlton et al. 1989). The results (fig. 9) demonstrated that DNA from the mother's fibroblasts at passage 13 retained several distinctive bands also found in the proband's fibroblasts. Therefore, the results were consistent with the conclusion that the fibroblasts in the passage 13 cultures were from the mother. The data in figure 9 are also consistent with the initial identification of the proband's, the mother's, and the father's cell lines.

Attempts to clone individual fibroblasts from the mother were unsuccessful. Also, attempts to use DNA from individual cells as templates for the polymerase chain reaction were unsuccessful because the sensitivity of the polymerase chain reaction at the level required made it difficult to rule out contamination by exogenous human DNA in the assays.

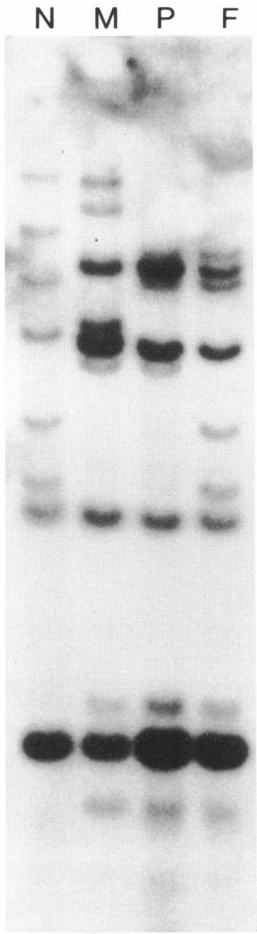

Figure 9 Southern blot analysis with a DNA probe for a hypervariable locus on the X chromosome (14). N = DNA from an unrelated normal control; M = mother's DNA from passage 13 fibroblasts; P = proband's DNA from cultured fibroblasts; F = father's DNA from cultured fibroblasts.

Ratio of Mutated to Normal Alleles in the Mother's Leukocytes

Further experiments were carried out on peripheral blood leukocytes from the mother. DNA from the mother's leukocytes and DNA from the proband's fibroblasts were processed in parallel, slot blotted onto the same filters, and hybridized with the same probes so as to obtain comparable values for the ratio of mutated to normal allele. As indicated in table 1, reproducible results were obtained in assays of several dilutions of the same DNA. Also, it was possible to standardize the assay sufficiently to obtain similar values from two separate experiments. The results demon-

Table 1

Ratio of Mutated to Normal Allele in Peripheral Blood Leukocytes from Proband's Mother

	DNA[a] (ng)	^{32}P Hybridized (cpm)		Adjusted Mutant-to-Normal Allele Ratio[b]	Mean ± SD
		ASO-Gly^{904}	ASO-Cys^{904}		
Experiment 1:					
Proband's fibroblast DNA	200	1,880	7,178	1.35	1.32 ± .087
	100	980	3,581	1.25	
	50	375	1,350	1.25	
	25	117	489	1.43	
Mother's leukocyte DNA	200	3,185	5,642	.63	.68 ± .048
	100	1,311	2,351	.63	
	50	676	1,398	.71	
	25	259	514	.71	
Experiment 2:					
Proband's fibroblast DNA	200	6,266	7,814	1.25	1.36 ± .132
	100	3,490	4,448	1.27	
	50	1,826	2,476	1.36	
	25	873	1,345	1.54	
Mother's leukocyte DNA	200	8,753	6,461	.74	.67 ± .062
	100	5,397	3,304	.61	
	50	2,159	1,528	.71	
	25	954	597	.63	

[a] Initial amounts of DNA from the polymerase chain reaction, as estimated by intensity of fluorescent bands on agarose gel containing 0.1% ethidium bromide. The sample was then serially diluted for slot-blot hybridization as shown in figs. 7 and 8.

[b] For the polymerase chain reaction, 100 ng of DNA was used as template in experiment 1 and 500 ng was used as template in experiment 2. In experiment 1, the specific activity of the ASO-Gly^{904} probe was 2.2×10^8 cpm/μg, and the specific activity of the ASO-Cys^{904} probe was 6.2×10^8 cpm/μg. Therefore, the ratios were adjusted for the differences in specific activity. In experiment 2, the specific activities were essentially the same (2.5×10^8 and 2.4×10^8 cpm/μg, respectively). Therefore, no adjustments were made.

strated that the ratio of mutated to normal allele in the mother's leukocyte DNA was about half the value in fibroblast DNA from the proband.

Attempts were also made to assay individual hairs from the mother by using the polymerase chain reaction and allele-specific oligonucleotides (Higuchi et al. 1988). Seven hairs were positive with both the normal and the mutated oligonucleotide, and one was positive only with the normal oligonucleotide. However, occasional water blanks also gave positive signals with the normal oligonucleotide, even though extensive precautions were taken and even though the experiments were repeated several times in a laminar flow hood.

Discussion

The results here establish that the mildly affected mother of a proband with a lethal variant of OI had the same mutation in the proα1(I) gene for type I procollagen as did the proband (Constantinou et al. 1989, 1990). In the proband, both the mutation and the consequences of the mutation in terms of its effects on the structure and function of procollagen were similar to those of a series of other single base mutations that cause lethal or severe variants of OI (Prockop and Kivirikko 1984; Cheah 1985; Byers et al. 1988*a*; Constantinou et al. 1989; Prockop et al. 1989, 1990). Therefore, the lack of fractures, lack of any decrease in bone density, and lack of evidence of severe OI in the proband's mother was initially perplexing (Constantinou et al. 1989, 1990).

The results here demonstrated that cells from the mother contained the mutated allele, but there was a progressive decrease of expression of the mutated allele with passage number of her fibroblasts. At the same time, there was a progressive decrease in the relative amount of mutated to normal allele in the mother's fibroblasts. The mutated allele was also present in hair DNA and leukocyte DNA from the mother, but the ratio of mutated to normal allele in the mother's leuko-

cytes was about half the ratio in DNA from the patient's fibroblasts. It was not possible to demonstrate directly that some of the mother's cells did not contain the mutated allele. Therefore, the results do not rigorously exclude the possibility that the mother had a very mild OI phenotype because of events such as gene conversion that generated some normal diploid fibroblasts in her tissues or selection in vivo for fibroblasts synthesizing more normal than mutated procollagen (see Goldring et al. 1990). The simplest explanation for the data, however, is that the mother was a mosaic for the same mutation that produced a lethal phenotype in one of her three children.

The observation that the proband's fibroblasts consistently grew more slowly in culture than did the mother's is also consistent with somatic cell mosaicism. In OI fibroblasts, mutated type I procollagens frequently are secreted poorly and accumulate within the rough endoplasmic reticulum and other membranous compartments (Prockop and Kivirikko 1984; Cheah 1985; Byers et al. 1988*a;* Prockop et al. 1989). Poor secretion of the mutated type I procollagen was demonstrated here for the mother's fibroblasts and elsewhere for the proband's (Constantinou et al. 1989). Such accumulation of abnormal type I procollagen may well lead to slower replication, in culture, of the cells containing the mutated gene and, therefore, to overgrowth by normal fibroblasts. Such selective advantage for survival of normal cells over cells containing mutated genes has been seen in a number of other genetic defects (Goodship et al. 1988; Hall 1988; Migeon et al. 1989). A selective loss of cells containing the mutated allele may well explain why the mother appeared to have a mild OI phenotype as a child (fig. 1) but lost most of the features in adulthood. Genetic data on one family with lethal OI suggested that the mother had germ-line mosaicism for the mutation (Byers et al. 1988*b*). Also, as the current studies were being completed, two abstracts were presented describing two examples of mosaicism in parents of children with OI (Cohn et al. 1989; Wallis et al. 1989).

Somatic cell mosaicism has been recognized for a long time (Stern 1968; Hall 1988). Since ancient times artists have expressed, as fantasies in the form of hermaphrodites or gynanders, human beings having both male and female traits (Stern 1968). Gynanders produced by cell or tissue mosaicism are well recognized in man, mice, *Drosophila,* bees, butterflies, and yeast (Stern 1968). Also, because of inactivation of one X chromosome, normal women are somatic cell mosaics. Somatic cell mosaicism of autosomal traits has also been noted in most species (Shun-Shin 1954; Stern 1968; Happle 1987; Hall 1988; Riccardi and Lewis 1988). The association of somatic cell mosaicism with the presence of the same mutation in the germ line, as is apparently seen here, is consistent with evidence from chimeric mice (Mintz et al. 1973) and transgenic mice (Soriano and Jaenisch 1986; Wilkies et al. 1986), indicating that the germ line is partitioned from somatic cells after the 15-cell stage of development of the inner cell mass of mouse embryos (Soriano and Jaenisch 1986). However, the stage at which this partitioning occurs in man has not been established.

The probable presence of somatic cell mosaicism in the mother of the proband examined here has implications for other probands and families with OI. One of the intriguing observations in OI is that many families show phenotypic variability (Prockop and Kivirikko 1984; Cheah 1985; Byers et al. 1988*a;* Constantinou et al. 1989, 1990; Prockop et al. 1989, 1990). Some of the phenotypic variability may be explained by somatic cell mosaicism in the first individual in the family with the disease.

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