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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

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Summary

Fibroblasts from a man with a mild form of osteogenesis imperfecta (OI) and from his son with perinatal lethal OI (OI type II) produced normal and abnormal type I procollagen molecules. The abnormal molecules synthesized by both cell strains contained one or two proα1(I) chains in which the glycine at position 550 of the triple-helical domain was substituted by arginine as the result of a G-to-A transition in the first base of the glycine codon. Cells from the mother produced only normal type I procollagen molecules. By allele-specific oligonucleotide hybridization to amplified genomic sequences from paternal tissues we determined that the mutant allele accounted for approximately 50% of the COL1A1 alleles in fibroblasts, 27% of those in blood, and 37% of those in sperm. These findings demonstrate that the father is mosaic for the potentially lethal mutation and suggest that the OI phenotype is determined by the nature of the mutation and the relative abundance of the normal and mutant alleles in different tissues. Furthermore, the findings make it clear that some individuals with mild to moderate forms of OI are mosaic for mutations that will be lethal in their offspring.

Introduction

Osteogenesis imperfecta (OI) is a heterogeneous group of inherited disorders characterized by an excessive tendency to fracture and the variable involvement of extraskeletal tissues including teeth, sclerae, and ligaments. Forms of the disorder range from death in the perinatal period, through marked short stature and severe deformity, to normal life span and little disability (Sillence et al. 1979; Smith et al. 1983). The clinical heterogeneity appears to reflect the effects of mutations in two collagen genes (COL1A1 and COL1A2) which encode the chains of the type I collagen, the nature and location of those mutations and, possibly, mutations in non-collagen genes (see Byers et al. [1988*a*] and Byers [1989] for reviews).

Although there is clinical overlap among the nonlethal forms of OI (types I, III, and IV) and intrafamilial variability of OI is well recognized, the occurrence of lethal and nonlethal forms of OI in the same family as a result of the same mutation appears to be very rare. One such instance was recognized in a survey of the inheritance of the perinatal lethal form of OI, OI type II, where the father of an infant was found to have features of OI type IV (Byers et al. 1988*b*).

We have now investigated the molecular basis of the variable expression of OI in this nuclear family. The infant was heterozygous for a mutation resulting in the substitution of the glycine residue at position 550 of the triple helix of the α1(I) chain by arginine. The father was found to be mosaic for this mutation so that about half the COL1A1 alleles (which encode the proα1(I) chains of type I procollagen) in fibroblasts contained the mutation, but the proportion in blood and

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sperm was lower. These findings support the concept that the phenotypic expression of a genetic disease reflects the nature of the mutation, its relative abundance, and its expression in target tissues. Furthermore, this evidence raises the concern that some individuals who apparently have new dominant mutations that produce OI are, in fact, mosaic for mutations that will be lethal in their offspring.

Material and Methods

Clinical History

The proband was the second child born to a 39-year-old man and his 34-year-old wife. The infant was born at 35 wk gestation and weighed 2.0 kg. The child had the characteristic appearance of OI type II, and radiographs demonstrated markedly diminished calvarial mineralization, beaded ribs, platyspondylia, short broad femoral and humeral bones, bowing of the short tibiae and fibular bones, and generalized osteopenia. The infant was included in a previous survey of OI type II (patient 35 [84–170] in table 1 of Byers et al. [1988*b*]).

The father was a healthy 44-year-old man at the time of a recent physical examination. He was the second child of a nonconsanguineous couple. His mother developed severe scoliosis during her teenage years, but has no other skeletal anomalies or health problems. His father had no skeletal anomalies, and died from a cardiac arrest at age 81 years. His sister has no skeletal anomalies.

He was born following a forceps delivery and weighed 2.87 kg. He was noted at birth to have a large, triangular-shaped head, and was thought to have hydrocephalus. No broken bones were noted. His head remained disproportionately large throughout life and his linear growth was slower than normal.

He broke his right collar bone while playing tag at age 8 years; the bone healed within the expected period of time, without disfigurement, and has never rebroken. He has not broken any other bones. His primary and secondary teeth were small and he had many dental caries. His hearing and vision are normal.

He was treated twice weekly with human growth hormone from age 8 to age 14 years. His height was 106.6 cm at the beginning of treatment and 148.6 cm at the end of treatment; his height has not increased since stopping treatment. The growth hormone administration was associated with early sexual maturation (pubic hair growth began at age 8 years, shaving at age 9 years, and genital maturity by age 10 years) and improved physical strength.

On physical examination at age 44, the father was 148.6 cm and weighed 61.8 kg. His head circumference was 61.0 and span was 147 cm. The skull shape was triangular. The sclerae had a bluish hue. The midface was small and flat and the philtrum hypoplastic. He had a normal number of small teeth which had a greyish discoloration. His neck was short and he had a barrel-shaped chest. No heart murmur was heard. His spine was straight. The genitalia were normal and he had an unrepaired right inguinal hernia. There was no limitation of movement of the extremities and there was no bowing or angulation of the long bones. The hands and feet were normally proportioned. The fingers and toes were hyperextensible. Neurological examination was normal.

Preparation and Electrophoretic Analysis of Procollagens and Collagens

Fibroblast cell strains were established from explants of skin from the parents and the infant with OI type II, with appropriate consent. Cells from unrelated healthy subjects served as controls. Cell cultures were maintained under standard conditions in Dulbecco-Vogt Modified Eagle Medium (DMEM, GIBCO) as described elsewhere (Bonadio et al. 1985). Labeling proteins with 2,3,4,5-[^{3}H]proline (Amersham; 101 Ci/mmol), harvesting of medium and cell-layer proteins, and SDS-PAGE of the labeled proteins were carried out as described elsewhere (Bonadio et al. 1985). Cyanogen bromide (CNBr) peptides of pepsin-treated procollagen were separated by isoelectric focusing in a vertical slab gel as described elsewhere (Benya 1981; Cole and Chan 1981; Bonadio et al. 1985) and then separated in a second dimension by SDS-PAGE.

DNA Sequence Determination

Total RNA was prepared from dermal fibroblasts of the father and of the fetus with OI type II (Chromcgynski and Sacchi 1987; Greenberg 1987). Ten micrograms of RNA were precipitated with 1 μg of a *Sal*I-tailed oligonucleotide primer, 709, located 3′ to the region encoding α1 (I) CB3 (amino acid residues 402–551 of the triple helix); the sequence was 5′ CTTGTCGACTGG-AAGACCAGCTGCACCAC 3′ coding for amino acids 555–561 of the triple helix. The cDNA was prepared as described elsewhere (Maniatis et al. 1982; Willing et al. 1990). An *Eco*RI-tailed primer, 708, located 5′ to the region encoding α1(I)CB3 (sequence: 5′ GGGAATTCCCACCTGGTGCCCGTGGTCA 3′; amino acids 392–398 of the triple helix), and primer 709 were used to amplify cDNA synthesized from the α1(I)

mRNA spanning the α1(I) CB3 domain using the polymerase chain reaction (Saiki et al. 1988). Five picomoles of the appropriate oligonucleotide primers per reaction and the buffers specified in the Perkin Elmer–Cetus DNA amplification kit were used, except that the reaction mixture had a final concentration of 4 mM $MgCl_2$ and 10 mM dithiothreitol. Thirty cycles of 1.5 min at 94°C, 2 min at 55°C, and 3 min at 72°C were performed. The amplified cDNA products were separated on a 1% LMP agarose gel (Bethesda Research Laboratories), and the appropriate 527-bp fragment was excised and purified (Benson 1984). The amplified cDNA fragment was digested with *Eco*RI and *Sal*I, and 30 ng was ligated to 100 ng of similarly cleaved M13mp18. Single-stranded DNA was prepared from clones containing inserts of the correct size (Messing et al. 1984), and the DNA was sequenced by the dideoxy-chain-termination method (Sanger et al. 1979) with T7 polymerase (Sequenase; United States Biochemical).

Hybridization of Allele-specific Oligonucleotides to Genomic DNA

Genomic DNA was prepared using standard procedures from the parental and infant fibroblastic cell strains and from peripheral blood leukocytes and sperm samples from the father. The genomic DNA sequence containing the mutation site was amplified using primers 709 and 705 (sequence: 5′ TGGAGGTCCCGGTAGCCAGGGCGCCCCTGG 3′; amino acids 537–547 of the triple helix), located at the 3′ and 5′ ends of exon 32 of the COL1A1 gene, respectively. Serial twofold dilutions of the 82-bp fragment were prepared in 33.3% (vol/vol) formaldehyde (using a standard solution of 37% formaldehyde). The samples were heated for 4 min at 100°C, immediately applied to a nylon filter (Nytran; Schleicher and Schull) in a slot blot apparatus, and the filter was baked for 2 h at 80°C. Duplicate filters were prepared for each sample, prehybridized for 4 h, and then hybridized overnight at 52°C using a standard procedure (Maniatis et al. 1982) with either the mutant or normal [γ-^{32}P]ATP (3,000 Ci/mmol; Amersham) end-labeled allele-specific oligonucleotides (normal, 5′ TGGCCTTCAGGGAATGCCTGG 3′; mutant, 5′ TGGCCTTCAGAGAATGCCTGG 3′). The filters were washed in 6 × SSC and 0.5% SDS at room temperature, then in 6 × SSC alone at room temperature, and then in 6 × SSC at 68°C for 10 min. The filters were wrapped in Saran Wrap and exposed to Kodak XAR-5 X-ray film for 4–24 h. Under these conditions there was no cross-hybridization of the normal or mutant oligonucleotides to cloned DNA of the mutant or normal alleles, respectively (results not shown). The absorbance of the resultant bands on the film was determined by densitometry (Zenich Scanning Densitometer, Model SLR-504-XL, Biomed Instruments). A curve was generated to determine the density values over which there was linearity, and experimental measurements within that range were used to determine relative ratios. The relative frequencies of the mutant COL1A1 allele were calculated from the ratio of the densities of the bands obtained when hybridizing with the mutant-allele oligonucleotide versus the combined densities of the bands obtained when hybridizing with the mutant or the normal-allele oligonucleotides. The proportion of the mutant allele was expressed as a percentage. The frequency calculations were uncorrected for differences in the specific radioactivities and hybridization efficiencies of the oligonucleotides. These differences were corrected for and normalized by using, as a standard, the results from the presumed heterozygous infant in the calculation of the allele frequencies in the tissue from the father.

Results

The Father and the Fetus Both Have a Charge Shift in α1(I)CB3 of Type I Procollagen

Fibroblastic cells from the infant with OI type II and the infant's father produced normal and abnormal type I procollagen molecules. The chains in the abnormal molecules were delayed in electrophoretic mobility, and the abnormal molecules were preferentially retained within the cells (fig. 1). Cells grown from the mother of the fetus produced type I procollagen molecules that contained only normally migrating chains. Two populations of CNBr peptides of the α1(I) chains of type I procollagen were synthesized by the cells from the father and the fetus: a normally migrating population and a population of peptides that were overmodified amino-terminal to α1(I)CB3. The α1(I)CB3 peptide contained both a normally charged population and one with a more basic isoelectric point (fig. 2).

The Father and the Fetus Both Have the Same Point Mutation Producing a Single Amino Acid Substitution

The COL1A1 cDNA synthesized from fibroblastic mRNA from the father and the fetus had a G-to-A transition in the first nucleotide of the codon for amino acid residue 550 of the triple-helical domain of the α1(I) chain in about half the clones that were sequenced (fig. 3). This point mutation resulted in the substitution of glycine by arginine.

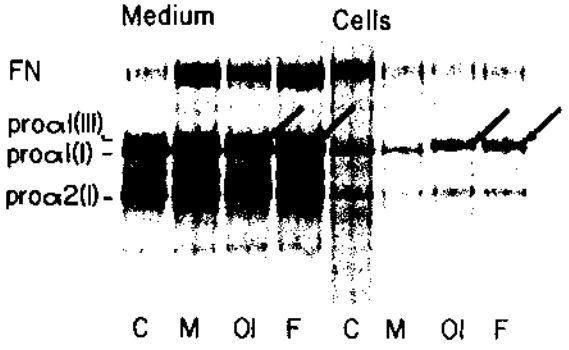


Figure 1 Proα chains of type I collagen separated under reducing conditions. The arrows indicate the populations of overmodified proα1(I) chains present in the medium and cell layers of the OI type II cell strain (OI) and the father (F) but not seen in the control (C) or the mother (M). FN = fibronectin.

The Father is Mosaic for the Point Mutation

Allele-specific oligonucleotides were used to estimate the relative frequency of the normal and mutant COL1A1 in genomic DNA isolated from several paternal tissues (fig. 4). The relative proportions of the mutant allele were in the range of 46%–50% of the COL1A1 alleles in fibroblasts, 26%–27% of those in blood, and 36%–40% of those in sperm.

Discussion

For point mutations that result in substitutions for glycyl residues within the triple-helical domain of the α1(I) chain of type I collagen there appears to be a predictable relationship between the nature and location of the substitution and the resulting phenotype (Byers 1989; Starman et al. 1989). In general, as the same substitution is moved toward the amino-terminal end of the triple helix, the phenotype becomes milder. For example, the substitution of cysteine for glycine at positions 988, 904, 748, and 718 is lethal, at 526 produces OI type III, at 175 produces OI type IV, and at 94 produces OI type I (Starman et al. 1989). Similarly, arginine substituted for glycine at positions 847 (G. A. Wallis et al., submitted), 667 (Bateman et al. 1988; in a personal communication, J. F. Bateman stated that, in Bateman et al. [1988], "substitution of arginine for glycine 664" should read "substitution of arginine for

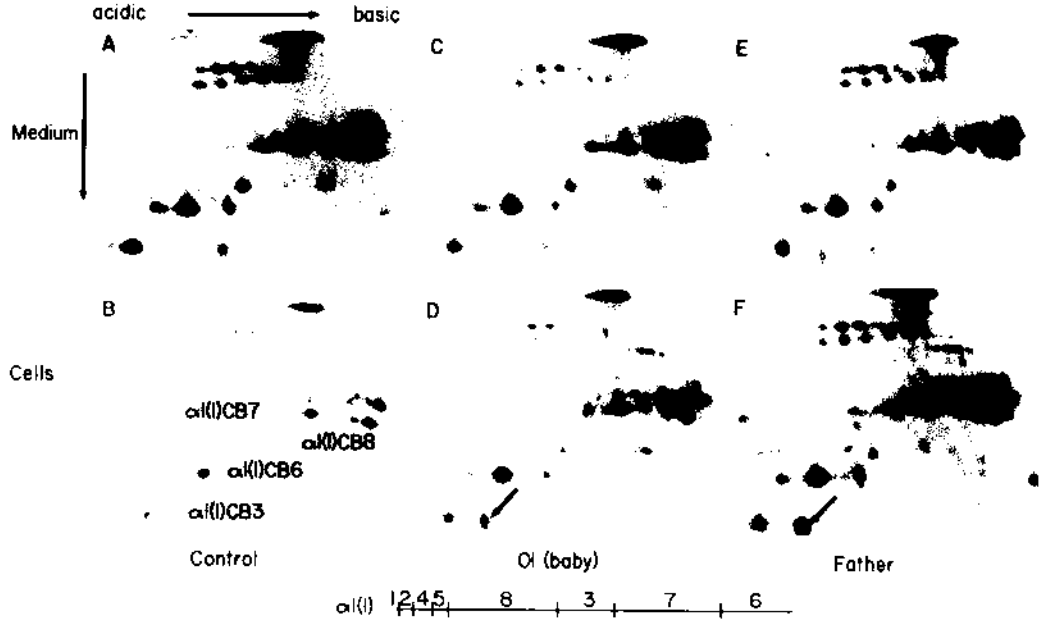


Figure 2 Two-dimensional maps of CNBr peptides. Peptides were separated by isoelectric focusing in the first dimension and by SDS-PAGE in the second. Panels represent the medium and cell-layer peptides after labeling of cell strains from a control, father, or infant (OI) for 18 h with [^{3}H]proline. The additional basically charged α1(I) CB3 peptide synthesized by the paternal and OI cell strains is indicated by an arrow. The line diagram below the panels indicates the arrangement of the CNBr peptides of the α1(I) chain. The reason for the apparent lower molecular weight of the basically charged α1(I) CB3 peptide relative to its normal charged counterpart is unknown. This phenomenon has been observed in other reported glycine-to-arginine mutations in collagenous peptides (Bateman et al. 1988; Wallis et al., submitted).

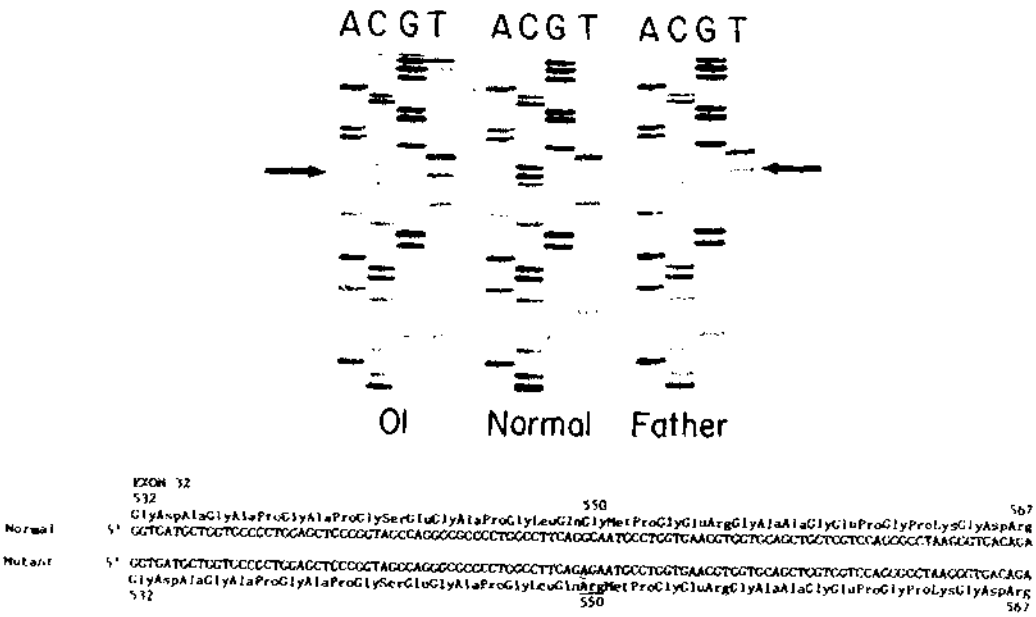


Figure 3 Sequence of the COL1A1 mutation. *Top*, cDNA sequencing gels of the normal and the mutant COL1A1 alleles from the father and the mutant COL1A1 allele from the infant (OI). The arrows mark the site of the mutation. The G-to-A transition occurs at the first base of codon 550 within exon 32. The cDNA sequence is of the antisense strand. *Bottom*, DNA and protein sequences surrounding the mutation site. The G-to-A transition results in the substitution of arginine for glycine at amino acid position 550 of the triple helix of α1(I). The first glycine of the triple helix is designated residue 1.

glycine 667) and 391 (Bateman et al. 1987) are all lethal, whereas substitution at position 154 (C. J. Pruchno et al., submitted) produces the OI type III phenotype.

Thus, the substitution of arginine for glycine at position 550 in α1(I) is consistent with the lethal phenotype found in the proband. However, this mutation would also be expected to produce a lethal phenotype in the father in the absence of ameliorating mutations or some other special circumstance. The abnormal type I procollagen molecules synthesized by cells from the father and from the proband were overmodified to the same degree and extent, were retained within the cell in the same manner, and had the same decreased melting temperatures (results not shown). While phenotypic variability in the family might be explained by the presence of a protective mutation in the father's tissues (type unknown) the recognition of different frequencies of the mutant allele in tissues suggests that the milder phenotype in the father reflects the mosaic distribution of the mutant allele in his tissues.

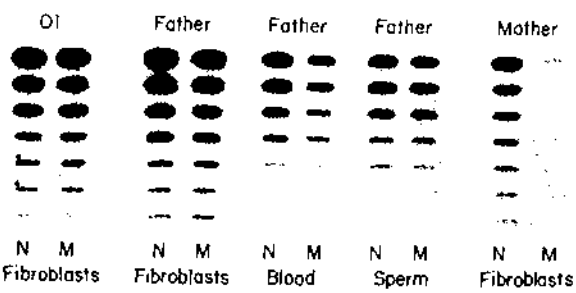


Figure 4 Allele-specific oligonucleotide hybridization to genomic DNA isolated from fibroblasts of the mother and infant (OI) and from fibroblasts, blood, and sperm of the father. The DNA was hybridized to either the normal (N) or the mutant (M) oligonucleotide.

A substantial proportion of the father's somatic cells (fibroblasts and leukocytes) and germinal cells contained the mutant allele, indicating that the mutation occurred during the father's embryonic development, prior to the segregation of fibroblast, germline, and hematopoetic lineages. The proportion of cells with the mutant allele to cells with the normal allele in the paternal osteoblasts has not been determined, but there must be sufficient cells with the mutant allele in bone and other tissues to cause the clinical manifestations of OI apparent in the father. The proportion of cells with the mutant allele required to produce a phenotypic effect is not known, and the phenotypic differences may well result if the cells synthesizing abnormal molecules are not uniformly distributed in the matrix. Experiments

in transgenic mice suggest that expression of a mutant collagen allele bearing a point mutation (α1[I]gly859 to cys) at a level as low as 10% of the total α1(I) mRNA can produce a lethal phenotype (Stacey et al. 1988). Presumably, those alleles are expressed in all cells in every tissue, so that 19% of the molecules (assuming random assortment of products) produced by every cell would be abnormal. In mosaic individuals, however, only some cells express the mutant allele while others express only normal alleles. Thus, if 10% of alleles in a mosaic individual contained the mutation, then 15% of all molecules would be abnormal but only 20% of the cells would express the abnormal chain. As a consequence, collagen production by 80% of the cells would be normal, and any effects on secretion or fibrillogenesis would probably be limited to the local environment of the cells in the tissues that express the mutant allele.

We are aware of a dozen families in which recurrence of the OI type II phenotype in siblings is best explained by parental mosaicism for the mutant allele. In each family the parents are clinically normal. In one family that has been extensively studied, a father who has no clinical signs of OI is mosaic such that about one of seven alleles in sperm and one of five alleles in blood are mutant, but he has no detectable mosaicism in fibroblasts (Cohn et al. 1990). Thus, it is clear that the level of mosaicism and the expression of the mutant allele in target tissues contribute to the phenotypic effect (see also Constantinou et al. 1989).

Although most of the clinical heterogeneity in OI can be explained by different mutations in either of the two genes that encode the chains of type I procollagen, somatic mosaicism provides an additional source of intrafamilial variability. Our finding (and that of Constantinou et al. 1989) that mosaicism for an otherwise lethal mutation can result in a clinical phenotype similar to that of OI type IV complicates genetic counseling for individuals who appear to represent new dominant mutations. It is likely that most of these individuals have OI because they are heterozygous for a mutation in a collagen gene whereas others (an unknown proportion), like the individual described here, are mosaic as the result of a postzygotic mutation. The recurrence risk among sibs of such individuals would be the background risk, but the risk of having children with more severe forms of OI would depend on the proportion of mutant alleles in the germ line. As a consequence, it is important to consider the possibility of mosaicism in each individual with nonlethal OI who is the first affected individual in his or her family. As more lethal and nonlethal mutations that result in OI are characterized, and the relationship between genotype and phenotype becomes clear, it will be possible to provide better genetic counseling to individuals who appear to have OI as a result of new dominant mutations in the genes that encode the chains of type I collagen.

We note, finally, that genetic anticipation [the concept that a mutation becomes more severe with successive generations (see Hall [1988] for review)] may be explained in some nuclear families by somatic mosaicism.

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