Alternative Splicing in COL1A1 mRNA Leads to a Partial Null Allele and Two In-frame Forms with Structural Defects in Non-lethal Osteogenesis Imperfecta*

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We have identified a novel multiexon genomic deletion in one COL1A1 collagen allele that results in three alternative forms of mutant mRNA. This mutation occurs in a 9-year-old girl and her father, both affected with severe type III osteogenesis imperfecta (OI). We previously reported detection of a mismatch in their α1(I) amino acids 558–861 region by RNA/RNA hybrid analysis (Grange, D. K., Gottesman, G. S., Lewis, M. B., and Marini, J. C. (1990) Nucleic Acids Res. 18, 4227–4236). Single Strand Conformational Polymorphism further localized the mRNA mutation to the amino acids 579–679 coding region. At the gene level, polymerase chain reaction (PCR) amplification of patient leukocyte DNA from the exon 33–38 region yielded the normal 1004-base pair (bp) fragment and an additional 442-bp fragment. Sequencing of the shorter genomic PCR product confirmed the presence of a 562-bp deletion, extending from the last 3 nucleotides (nt) of exon 34 to 156 nt from the 3'-end of intron 36. The genomic deletion was also detected in the clinically normal grandmother, who was confirmed to be a mosaic carrier.

PCR amplification and RNase protection experiments were used to investigate the mRNA structure and occurrence of alternative splicing. One form of the mutant cDNA has a deletion with end points that are identical to the genomic deletion. This results in a combination deletion/insertion, with a deletion of amino acids 603–639 followed by an insertion of 156 nt from the 3'-end of intron 36. In addition, we found two alternatively spliced forms. One form uses a cryptic donor site in exon 34 and the exon 37 acceptor. The second form uses the normal exon 32 splice donor and exon 37 acceptor. Use of the cryptic donor results in a coding sequence that is out-of-frame. Both the retained intron form and the use of the exon 32 donor site result in coding sequences that are in-frame. This is the first report of a collagen defect in OI with alternative splicing generating both in-frame and out-of-frame forms of mRNA. Although the in-frame forms constitute more than 60% of the mRNA from the mutant allele, no mutant protein chain was identified. Collagen produced by cultured OI osteoblasts showed a significant increase in the relative amount of type III collagen but no mutant α1(I) chain.

Osteogenesis imperfecta (OI) is a heritable disorder of connective tissue; its most significant clinical feature is fragile bones that are susceptible to fracture from minimal trauma (1). The phenotypic spectrum of OI is described by the Sillence classification and ranges from perinatal lethal to minimal skeletal involvement. The full clinical spectrum of OI has been demonstrated to be caused by defects in the structure or synthesis of type I collagen, the most abundant protein of bone and skin (for reviews, see Refs. 2 and 3). In the majority of instances, the mild OI type I is caused by defects that effectively create a null α1(I) allele (4), such as a frameshift resulting in premature chain termination. In these cases, all the secreted collagen is structurally normal. The decreased total production of type I collagen results in an increase of the α1(III)/α1(I) ratio. In OI types II, III, and IV, there is an expressed structural defect in either the α1(I) or α2(I) chain (2). Over 100 of these defects have been molecularly delineated; 79% are point mutations resulting in the substitution of another amino acid for one of the glycine residues that occur in every third position of the chain, 11% are in-frame single exon splicing defects, and the small remainder comprises deletions, duplications, and insertions.

Intronic inclusions and multiexon deletions are among the less common types of collagen mutations that have been demonstrated in OI. Intronic inclusions have been described in three cases. When these sequences are in-frame and contain no stop codons, a non-collagenous region is inserted into the helical portion of the collagen chain. Since the fibrillar collagens are defined by uninterrupted repeats of the Gly-X-Y triplet, such insertions disrupt the essential nature of the molecule. A patient with moderately severe type IV OI has a 6-amino acid intronic insertion from intron 33 in one α2(I) allele (5) resulting from activation of a cryptic splice site. Another patient with type IV OI (6) has a defect at the +5 position of the intron 8 splice donor site in α1(I) that leads to a deletion of the 18 amino acids of exon 8 and insertion of 32 amino acids coded by intron 7. Finally, insertion of 75 bp of α1(I) intron 35 is associated with lethal type II OI phenotype (7).

One multiexon deletion has been described in each of the type I collagen α chains, both associated with a lethal phenotype. In α1(I), the well studied CRL 1262 has a deletion of exons 27, 28, and 29 resulting from an intron to intron recombination (8). The chains carrying the 84-amino-acid deletion are detected in the cells but are poorly secreted (9). In α2(I), another intron to intron recombination results in the loss of the 180 amino acids encoded by exons 34–40 (10). The short α2(I)

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In this paper, we describe a novel α1(I) mutation that occurs in a father and daughter affected with severe type III OI and, in mosaic form, in the paternal grandmother. The gene-level splicing generating both in-frame and out-of-frame forms of mRNA from this allele, with intronic inclusion and multiexon deletion, were demonstrated by PCR and RNA protection. This is the first report of a collagen defect in OI with alternative splicing generating both in-frame and out-of-frame forms of mRNA. This novel type of mutation provides insight into the clinical and biochemical consequences of the combination of a partial null α1 allele and interruptions in the helical region of collagen.

**MATERIALS AND METHODS**

**Case Report**—The patients with severe type III OI are a 9-year-old girl and her 37-year-old father. The clinically unaffected grandmother had no history of fractures and was of normal stature; her facies were mildly triangular. She died after a right-side intracranial hemorrhage at 36 months of age. She has had several dozen femur fractures and is severely deformed. The 9-year-old child is the 3500-g, 18-inch product of a term gestation. At birth, features of severe OI were noted, including soft calvaria and disorganized popcorn growth plates.

**Cell Culture**—Dermal fibroblast cultures were established from skin punch biopsies of the patients and their family members. Cells were grown in Dulbecco’s modified Eagle’s medium enriched with 10% calf serum plus 2.0 mM glutamine in the presence of 5% CO2. The cell lines (passages 5–10) and the control cell line (GMO 3349, passage 12–17) were incubated at 37 or 32 °C and labeled with [3H]proline (Amersham Corp.) or [35S]cysteine (50 μCi/well, Amersham Corp.) for about 16 h. Osteoblast primary cultures were established from surgical bone chips of the proband and normal control. Osteoblasts, released from bone chips using collagenase P (11), were cultured in a 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s F-12 medium, both without CaCl2, supplemented with 10% fetal bovine serum, 2 mM glutamine, and 25 μg/ml ascorbate in an atmosphere of 8% CO2. Osteoblasts were incubated at 37 °C and labeled with [3H]proline for 16–18 h.

**RT-PCR and SSCP Analysis**—Total RNA was isolated from cultured fibroblasts using the acid/phenol method (14). For SSCP analysis, the region coding for α1(I) aa579–679 was amplified by an RNA PCR (15) core kit (Perkin-Elmer) from patients and control total RNA in the presence of [32P]dCTP (10 μCi/ml, Amersham Corp.). The primers are 39-mer sense primers identical to the published sequences for nt 2388–2417, nt 2766–2795, and nt 2981–2990; the antisense primers are complementary to nt 2661–2690, nt 3048–3077, and nt 3291–3320, respectively (16). Upstream primers have an EcoRI linker and downstream primers have a HindIII linker. After cDNA synthesis, PCR was performed for 1 cycle of 1.5 min at 94 °C, 1 min at 68 °C, and 1 min at 72 °C followed by 40 cycles of 40 s at 94 °C, 40 s at 68 °C, and 40 s at 72 °C. The labeled PCR fragments were analyzed by SSCP (17) under two gel conditions: 1) MDE74 gel (Bio Chem) with 0.5 × Tris borate/EDTA at 4°C for 3 h at 40 V, and 2) MDE74 with 0.6 × Tris borate/EDTA at room temperature for 6 h at 30 V.

The fragment spanning aa 579–679 was subcloned into pGEM3Z. This subcloning yielded both the normal 300-nt fragment and the 345-nt fragment containing the insertion/deletion mutation. For the out-of-frame mRNA, we subcloned a 375-nt band that was generated by RT-PCR of the region coding for aa 482–679. The RT-PCR of the region coding for aa 482–679 was sequenced and a sense primer identical to nt 2097–2126. The PCR was performed as described above.

**Genomic DNA**—PCR amplification of leukocyte DNA was done using the same primers used for the cDNA subclones (upstream primer is located in exon 33/34 and downstream primer is complementary to exon 38). PCR conditions were as follows: 1 cycle of 5 min at 95 °C and 5 min at 68 °C to denature genomic DNA; then 1 cycle of 2 min at 94 °C, 1 min at 68 °C, and 1 min at 72 °C; then 10 cycles of 1 min at 94 °C, 1 min at 68 °C, and 1 min at 72 °C; and finally 30 cycles of 1 min at 92 °C, 1 min at 68 °C, and 1 min at 72 °C. The PCR products were examined on a 1.5% agarose gel. The 452-nt genomic PCR fragment was subcloned into pGEM3Z vector.

**Sequencing**—Subclones of fragments spanning aa 579–679 and 482–679 of cDNA of the patients and genomic DNA were sequenced using the dideoxy chain-termination method (18) (Promega fmolTM kit). The primer for sequencing the retained intron, the out-of-frame mRNA, and the genomic DNA was the pUC forward primer (Promega). For the

**Fig. 1. X-rays of long bones and thoracic vertebrae of the proband.** A, lower long bones of the 9-year-old girl with OI type III. The long bones are extremely osteoporotic with poor modeling and disorganized popcorn growth plates. B, all thoracic vertebrae are severely compressed.
RESULTS

Collagen Protein Analysis—The procollagen synthesized by the dermal fibroblasts of the two patients and family members was examined by SDS-urea-polyacrylamide gel electrophoresis. The patients and the clinically normal grandmother (Fig. 2, panel A) have normally migrating pro-α1(I) and pro-α2(I) chains, as well as an additional band that migrates slower than pro-α1(I) and is well secreted by the cells. The quantity of the slow band was less in the procollagen of the grandmother than in that of her son and granddaughter. When we originally examined the collagen synthesized by the fibroblasts of the child (21), we assumed that this slow band would represent an overmodified α1(I) chain because of the phenotype of the child, the migration position of the band, and its abundance relative to normally migrating α1(I). This extent of electrophoretic delay would be expected to involve overmodification of two-thirds to three-quarters of the helical region.

This band was very pepsin-sensitive. Even 15 s of pepsin digestion (Fig. 2, panel B) yielded collagen containing only normally migrating chains. We subjected the α1(I) and α2(I) collagen chains to cyanogen bromide digestion. All the CNBr peptides from the pepsin-derived collagen chains migrated normally (Fig. 2, panel C). We also examined the CNBr peptides of the slow band from the procollagen gel and demonstrated that it was pro-α1(III) chain rather than an overmodified α1(I). Thus, these cells have an increased α1(III)/α1(I) ratio. On the pepsin-digested collagen gel (Fig. 2, panel B), the α1(III) and α1(I) chains are co-migrating, accounting for the disappearance of the slow band.

Detection of Collagen Mutation in α1(I) mRNA—Because this mutation did not result in helix overmodification, no protein localization was possible, and the entire α1(I) mRNA was screened by RNA/RNA hybrid analysis. We previously reported detection of a mismatch in both patients in the region coding for aa 558–561 (21). The 910-nt riboprobe (PES), spanning exons 32–43, that was used for this analysis was digested to fragments with apparent sizes of 160 and 750 nt (Fig. 3, panel B).
The mutation was further localized by performing SSCP analysis of three overlapping PCR fragments. The PCR fragment spanning the subregion coding for aa 579–679 of α1(I) mRNA was amplified from OI and control (C) total RNA in the presence of [32P]dCTP and analyzed by SSCP under two gel conditions. Arrows indicate the additional bands with strikingly abnormal migration. TBE, Tris borate/EDTA.

The mutation was further localized by performing SSCP analysis of three overlapping PCR fragments. The PCR fragment spanning the subregion coding for aa 579–679 in exons 33–38 (Fig. 3, panel B) yielded additional bands with strikingly abnormal migration under two electrophoretic conditions (Fig. 4). This PCR fragment was subcloned for further analysis. The alleles could be distinguished by differences in the gel electrophoretic mobility of the PCR inserts, as well as by SSCP.

**Sequencing the cDNA of the Patient**—To determine the cDNA mutation of the proband, duplicate subclones of both alleles were sequenced. For both patients, the sequences of one allele were normal (Fig. 5). In the second allele (Fig. 5), the last 3 nt at the 3'-end of exon 34 were deleted, as were the sequences coding for exons 35 and 36. An insertion of the last 156 nt at the 3'-end of intron 36 was joined to the exon 34 sequences. Normal collagen sequences resumed with exon 37 (Fig. 3, panel B). The sequences of the patients were identical to the published sequence of human COL1A1 intron 36 (22) except that there were 3 additional nt, CCA, located 83 nt from the 5'-end of the intron. We also found 2 single nt changes, C→A and T→G at positions 81 and 100, respectively, of the intron.

**Alternative Splicing: PCR Detection, Sequencing, and Quantification by RPA**—Two different alternatively spliced mRNAs were detected by RT-PCR and further defined by sequencing (Fig. 5). The RT-PCR of the region coding for aa 579–679 gave rise to two fragments, a 300-nt band corresponding to the normal allele and a 130-nt band. Sequencing of the subcloned shorter product revealed a deletion of 8 nt at the 3'-end of exon 34 and all of exons 35 and 36 as a consequence of utilization of a cryptic donor site in exon 34 and the normal exon 37 acceptor. This mRNA encoded an out-of-frame sequence with a stop codon at the second amino acid of exon 37, generating a 779-aa α1 chain lacking the carboxyl-terminal helix incorporation region. The RT-PCR of the region coding for aa 482–679 produced a shorter 375-nt band in addition to the normal amplification product of 681 nt. The subcloning and sequencing of this fragment revealed a multiexon skipping of exons 33 through 36 (Fig. 5) generated by the use of the regular exon 32 splice donor and exon 37 splice acceptor. The resulting in-frame sequence encoded an α1(I) mutant chain 72 aa shorter than the normal α1(I) chain.

We used a ribonuclease protection assay to quantify the relative amounts of the different mRNA forms. Radiolabeled antisense riboprobes synthesized from the three subcloned mutant transcripts were hybridized with proband total RNA (Fig. 6). Full-length probe protection by the mutant forms yielded the expected fragments of 345 nt for the retained intron form, 375 nt for the in-frame mRNA, and 183 nt for the out-of-frame form. Densitometric analyses of the gels were performed to...
The smaller product is compatible with a genomic deletion of both probands, the major product is a 445-bp fragment (Fig. 7). Though a small amount of the predicted product is detected in the form of normal DNA should generate a 1007-bp fragment. Amplification was performed with the same primers used to the patients and family members, including the clinically un- mutation, we used PCR amplification of leukocyte DNA from the family Members—

To determine the gene level structure of the scriptswerehybridizedagainstthetotalRNAoftheaffectedfather((shown in Fig. 3) complementary to the three different mutant tran-
forms, normalizing the values obtained by the specific activity

PCR and Sequencing of Genomic DNA of Proband and Family Members—To determine the gene level structure of the mutation, we used PCR amplification of leukocyte DNA from the patients and family members, including the clinically un-affected grandmother and her three daughters. Genomic PCR amplification was performed with the same primers used to amplify the cDNA fragment spanning aa 579–679. Amplification of normal DNA should generate a 1007-bp fragment. Although a small amount of the predicted product is detected in both probands, the major product is a 445-bp fragment (Fig. 7). The smaller product is compatible with a genomic deletion of the region from exon 34 to intron 36 (Fig. 3, panel A). The discrepancy in the quantity of the two products is most likely due to preferential amplification of the smaller product. The smaller mutant fragment was also detected in leukocyte DNA of the unaffected grandmother; the larger normal fragment was more abundant in her DNA than in that of her son and granddaughter. This suggests that she had relatively fewer cells containing the deleted allele and is a mosaic for the deletion. The deletion was not detected in the three clinically normal sisters of our adult patient. The shorter PCR fragment amplified from the leukocyte DNA of the grandmother was subsequently subcloned and shown to contain the identical deletion present in her son and granddaughter, thus confirming her carrier status.

The shorter genomic PCR fragment was subcloned from both patients. Sequencing confirmed the presence of a 552-bp deletion extending from the end of exon 34 to the middle of intron 36. The deletion end points are identical in genomic DNA and the cDNA-retained intron form (Fig. 5).

Analysis of α1(I) Chain Synthesis in Cell Culture—Two of the three forms of mutant α1(I) chain were in-frame, the form resulting from alternative splicing with the exon 32 donor and the form with the retained intron. In addition, they have the carboxyl-terminal alignment region. We verified that the inserted intron sequences did not interfere with translation by using a coupled in vitro transcription/translation system to demonstrate that the portion of the mutant allele spanning aa 430–732 was translatable. We detected an approximately 35-kDa protein product from the retained intron mutant template; no product was detected in the negative control (data not shown).

We examined the α1(I) chains for the presence of the two in-frame forms of the mutant protein. The abnormal portion of both of these forms would be contained entirely within the CB7 peptide (Fig. 3, panel C). CB7 normally contains 271 aa. In the exon 33–36 deletion form, CB7 would contain 199 aa, making it hard to detect in comparison with the 191-aa CB6. In the intronic insertion form, CB7 would contain 286 aa. However, the intronic sequences contain an additional methionine. Cysteine digestion would be expected to generate a 235-aa fragment, migrating between the CB6 and CB8 peptides. This 235-aa fragment should contain two cysteine residues that are not present elsewhere in normal α1(I). No mutant forms of CB7 were detected with either [3H]proline or [35S]cys-

We examined the possibility that the mutant chain might survive processing to collagen by the pericellular enzymes. OI and control cells were labeled for 24 h, and the processing to collagen was followed for 5 days (Fig. 8, panel A). We noted that the OI procollagen was processed faster than the normal control; the proportion of procollagen processed to collagen at 1 day by the OI cells was equivalent to the processing completed by normal cells at 2 days. Cysteine digestion of each of the bands indicated in Fig. 8 (panel A) yielded only various processed forms of α1(I) or α1(III) (data not shown). Repetition of the processing experiment with [35S]labeling did not detect mutant α1(I) chain (data not shown).

We also examined collagen chains labeled during brief pulse-chase studies (Fig. 8, panel B). Control and OI cells were
labeled with $[^3]H$proline or $[^35]S$cysteine for 3 h and then chased with cold medium. Even in the collagen harvested from the 30- and 60-min chase points, no mutant chain was detected. We did note that the rate of collagen secretion by the OI cells was about half that of the normal control cells.

In addition, we considered the possibility that either of the mutant chains might be more stable at a reduced temperature. We repeated the overnight labeling with $[^3]H$proline or $[^35]S$cysteine, as well as the pulse-chase and cell processing experiments at 32 °C. Furthermore, we labeled cells in the presence of a,$a$-dipyridyl at both 37 and 32 °C. Mutant protein was not detectable under any of these conditions.

**Analysis of Collagen Protein Synthesized by Osteoblasts**

The gel electrophoresis of procollagen produced by osteoblast cultures from the affected child showed a pattern similar to that seen for the fibroblast collagen of the patient (Fig. 9). The additional band migrating slower than pro-$a$-I(III) chain was confirmed to be a pro-$a$-I(III) chain by CNBr digestion (Fig. 9). In the collagen synthesized by normal control osteoblasts, type III collagen is undetectable. The ratio of type III to type I collagen in the fibroblasts and osteoblasts of the proband is approximately equal in the fibroblasts and osteoblasts of the proband.

**DISCUSSION**

We have described here a novel collagen mutation that occurs in a father and daughter with severe type III OI. The mutation is a 562-bp exon to intron genomic deletion in one COL1A1 allele. The deletion begins with the final codon of exon 34 and ends within intron 36. We have examined the regions surrounding both ends of the deletion and find no appropriate sites for homologous crossover. Furthermore, the exon to intron end points of the deletion are most compatible with a mechanism of illegitimate recombination; the GC-rich collagen exons and the AT-rich introns have only rare sequence homologies. The $a$-I(III) transcript produced by the mutant allele is alternatively spliced, resulting in three distinct forms. One form of the mRNA has end points that are identical to those of the genomic deletion. This results in a deletion/insertion mutation in which there is a deletion of 37 aa, encoded by the last triplet of exon 34 and exons 35 and 36, and also an insertion of 156 bp from the 3’-end of intron 36. The inserted sequence is in-frame and contains no stop codons; it encodes a non-collagenous hinge of 52 amino acids that would be inserted after residue 602 in the $a$-I(III) chain. A second in-frame form of the mutant mRNA results from alternative splicing that utilizes the normal exon 32 donor and exon 37 acceptor. This form is a simple deletion of aa 568–640 of the $a$-I(III) chain. Together, the in-frame forms comprise approximately 60% of the mutant mRNA detectable in fibroblasts. The third form of the mutant mRNA also results from alternative splicing but is out-of-frame. In this case, a cryptic splice donor in exon 34 is used, and the protein product should be prematurely terminated in exon 37. The cryptic splice donor matches the donor consensus sequence from positions –2 to +4. The proportions in which the cryptic donor and the normal exon 32 donor are used may depend on both general splicing factors, such as the match to the donor consensus sequence, and collagen-related factors. Collagen exons have a “standard size” and code for 18 residues of protein; there are a few double size exons. Exon 33/34 is a double size exon. The addition of the 52 aa residues encoded by the retained intron would make a quadruple size exon. Although this would be expected to favor the use of the cryptic donor in exon 34, we saw approximately equal use of the more proximal cryptic site and the more distal natural site. This is the first report of a collagen defect in OI with alternative splicing generating both in-frame and out-of-frame forms of mRNA.

The dermal fibroblasts of the clinically unaffected paternal grandmother also synthesized collagen with the same electrophoretic abnormalities as those seen in her son and granddaughter although the $a$-I(III) band was less abundant in the grandmother. In her fibroblasts and leukocytes, the abnormal genomic deletion was easily detected by PCR amplification. Because of the size of the deletion, PCR amplification occurs preferentially from the mutant allele, and it is difficult to quantitate the proportions of normal and mutant alleles. Nonetheless, substantially more normal allele product was seen in the grandmother than in the affected individuals. In addition, the presence of the deletion in her leukocyte DNA was verified by subcloning and sequencing. The presence of the mutation in lower proportions than in the affected individuals identifies the grandmother as a mosaic carrier. Given her multietissue expression of the mutant allele, it is interesting to note that her physical involvement was limited to triangular facies, which...
did not resemble those of her unaffected daughters, and light blue sclerae. We were able to provide reassurance to the unaffected daughters that they were not carriers in spite of their concerns about relatively loose joints and blue sclerae. This is an excellent illustration of how mild phenotypic features within the normal range may be overinterpreted because of concern about a familial disorder.

The mutation in this family provides a complex illustration of two of the more unusual types of collagen mutations in OI, that is, non-collagenous insertions and large multiexon deletions. Although the mRNA form with an intronic insertion comprises less than 10% of the mutant message, this proportion is sufficient for a significant contribution to phenotype. Non-collagenous insertions have been associated with phenotypes ranging from lethal to moderately severe. A 6-amino acid insertion in the middle of the α2(I) chain is associated with a mild outcome (5), and the mutant chains are easily detected. In α1(I), insertion of 32 amino acids from intron 7 is clinically moderately severe (6). The type I collagen is very protease-sensitive, but a band with faster migration can be detected in cultures grown at 32°C. Finally, a 25-amino acid insertion from intron 35 of α1(I) is sufficiently stable to be detectable by CNBr digestion and has a lethal phenotype (7). For these cases, phenotype seems to be related to two factors: 1) the net change in the length of the α chain resulting from the deletion/insertion with longer net change having a more severe outcome and 2) the position along the α chain with more amino-terminal insertions having a milder outcome. In the middle of the chain, one might speculate that stable helices can form on both sides of the insertion and may have the effects of looping out the non-collagenous region and exposing it to proteolytic enzymes.

The predominant form of our mutant α1(I) mRNA contains a 4-exon deletion. One such large deletion has been previously described for each of the type I α chains, and in both cases the phenotypic outcome was lethal. In α2(I), an intron to intron deletion of 7 exons removed 180 amino acids from the helical region (10). The mutant chains were detectable in the cells; the thermal stability of helices containing the mutant chains was reduced dramatically to 34°C. In α1(I), an intron to intron deletion of 3 exons deleted 84 amino acids (8). The mutant chains were detectable but were poorly secreted from the cells (9).

The relationship of protein structure to phenotypic outcome in the case reported here is complex. The phenotype itself is quite consistent in the two affected individuals. Our inability to detect either of the two in-frame forms of the mutant protein suggests that helices containing them are exquisitely protease-sensitive. It is also possible that the retained intron mRNA is selectively retained in the nucleus, limiting protein production from that form. From the experience with non-collagenous insertions and multiexon deletions described above, we would have predicted a lethal outcome for this mutation. Our model for the correlation of molecular defect and phenotype in our probands is that of a functional combination of lethal type II and the very mild type I OI in which the "type I component" moderates the lethal outcome to a severe outcome compatible with life into adulthood. In type I OI, a null α1(I) allele results in reduced collagen production. In the case presented here, the out-of-frame form of the mRNA reduces the protein production of the mutant chain allele by 40%. The in-frame forms of the mutant α1(I) chain provide the "type II OI component." They appear to exert most of their effect by "protein suicide," causing the degradation of helices into which they have incorporated. A small amount of mutant protein may be secreted into matrix.

Type III collagen synthesis is dramatically increased in both the fibroblasts and osteoblasts of our patients. It is possible that the increased amount of type III collagen, which is normally undetectable in bone matrix, has a substantial impact on clinical outcome. Increased type III collagen may alter the fibril diameter or higher order structure of the collagen scaffolding. Interestingly, an increased α1(III)/α1(I) ratio is characteristic found in type I OI and has also been noted in the bone tissue of two cases of lethal OI (23). Futhermore, osteoblasts synthesizing and degrading substantial quantities of mutant type I collagen helix would be expected to secrete altered quantities of non-collagenous components of bone extracellular matrix (24), such as reduced osteonectin or increased bone sialoprotein. Changes in the proportions of non-collagenous components could contribute to the severe non-lethal outcome, and studies in this direction are planned.

This case also has implications for antisense approaches to the gene therapy of dominant disorders such as OI. Eliminating 40% of the mutant mRNA from protein production may be sufficient to moderate a lethal mutation into a severe non-lethal condition. Undoubtedly, the extent of clinical modulation will vary with the particular mutation and the proportion of reduction of the mutant chain, but this case provides additional encouragement for the potential effectiveness of an antisense approach.

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