Patients with OI/EDS form a distinct subset of osteogenesis imperfecta (OI) patients. In addition to skeletal fragility, they have characteristics of Ehlers-Danlos syndrome (EDS). We identified 7 children with types III or IV OI, plus severe large and small joint laxity and early progressive sclerosis. In each child with OI/EDS, we identified a mutation in the first 90 residues of the helical region of α1(I) collagen. These mutations prevent or delay removal of the procollagen N-propeptide by purified N-proteinase (ADAMTS-2) in vitro and in pericellular assays. The mutant pN-collagen which results is efficiently incorporated into matrix by cultured fibroblasts and osteoblasts and is prominently present in newly incorporated and immaturity cross-linked collagen. Dermal collagen fibrils have significantly reduced cross-sectional diameters, corroborating incorporation of pN-collagen into fibrils in vivo. Differential scanning calorimetry revealed that these mutant collagens are less stable than the corresponding procollagen, which is not seen with other type I collagen helical mutations. These mutations disrupt a distinct folding region of high thermal stability in the first 90 residues at the amino end of type I collagen and alter the secondary structure of the adjacent N-proteinase cleavage site. Thus, these OI/EDS collagen mutations are directly responsible for the bone fragility of OI and indirectly responsible for EDS symptoms, by interference with N-propeptide removal.

Osteogenesis imperfecta (OI) is a genetic disorder of connective tissue characterized by bone fragility, growth deficiency, and blue sclerae (1, 2). Defects in type I collagen are well known to cause the full clinical range of OI (3, 4). Haploinsufficiency for type I collagen, caused by a null mutation, is well known to cause the full clinical range of OI (3, 4). Defects in type I collagen are indirectly responsible for OI/EDS collagen mutations, which are directly responsible for the bone fragility of OI and indirectly responsible for OI/EDS collagen mutations.

Mutations Near Amino End of α1(I) Collagen Cause Combined Osteogenesis Imperfecta/Ehlers-Danlos Syndrome by Interference with N-propeptide Processing*

Received for publication, December 30, 2004, and in revised form, February 18, 2005 Published, JBC Papers in Press, February 22, 2005, DOI 10.1074/jbc.M414698200

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¶ The abbreviations used are: OI, osteogenesis imperfecta; EDS, Ehlers-Danlos syndrome; DMEM, Dulbecco’s modified Eagle’s medium; RT, reverse transcriptase.

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in exon 6 is intact. Thus, OI/EDS and EDS VII are shown to have a common mechanism for EDS symptoms.

** EXPERIMENTAL PROCEDURES **

**Cell Culture—**Skin fibroblast cultures were established from dermal punch biopsies. Fibroblasts were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum and 2 mM glutamine in the presence of 5% CO₂. Osteoblast primary cultures were established from surgical bone chips using the method of Robey and Termine (29). In brief, osteoblasts were released from bone chips by digesting for 2 h at 37 °C with 0.3 units/ml collagenase P in serum-free medium, and grown in 45% low-calcium DMEM, 45% low-calcium Ham’s F-12 Kinease medium (Biofluids, Rockville, MD), 25 µg/ml ascorbate, and 10% fetal bovine serum in the presence of 8% CO₂.

**Steady State Collagen Synthesis—**To label procollagens, confluent fibroblast cultures of probands and control cells (ATCC 2127, American Type Culture Collection, Manassas, VA) were incubated for 2 h in serum-free medium containing 50 µg/ml ascorbic acid, followed by incubation with 260 µCi/ml of 3.96 TBq/mmol [3H]proline for 24 h. Procollagens were harvested from media and cell layer and precipitated with ammonium sulfate; collagens were precipitated by peptide digestion (50 µg/ml) of procollagen samples, as previously described (30).

**Mutation Identification—**Total RNA was isolated from cultured fibroblasts of patient and control cell lines using TRIReagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer’s directions (31). The region of the α1(I) collagen mRNA corresponding to exons 5–12 was amplified by reverse transcription-polymerase chain reaction (RT-PCR). Total RNA (1 µg) was reverse transcribed with 50 units of murine leukemia virus RT (Applied Biosystems, Foster City, CA) using an antisense primer complementary to nucleotides 813–842 of the cDNA sequence (GenBank™ AF017178) in exon 12 (5′-CCAGCAGGACACGATCTCCCTTGACACCA-3′). The RT reaction was used as a template for PCR with a sense primer corresponding to nucleotides 377–406 of cDNA sequence and located in exon 5 (5′-CTCGCGCAAGATGGGACGACACCTGCT-3′). PCR used 0.1 mM dNTP, 2.5 units of AmpliTaq, and 1× PCR Buffer II (Applied Biosystems). PCR cycling conditions were as follows: 94 °C for 5 min; then 35 cycles of 1 min at 94 °C, 1 min at 65 °C, and 1.5 min at 72 °C; and finally 7 min at 72 °C. RT-PCR products were sequenced directly on a Beckman Coulter CEQ2000 DNA Sequencer (Beckman Fullerton, CA) according to the manufacturer’s protocol.

**Pericellular Processing—**Processing of procollagens secreted by fibroblasts was examined by labeling confluent cells from probands and control with 260 µCi/ml of 3.96 TBq/mmol [3H]proline for 24 h and then replacing the media with DMEM containing 2 mM non-radioactive proline and 10% fetal bovine serum. Media from independent wells were harvested at 24-h intervals over a 5-day period as previously described (25). Media procollagen samples from fibroblasts were precipitated with ammonium sulfate and electrophoresed on 6% polyacrylamide–urea–SDS gels.

**Matrix Deposition—**Proband and control fibroblasts and osteoblasts were grown to confluence and stimulated every other day for 11 days (fibroblasts) or 9 days (osteoblasts) with fresh DMEM containing 10% fetal bovine serum and 100 µg/ml ascorbic acid. Cultures were then incubated for 24 h with 260 µCi/ml of [3H]proline in serum-free medium. Medium was collected and procollagen samples were precipitated with ammonium sulfate. Matrix collagens were serially extracted at 4 °C as previously described (32). Matrix collagen samples from fibroblasts were precipitated with ammonium sulfate and electrophoresed on 6% polyacrylamide–urea–SDS gels.

**Transmission Electron Microscopy of Proband Dermal Fibrils—**A dermal biopsy was obtained from each proband and from a control matched for age and race. The samples were fixed in 2.5% glutaraldehyde and then treated with 1% osmium tetroxide followed by en bloc staining with 2% uranyl acetate. After dehydration, the tissue was infiltrated with Spurr’s plastic resin. 600–800-Å sections were obtained with an AE Reichert Ultracut ultracmicrotome mounted on copper grids and stained with lead citrate. The stained grids were examined in a Zeiss EM10 CA transmission electron microscope and representative areas were photographed (JFE Enterprises, College Park, MD).

**RESULTS**

**Phenotype of OI/EDS Patient Group—**The seven probands in this study have a distinct OI/EDS phenotype (Table I). All seven first came to medical attention for symptoms of osteogenesis imperfecta. All have types III or IV OI, with multiple fractures of long bones; the children with G25V, G76E, and G88E had bone deformity sufficient to require osteotomy procedures. Their L1–L4 DEXA z-scores range from −3.0 to −5.2. All have the significant short stature of OI and a height age that ranges from 20 to 80% of the mean height for their chronological age. Also characteristic of the relatively shorter lower extremities in OI, most have arm span significantly greater than length. All probands have strikingly blue sclerae.

The symptoms of EDS are notably more severe than the mild to moderate joint hyperextensibility frequently found in OI. In addition to significant hyperextensibility of large and small joints (Fig. 1), these probands have laxity of paraspinal ligaments. This results in early scoliosis without vertebral compressions. The scoliosis is rapidly progressive and unresponsive
to bracing. Spinal fixation has been required by the mid-teenage years.

Collagen Biochemistry and Mutation Detection—Because the probands have clinically significant osteogenesis imperfecta, we examined the type I collagen synthesized by their dermal fibroblasts electrophoretically on SDS-urea-PAGE. Proband steady state media and cell layer collagen did not have delayed electrophoretic migration, as would be expected with the well known overmodification of type I collagen chains frequently seen in OI (Fig. 2A). Proband 1 had a leading edge in both media and cell layer α1(I) bands and proband 2 had a leading edge in the α2(I) band isolated from the cell layer.

The normal collagen electrophoresis results prompted us to screen these patients for collagen mutations at the amino end of the helical region of either α1(I) or α2(I), which would not be expected to cause overmodification. Direct sequencing of RT-PCR products spanning exons 5–12 of both α1 chains revealed that all 7 probands had mutations located in exons 7–11 of COL1A1 (Fig. 2B), causing structural abnormalities in the 90 residues at the amino end of the α1(I) protein chain. Proband 1 has a mutation (IVS7+4 A > T; g.3756A>T) that causes skipping of exon 7 from the mutant transcript. The leading edge seen after pepsin digestion on SDS-PAGE is most likely the consequence of normal length α2(I) chain looping out of helices containing a shorter mutant α1(I) chain(s). The remaining 6 probands are heterozygous for glycine substitution mutations.

### Table I

**Clinical characteristics of OI/EDS subjects**

| OI characteristics | EDS characteristics |
|--------------------|--------------------|
| **Sex** | **CA** | **OI type** | **HA** | **Proportions** | **DEXA Z** | **Sclera** | **Beighton score** | **Scoliosis (degree)** | **Spinal fixation** |
| **Sex** | **CA** | **OI type** | **HA** | **Proportions** | **DEXA Z** | **Sclera** | **Beighton score** | **Scoliosis (degree)** | **Spinal fixation** |
| Exon 7 skipping | M | 23 | IV | 12.3 | US: LS | 1:1.34 | 1:0.91 | −4.9 | 4 | Large joints | Severe | Braced |
| G13D | F | 15.75 | IV | 10 | 1:1.34 | 1:0.91 | −4.9 | 4 | Large joints | Severe | Braced |
| G25V | F | 18 | IV | 4.25 | 1:1.8 | 1:0.69 | −4.3 | 4 | 9 | 50 | 9 |
| G25V | F | 2.9 | IV | 1.5 | 1:1 | 1:1 | −4.3 | 4 | 9 | 50 | 9 |
| G34R | M | 0.6 | III/IV | 0.6 | 1:1.79 | 1:0.73 | −5.2 | 3 | 5 | 35–40 | None |
| G76E | F | 16.2 | III | 3 | 1:0.79 | 1:0.73 | −3.8 | 3 | 7 | 50 | 16 |
| G88E | F | 23 | III/IV | 9.5 | 1:0.73 | 1:0.73 | −3.0 | 3 | 7 | 50 | 16 |

**a** Chronological age (years).

**b** Height age (years); age for which the height is at the 50th percentile.

**c** Upper segment (trunk, crown to pubis) to lower segment (legs, pubis to heel) ratio.

**d** Arm span to height ratio.

**e** Age matched z-score (S.D. units) for bone density at the lumbar spine (L1–L4).

**f** Sclerae are scored on a scale from 1 (tinge blue) to 4 (dark blue).

**g** Beighton score of joint hyperextensibility ranges from 1 to 9; (a) passive apposition of the thumb to the flexor aspect of the forearm (1 point each thumb), (b) passive dorsiflexion of the fifth finger beyond 90° (1 point each hand), (c) hyperextension of the elbow beyond 10° (1 point each elbow), (d) hyperextension of the knee beyond 10° (1 point each knee), (e) forward flexion of the trunk with knees fully extended so that palms of the hands rest on the floor (1 point).

**h** Degree of thoracolumbar scoliosis as measured on AP radiograph.

**i** Patient has had 2 fusion/fixation surgeries with hardware placement. The first (9/2001) occurred after the curve reached 45°. Hardware failure occurred 4 months later; it was removed in 1/2002. A subsequent fusion with fixation and graft was completed in 11/2002, after the curve had progressed to 90°. At this time, the second fusion/fixation is holding.

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**Fig. 1. Hyperextensibility component of α1(I) OI/EDS.** a and b, large (hips) and small joint hyperextensibility of proband with G13D mutation. Note the orthotic rings on the fingers in b to prevent hyperextension and dislocation of the interphallangeal joints. c and d, small joint hyperextension of probands with G76E (c) and G88E (d) mutations.
tions, located at Gly13, Gly25 (2 patients), Gly34, Gly76, and Gly88, respectively. The G13D substitution presumably disrupts the collagen helix sufficiently to expose the amino end of the α2(I) chain to pepsin digestion, generating the leading edge seen on the α2(I) band. Only RT-PCR products of the expected size were obtained from both α1(I) and α2(I) transcripts in the G13D case; sequencing ruled out a second mutation or use of a cryptic splice signal.

**FIG. 2.** *Steady-state collagen and mutation sequences in α1(I) OI/EDS.* a, Type I collagen synthesized by proband dermal fibroblasts was harvested separately from media and cell layer and electrophoresed on SDS-urea-PAGE. Lane numbers in panel a correspond to proband numbers in panel b. Arrows indicate the fast migrating component of α2(I) band in cases with mutations in α1(I) exon 7. Unfolding at the extreme amino end of the helix presumably exposes a protease-sensitive site in α2(I). b, Sequencing of proband fibroblast cDNA delineated heterozygous mutations in exons 7–11 of the α1(I) chain in all seven probands, as indicated.
Because of random association of pro-
N-proteinase cleavage site in exon 6 is intact in all cases.

by purified N-proteinase (Fig. 3), although the sequence of the
in vitro

nal Processing—

The mutations causing OI/EDS interfere with

processing of the N-propeptide of proband procollagen
in fibroblast matrix, but about one-third of total
pN-collagen is greater in the matrix deposited by cultured
osteoblasts than fibroblasts in the four probands for whom
osteoblasts were available (G13D, G25V, G76E, and G88E).

For the G88E mutation, very little pN-collagen was detected
pN form in osteoblast matrix. The matrix deposition assays
also demonstrate that pN-collagen is efficiently cross-linked
into matrix, rather than accumulating in the neutral salt
extract.

Reduced Cross-section of Dermal Fibrils Corroborates pN-
collagen Incorporation into Matrix in Vivo—In dermal fibrils
from patients with EDS VII, the incorporation of pN-collagen
into the fibril has been shown to result in the reduction of the
fibril diameter (38), most likely by steric hindrance. Examin-
a tion of dermal fibril diameter in our OI/EDS probands provides
indirect evidence that pN-collagen is incorporated into OI/EDS
matrix in vivo. The diameters of the dermal fibrils of all the
OI/EDS probands are significantly smaller than those of age-
matched control fibrils (Fig. 5 and Table II). In all probands
except one, the fibrils have about 1⁄3 the diameter of control
fibers (Fig. 5 and Table II). In all probands except one, the fibrils have about 1⁄3 the diameter of control
fibers. The fibrils with G76E mutant collagen are closer to
normal diameter (~75%), but are still significantly smaller.
Very few cauliflower forms were seen in OI/EDS fibrils. Fur-

Amino End α1(I) Collagen Mutations Cause OI/EDS

Mutations Interfere with in Vitro and Pericellular NH2-termi-
nal Processing—The mutations causing OI/EDS interfere with
in vitro processing of the N-propeptide of proband procollagen
by purified N-proteinase (Fig. 3), although the sequence of the
N-proteinase cleavage site in exon 6 is intact in all cases.
Because of random association of pro-α chains during procolla-
gen assembly, heterozygous patients generate 25, 50, and 25%,
respectively, of procollagen molecules containing two, one, and
no mutant pro-α1(I) chains. From probands heterozygous for
mutations in exon 7 (ΔE7 and G13D), only about 25% of pro-
α1(I) chains were processed in vitro. Apparently, these muta-
tions prevent processing of the N-propeptide from all helices
that contain one or two copies of the mutant pro-α1(I) chain,
and only the 25% of helices with two normal pro-α1(I) chains
could be processed. The procollagens with mutations in exons
8–11 (G25V, G34R, and G76E) underwent 70–88% cleavage,
suggesting that helices with one or two normal pro-α1(I) chains
were processed, whereas the 25% of helices containing two
mutant chains could not be cleaved. The procollagen containing
the G88E substitution was completely cleaved, but at a reduced
rate compared with normal procollagen. Mutations located
more distal to the cleavage site, at G121D and G136R, had
complete in vitro cleavage with normal kinetics.

We also examined the conversion of procollagen to collagen by
the pericellular processing enzymes in a cell culture assay, by
following the conversion of a pulse of [3H]procollagen to collagen
over 5 days (data not shown). The procollagen secreted by all
seven of the OI/EDS probands showed delayed processing to
collagen by the pericellular enzymes, consistent with the in vitro
processing results. In comparison with processing of normal con-
trol procollagen, amino propeptide processing is substantially
delayed in probands 1 and 2 (exon 7 mutations), delayed to a
lesser extent in probands 3 and 5 (exon 8 mutations), and only
modestly delayed in probands 6 and 7 (exon 11 mutations).

pN-collagen Deposited in Matrix in Fibroblast and Osteo-
blast Cultures—The pN-collagen present in the media of cul-
tured fibroblasts and osteoblasts is incorporated into the
matrix deposited by those cells (Fig. 4). In each proband, we
see a substantial increase of pN-collagen in these fractions,
as compared with control. For mutations in exon 7 (ΔE7 and
G13D), the amount of pN-α1(I) is equivalent to the amount of
fully processed α1(I) chain. For mutations in exon 8 (G25V
and G34R), pN-α1(I) in fibroblast extracts is about 20–30% of
the amount of fully processed α1(I). Mutations further from
the amino end of the collagen helix (G76E and G88E) have
relatively less pN-α1(I) in the fibroblast matrix. Two glycine
substitution mutations beyond the first 90 residues of the
chain, at G121D and G136R, do not have levels of pN-collagen
greater than control. It is noteworthy that the proportion of
pN-collagen is greater in the matrix deposited by cultured
osteoblasts than fibroblasts in the four probands for whom
osteoblasts were available (G13D, G25V, G34R, and G88E).

For the G88E mutation, very little pN-collagen was detected
in fibroblast matrix, but about one-third of total α1(I) was in
pN form in osteoblast matrix. The matrix deposition assays
also demonstrate that pN-collagen is efficiently cross-linked
into matrix, rather than accumulating in the neutral salt
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Reduced Cross-section of Dermal Fibrils Corroborates pN-
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normal diameter (~75%), but are still significantly smaller.
Very few cauliflower forms were seen in OI/EDS fibrils. Fur-

FIG. 3. N-propeptide cleavage in vitro. In vitro N-propeptide cleavage by purified bovine N-proteinase. Open and filled symbols in each graph represent normal control and mutant protein co-processed as described under "Experimental Procedures." The error bars represent S.D. from two to four experiments. Measurements were corrected for unprocessed type III collagen, which may introduce an additional error of up to ~5%.
Therefore, the borders of their fibrils were regular, unlike the irregular margins frequently seen in EDS VII.

**Calorimetry Demonstrates Disruption of a Distinct Folding Region**—Differential scanning calorimetry provides insight into the structural basis of OI/EDS. Typical DSC tracings (thermograms) for procollagen and collagen are illustrated in Fig. 6A for the control protein and two classical type IV OI mutations (G121D and G136R), which do not result in N-propeptide retention or cause EDS symptoms. The thermograms of normal procollagen and collagen each have a single peak of protein denaturation. Mutations in the helical region of collagen may result in one or two additional DSC peaks with reduced stability, representing denaturation of molecules with one and two mutant chains, respectively, in addition to the normal peak for molecules with no mutant chains. However, regardless of their specific shape, thermograms for each procollagen and the corresponding collagen are usually identical, because the propeptides do not contribute to overall stability of normal and most mutant molecules.

In contrast, the DSC of the OI/EDS probands is distinctive, with procollagen and collagen tracings that differ from each other: (i) a single procollagen peak in ΔE7 and G13D splits into normal and reduced stability collagen peaks; (ii) the reduced stability procollagen peaks in G25V and G34R shift to lower temperature in collagen; (iii) an additional low-temperature peak appears in the collagen of G76E; and (iv) the thermograms of G88E procollagen and collagen have a reciprocal change in the heights of the low and normal stability peaks.

This difference in thermal stability with and without N-propeptide suggests that mutations in the first 90 residues at the amino end of the collagen helix disrupt a distinct folding region that anchors the collagen triple helix (Fig. 7, A and B). The presence of the N-propeptide in these mutant molecules compensates for the loss of helical stability, resulting in procollagen that is more stable than collagen. The anchor region is bordered by a low-stability microunfoldling region (Fig. 6B); mutations beyond the first 90 residues at the N-proteinase cleavage site are intact. This abnormality was detected in both pericellular assays and in vitro with purified proband procollagen and N-proteinase. In vitro, mutations closest to the amino end in exon 7 prevented N-propeptide cleavage from 3/4 of procollagen molecules, presumably those containing one or two mutant chains. Mutations further from the N-proteinase site (exons 8–11) prevented N-propeptide cleavage from 1/4 to 1/10 of secreted procollagen, most likely only molecules containing two mutant chains. The most remote OI/EDS mutation at the end of exon 11 (G88E) delayed the kinetics of propeptide removal.

We demonstrated that the resulting pN-collagen is incorporated into matrix deposited by cultured fibroblasts and osteoblasts of the probands. The amount of pN-collagen seen in the matrix deposited by fibroblasts is consistent with the in vitro processing results; mutations with impaired processing of a fraction of the α1(I) chains have a greater proportion of pN-α1(I) in matrix than do mutations with a simple delay in processing. Interestingly, osteoblast matrices always have more pN-collagen than the corresponding fibroblast matrix. This is most dramatically seen in the case of G88E, in which at least one-third of total osteoblast α1(I) is in the pN-form and
Collagen dermal fibrils from OI/EDS probands compared with age-matched controls. OI/EDS fibrils have smaller diameters than do controls. Fibril borders of OI/EDS probands were regular; only a few cauliflower forms were detected. Size distribution of 200 fibril diameters is plotted in the right-hand column.
very little pN-collagen is seen in the fibroblast matrix. This difference between fibroblast and osteoblast matrices may reflect the lower activity of N-proteinase in bone than skin tissue (39). It may also reflect the relatively greater modification of osteoblast than fibroblast collagen (40) and the slower cleavage of the conformation-dependent cleavage site that is known to occur with overmodified collagen even in the absence of a collagen structural mutation (41). We also present evidence strongly supportive of pN-collagen incorporation into fibrils in vivo. The dermal fibrils of all seven probands have a strikingly smaller diameter than those of age-matched controls. This is similar to the findings in fibrils of EDS VIIA and -B patients who are known to have pN-collagen in fibrils (13–15, 22, 23), although the α1(I) OI/EDS probands do not have fibrils with irregular borders.

The OI/EDS region has a structural basis. Analysis of collagen amino acid sequence (Fig. 6B) shows that the first 85 residues form a highly stable region that serves as the anchor for the amino end of the collagen triple helix. On its carboxyl end, it is bordered by a microunfold region of 15 residues containing no proline or hydroxyproline and a chymotrypsin-sensitive site (42). The DSC data presented here indicate that mutations in the first 90 amino acids disrupt the N-anchor region by destabilizing the NH₂-terminal end of the triple helix and cause structural changes that extend into the propeptide. These structural changes are a distinct feature of OI/EDS. They are most striking for mutations closer to the extreme amino end of the helix and become more subtle as mutations approach the microunfold region. We did not observe similar effects for G121D and G136R as well as several other glycine substitutions beyond the first 90 residues (data not shown).

![Figure 6](image)

**Fig. 6. Structural basis of OI/EDS region.** A, differential scanning calorimetry (DSC) thermograms of procollagen and collagen peaks on each tracing correspond to apparent denaturation temperatures. All three possible peaks for molecules with no, one, and two mutant α1(I) chains are clearly distinguishable in the G76E thermogram. In other thermograms two or all three peaks strongly overlap and become indistinguishable. B, amino acid sequence of the amino-terminal triple helical fragment of human collagen. Proline (P) and hydroxyproline (O) residues required for triple helical stability are underlined and highlighted in green. Based on their content, the first 85 residues are expected to form a stable triple helix that anchors the amino end of the molecule. The 15-amino acid stretch on the carboxyl end of this N-anchor (highlighted in yellow) contains no proline or hydroxyproline and is expected to be highly flexible. It is homologous to a known microunfold region in mouse and rat tail tendon collagens (42).

| Proband | Proband fibril diameter | Control fibril diameter |
|---------|-------------------------|-------------------------|
| α1(I) G13D | 61.0 ± 5.0 a | 94.2 ± 9.3 |
| α1(I) G25V | 50.9 ± 5.8 a | 81.8 ± 7.8 |
| α1(I) G25V | 50.4 ± 5.1 a | 81.6 ± 10.1 |
| α1(I) G34R | 59.1 ± 5.3 a | 81.6 ± 10.1 |
| α1(I) G76E | 70.7 ± 5.6 a | 81.8 ± 7.8 |
| α1(I) G88E | 67.3 ± 6.6 a | 106.9 ± 10.2 |

* Denotes p < 0.001.
Their processing, matrix deposition, and DSC were typical for type I collagen helical mutations causing OI. Apparently, the flexible microunfolding region prevents propagation of structural defects caused by such mutations into the N-anchor. Thus, the mutations that are responsible for an OI/EDS phenotype play both direct and indirect roles. As collagen structural mutations, they are directly responsible for the OI symptoms of the probands. By interfering with N-propeptide
processing, they are indirectly responsible for the EDS symptoms by a mechanism similar to EDS VIIA and -B (Fig. 7, C–E). In EDS VIIA and -B, however, the retention of the N-propeptide is caused by a deletion of the N-proteinase cleavage site. In OI/EDS, the cleavage site is intact. Instead, resistance to cleavage must be based on unfolding of the high-stability region at the amino end of the helix extending into the propeptide. Cleavage of the N-proteinase site requires an intact helical trimer (43–45) and maintenance of the normal configuration of the site itself, with an 18-amino acid hairpin-loop preceding the short a helix in which cleavage occurs (46). Although the presence of the N-propeptide provides the overall thermal stability detected by DSC, the N-proteinase cleavage site must be in an inaccessible configuration in the OI/EDS procollagens. There is experimental evidence supporting a normal procollagen configuration in which the N-propeptide is folded back over the end of the helical region (47–49). Our data supports unfolding of the N-proteinase cleavage site but does not shed new light on the normal configuration of the N-propeptide itself.

Children with OI/EDS mutations in α1(I) have a distinct phenotype, with joint hyperlaxity and early progressive scoliosis, as well as type III and IV OI symptoms, including fractures, osteoporosis, short stature, and blue sclerae. Within the α1(I) OI/EDS patient group, those with exon 7 mutations have relatively more N-propeptide retention, more severe EDS, and mild to moderate OI, whereas those with exon 11 mutations have less N-propeptide retention, milder EDS, and more severe OI. It is interesting that the OI/EDS symptoms of these patients are different from the clinical presentation of mutations at the amino end of the α2(I) chain. The patients with mutations in α2(I) have a presentation that is similar to EDS VIIA and -B, with severe hyperextensibility of large and small joints and bilateral congenital hip dysplasia (24–28). They have mild manifestations of OI, limited to osteoporosis and blue sclerae. The distinction in phenotype between EDS VII/α2(I) OI/EDS and α1(I) OI/EDS suggests that there is a difference at the level of the collagen fibril or the matrix. It is important to note that the mutations of the two sets are of different types. The EDS VIIA/B and α2(I) OI/EDS cases all involve total or partial loss of an exon or a large duplication. This will result in a shift of register of the mutant chain with respect to the remaining two register of the chains in a trimer with respect to each other. The patients with mutations in α2(I) have a presentation that is similar to EDS VIIA and -B, with severe hyperextensibility of large and small joints and bilateral congenital hip dysplasia (24–28). They have mild manifestations of OI, limited to osteoporosis and blue sclerae. The distinction in phenotype between EDS VII/α2(I) OI/EDS and α1(I) OI/EDS suggests that there is a difference at the level of the collagen fibril or the matrix. It is important to note that the mutations of the two sets are of different types. The EDS VIIA/B and α2(I) OI/EDS cases all involve total or partial loss of an exon or a large duplication. This will result in a shift of register of the mutant chain with respect to the remaining two register of the chains in a trimer with respect to each other.

Other factors that may contribute to the difference in phenotype are the location of the retained N-propeptides in the fibrils and their accessibility to cleavage by tissue proteinases. Holmes and co-workers (38) used scanning transmission electron microscopy to show that N-propeptides in EDS VIIA fibrils were in a bent-back configuration and were located in both gap and overlap zones on the fibril surface. Co-polymers of collagen and pN-collagen maintain a circular cross-section with resistance to mechanical shearing. Investigation of the structure and location of the retained N-propeptide in α1(I) versus α2(I) OI/EDS fibrils and its impact on tissue function will provide further insight into phenotype mechanisms.

Acknowledgments—J. C. M. and A. D. L. thank Drs. Gerber, Paul, and Cintas of the National Institutes of Health Clinical Center Department of Rehabilitation Medicine for productive discussions of joint laxity and scoliosis in OI. We are grateful to the NICHD patients (Gly13, Gly25, Gly25, Gly76, and Gly88) and their parents for long-standing and dedicated support of OI research. We acknowledge Drs. James Kooner and David Procop (Gene Therapy Center, Tulane University) for detection of the Gly34 mutation and the second mutation at Gly25. Dr. Jess Thoene (Human Genetics Program, Tulane University School of Medicine) referred the patient with the Gly34 mutation and facilitated sample procurement.

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