Bethlem Myopathy and Engineered Collagen VI Triple Helical Deletions Prevent Intracellular Multimer Assembly and Protein Secretion*

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Mutations in the genes that code for collagen VI subunits, COL6A1, COL6A2, and COL6A3, are the cause of the autosomal dominant disorder, Bethlem myopathy. Although three different collagen VI structural mutations have previously been reported, the effect of these mutations on collagen VI assembly, structure, and function is currently unknown. We have characterized a new Bethlem myopathy mutation that results in skipping of COL6A1 exon 14 during pre-mRNA splicing and the deletion of 18 amino acids from the triple helical domain of the a1(VI) chain. Sequencing of genomic DNA identified a G to A transition in the 1 position of the splice donor site of intron 14 in one allele. The mutant a1(VI) chains associated intracellularly with a2(VI) and a3(VI) to form disulfide-bonded monomers, but further assembly into dimers and tetramers was prevented, and molecules containing the mutant chain were not secreted. This triple helical deletion thus resulted in production of half the normal amount of collagen VI. To further explore the biosynthetic consequences of collagen VI triple helical deletions, an a3(VI) cDNA expression construct containing a 202-amino acid deletion within the triple helix was produced and stably expressed in SaOS-2 cells. The transfected mutant a3(VI) chains associated with endogenous a1(VI) and a2(VI) to form collagen VI monomers, but dimers and tetramers did not form and the mutant-containing molecules were not secreted. Thus, deletions within the triple helical region of both the a1(VI) and a3(VI) chains can prevent intracellular dimer and tetramer assembly and secretion. These results provide the first evidence of the biosynthetic consequences of structural collagen VI mutations and suggest that functional protein haploinsufficiency may be a common pathogenic mechanism in Bethlem myopathy.

Bethlem myopathy is a mild dominantly inherited disorder characterized by early childhood onset of generalized muscle weakness and wasting and, commonly, contractures of multiple joints (1, 2). Mutations resulting in Bethlem myopathy have recently been identified in three genes, COL6A1, COL6A2, and COL6A3, that code for subunits of the extracellular matrix protein collagen VI (3–5). The constituent collagen VI chains, a1(VI), a2(VI), and a3(VI), each contain a central triple helix-forming domain of repeating Gly-X-Y sequences, flanked by large N- and C-terminal regions primarily composed of ~200-amino acid subdomains that have homology to von Willebrand factor type A domains (6–8). Stable collagen VI monomers are formed when the three chains associate intracellularly and assemble into disulfide-bonded helical heterotrimers (9). However, in contrast to other collagens, these molecules are not secreted but assemble further within the cell into antiparallel overlapping dimers and then tetramers, which are stabilized by intermolecular disulfide bonds. Following secretion, tetramers link end-to-end to form characteristic beaded microfibrils (10, 11).

Three of the described Bethlem myopathy mutations are glycine substitutions within the triple helix of the a1(VI) and a2(VI) chains that interrupt the collagen Gly-X-Y amino acid repeat sequence (3), whereas a fourth mutation leads to a glycine to glutamic acid change within N2, one of the 10 N-terminal type A domains of the a3(VI) chain (4). However, the effect of these mutations on collagen VI biosynthesis, molecular assembly, and structure has not been determined. In the only patient in which the disease mechanism has been identified, Bethlem myopathy resulted from protein haploinsufficiency (5). A single base deletion in the a1(VI) mRNA introduced a downstream premature stop codon. The mutant mRNA was subjected to nonsense-mediated mRNA decay and was absent from patient fibroblasts and muscle. Reduced synthesis of a1(VI) chains limited the amount of collagen VI that could be assembled intracellularly into triple-helical molecules (a1(VI), a2(VI), and a3(VI)), and ultimately led to a matrix containing reduced amounts of structurally normal collagen VI.

In this study we have characterized a Bethlem myopathy mutation in the donor splice site of COL6A1 intron 14 that results in skipping of exon 14 during pre-mRNA splicing and the deletion of 18 amino acids from the triple helical domain of the a1(VI) chain. In addition, we have produced an a3(VI) cDNA expression construct containing a 202-amino acid deletion within the helical domain and stably expressed this construct in SaOS-2 cells. Biosynthetic analyses of the mutant collagen VI demonstrated that in contrast to the fibrillar collagens where molecules containing chains with deletions in the triple helical domain can be secreted and exert a severe dominant negative effect in the extracellular matrix, these collagen VI deletions interfere with formation of the precise multimeric structures critical for secretion of collagen VI and result in production of reduced amounts of functional collagen VI. These data provide the first evidence of the biosynthetic consequences of structural collagen VI mutations and suggest that functional
protein haploinsufficiency may be a common pathogenic mechanism in Bethlem myopathy.

**EXPERIMENTAL PROCEDURES**

**Clinical Summary**—The patient is a 32-year-old man. He and other members of his family have similar clinical histories of early onset slowly progressive muscle weakness in a limb-girdle distribution, with contractures of the ankles, elbows, and interphalangeal joints of the hands. Respiratory, cardiac, and facial muscles were normal. Bethlem myopathy was diagnosed based on the autosomal dominant family history and slowly progressive limb-girdle myopathy with prominent joint contractures.

**Production of Normal and Mutant α3(VI) cDNA Expression Constructs**—An α3(VI) expression construct containing protein domains N9–C5 (Fig. 1) was prepared by ligating previously characterized partial cDNA clones (6, 9). A 1.8-kilobase SacI-BstHI fragment encoding the signal peptide and domains N9, N8, and part of N7 was excised from clone FO19 and ligated into pGEM1zf+ (Promega). An 8-kilobase BamHI fragment encoding the remaining α3(VI) cDNA domains, N7–C5 was then inserted into the BamHI site of this subclone and a plasmid containing the insert in the correct orientation identified by digestion with XbaI. The entire α3(VI) cDNA was excised from pGEM1zf+ by cleavage at the S' SacI and N' NotI polynucleotide sites and ligated into the mammalian expression vector pCI-neo (Promega), which also contains the neomycin phosphotransferase gene conferring resistance to the antibiotic G418. The resulting expression construct, α3(VI) N9–C5, encoded the signal sequence, protein domains N9–C5 and the 3′-untranslated region and polyadenylation sequence (Fig. 1b).

To produce an α3(VI) expression construct containing a deletion of triple helical sequences, a 6.3-kilobase HpaI-Bst1107I fragment was subcloned into a Smal-cut pUC19 vector so that the SacII and NcoI sites within the insert would be unique. The plasmid was digested with NcoI, which cuts within the region coding for the triple helix, and the restriction site overhang was filled in with FdII DNA polymerase (Stratagene), and ligated with blunt-ended SacII linkers (Stratagene). The DNA was digested with SacII, which cuts toward the 5′ end of the triple helix in addition to the site within the synthetic linker, releasing a fragment of approximately 400 base pairs. The resultant larger band, which included pUC19 and 5.9-kilobases of α3(VI) cDNA, was gel purified and circularized. Individual clones were cycle sequenced (AmpliCycle™, Perkin-Elmer) using the primer 5′-AGAAAGCTTGCCTGTTGGGGTT-3′ corresponding to bases 5708–5727 of the α3(VI) cDNA (domains N9–C5, ATG at base 256 (6)). None of the clones contained the expected 399-base pair deletion; however, one clone contained a larger deletion of 606 base pairs that would result in the deletion of amino acids 6–207 of the α3(VI) triple helix. The 5.5-kilobase PmlI fragment of plasmid α3(VI) N9–C5 was replaced with the corresponding fragment containing the triple helical deletion to produce the expression plasmid α3(VI) N9–C5 Δh (Fig. 1c).

**Cell Culture and Transfection**—Human dermal fibroblasts were established from skin biopsies (12), and the human osteosarcoma cell line, SaOS-2 (13, 14) (ATCC HTB-85) was obtained from American Type Culture Collection. Cell cultures were maintained in Dulbecco's modified Eagle's medium containing 10% (v/v) fetal calf serum as described previously (12). SaOS-2 cells were transfected with the α3(VI) cDNA expression constructs using LipofectAMINE reagent (Life Technologies, Inc.) according to the manufacturer's protocol. Stably transfected cells were selected in growth medium containing 500 μg/ml G418 (Life Technologies, Inc.), and individual G418-resistant colonies were isolated and expanded into cell lines. G418 was removed from the culture medium after the fourth passage.

**COLLAGEN VI BIOSYNTHETIC LABELING AND ANALYSIS**—Primary skin fibroblasts and SaOS-2 cells were grown to confluence in 10-cm² dishes, incubated overnight in the presence of 0.25 mM sodium ascorbate, and then biosynthetically labeled for 18 h with 100 μCi/ml [35S]methionine (Translabel™ 1032 Ci/mmol, ICN Pharmaceuticals, Inc.) in 750 μl of methionine-free and serum-free Dulbecco's modified Eagle's medium containing 0.25 mM sodium ascorbate. The medium was removed to a sterile tube, and protease inhibitors were added to the following final concentrations: 1 mM phenylmethylsulfonyl fluoride, 20 mM N-ethylmaleimide, and 5 mM EDTA. The cell layer was solubilized in 50 mM Tris/HCl, pH 7.5, containing 5 mM EDTA, 150 mM NaCl, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, and 20 mM N-ethylmaleimide (cell lysis buffer). Cell lysates and medium samples were clarified by centrifugation, made up to 800 μl by weight with cell lysis buffer, and passed through a 0.45-μm filter (Millipore). Fibronectin, which co-immunoprecipitates with collagen VI and co-migrates with the α3(VI) chain on SDS-polyacrylamide gels, was removed by gelatin-Sepharose chromatography (Amersham Pharmacia Biotech). The column buffer was 50 mM Tris, pH 7.5, containing 150 mM NaCl, 5 mM EDTA, and 0.1% Nonidet P-40 (NET buffer). Collagen VI in the column flow-through was immunoprecipitated overnight at 4°C using a specific collagen VI antibody (Life Technologies, Inc.) (9) and 100 μl of 20% protein A-Sepharose (Amersham Pharmacia Biotech). The protein A-Sepharose beads were washed twice with NET buffer and then once with 10 mM Tris/HCl, pH 7.5, 0.1% Nonidet P-40 for 30 min each. Immunoprecipitated collagen VI was eluted into gel loading buffer at 65°C for 15 min and analyzed following reduction with 25 mM dithiothreitol by SDS-polyacrylamide gel electrophoresis on 5% (w/v) polyacrylamide gels. Collagen VI triple helical monomers, dimers, and tetramers were analyzed on 2.4% (w/v) acrylamide/0.5% (w/v) agarose composite gels under nonreducing conditions as described previously (5, 9). Radioactively labeled proteins were detected by fluorography (12) or imaged using a PhosphorImager (Molecular Dynamics, STORM™).

**RNA and DNA Isolation, Polymerase Chain Reaction, and Sequencing**—RNA was isolated from cultured fibroblasts using RNeasy™ (QIA-GEN). Total RNA (1 μg) was used for reverse transcription with an oligo(dT) primer followed by PCR (1) (Perkin-Elmer GeneAmp®). COL6A1 cDNA spanning bases 730–1582 (ATG at base 49 (3)) was amplified and either cycle sequenced directly (AmpliCycle™, Perkin-Elmer) or cloned into a Smal cut pGEM11zf+) vector and cycle sequenced; labeling was with [α-32P]dATP (2000 Ci/mmol, NEN Life Science Products). Genomic DNA was isolated from cultured fibroblasts. COL6A1 genomic DNA spanning exons 13–15 (15, 16) was amplified using primers corresponding to cDNA bases 1006–1125 and 1105–1124, and intron 14 was partially sequenced using a primer located within exon 14 (cDNA bases 1051–1070). Based on this sequence, a further sequencing primer within intron 14 (5′-CAGCAGGACCCGACACC-3′) was used to directly sequence across the donor splice site of intron 14, exon 14, and the acceptor splice site of intron 13.

The abbreviations used are: PCR, polymerase chain reaction; RT, reverse transcription.
Bethlem myopathy collagen VI was present as disulfide-bonded monomers (lane 3).

**RESULTS**

**Bethlem Myopathy Fibroblasts Produce Structurally Abnormal Collagen VI**—Because collagen VI is expressed not only in skeletal muscle, the main tissue affected in Bethlem myopathy but is also an abundant product of skin fibroblasts (17), which are readily accessible, control and patient fibroblasts were labeled overnight with [35S]methionine, and the collagen VI in the cell (C) and medium (M) fractions was immunoprecipitated and analyzed under reducing conditions on a 5% polyacrylamide gel (a) or without reduction on a composite 0.5% agarose-2.5% acrylamide gel (b). The migration positions of the individual collagen VI subunits a1(VI), a2(VI), and a3(VI) are indicated on the right in a, and the 200- and 97-kDa molecular mass standards on the left. In addition to normally migrating collagen VI chains, the Bethlem myopathy cell layer contained a band that migrated faster than the normal a1(VI) and a2(VI) and was not secreted (arrowhead, lane 4). The collagen VI disulfide-bonded triple-helical monomers, dimers, and tetramers are labeled in b. Intracellular Bethlem myopathy collagen VI was present as disulfide-bonded monomers (lane 3). When compared with control cells, Bethlem myopathy fibroblasts also showed reduced secretion of the normally migrating subunits (Fig. 2a, lane 4). This smaller protein was retained entirely within the cell layer and was not secreted into the medium (Fig. 2a, lane 5). When compared with control cells, Bethlem myopathy fibroblasts also showed reduced secretion of the normally migrating subunits (Fig. 2a, lane 4). The usual pathway of collagen VI biosynthesis involves the intracellular assembly of triple helical monomers containing all three chains to form disulfide-bonded dimers (6 chains) and then tetramers (12 chains), which are secreted from the cell and associate end-to-end to form microfibrils in the extracellular matrix (9, 18). Analysis of the collagen VI on nonreducing composite acrylamide-agarose gels demonstrated that in both control and Bethlem myopathy cultures collagen VI tetramers were the major secreted form (Fig. 2b). However, in contrast to the control, the vast majority of the intracellular collagen VI synthesized by Bethlem myopathy cells was present as disulfide-bonded monomers and had not assembled into dimers and tetramers (Fig. 2b, lane 3). Together these data suggested that the smaller mutant collagen VI subunit was able to associate with normal chains to form monomers but that these mutant-containing molecules could not assemble further into multimers and were retained within the cell.

The a1(VI) Chain Contains a Deletion within the Triple Helical Domain—The most likely explanation for the additional protein band in the Bethlem myopathy cells was the presence of a small deletion within the a1(VI) or the a2(VI) chain, and so we searched for such a change by RT-PCR of fibroblast RNA. Amplification of the a1(VI) triple helical domain produced two fragments of equal intensity (1153 and 1097 base pairs) in the Bethlem myopathy samples, whereas only the larger fragment was seen in the control (Fig. 3a). No mutations were detected when the a2(VI) triple helical domain was RT-PCR amplified (data not shown). To characterize the a1(VI) mRNA deletion, individual Bethlem myopathy RT-PCR products were cloned and sequenced (b). No mutations were detected when the a2(VI) triple helical domain was RT-PCR amplified (data not shown). To characterize the a1(VI) mRNA deletion, individual Bethlem myopathy RT-PCR products were cloned and sequenced (b), demonstrating that bases 1051–1104 corresponding to sequences coded by COL6A1 exon 14 were deleted from the mutant product. The cDNA and predicted amino acid sequences of the normal and mutant products are shown in c.

**Fig. 2. Electrophoretic analysis of collagen VI.** Control and Bethlem myopathy (BM) fibroblasts were biosynthetically labeled overnight with [35S]methionine, and the collagen VI in the cell (C) and medium (M) fractions was immunoprecipitated and analyzed under reducing conditions on a 5% polyacrylamide gel (a) or without reduction on a composite 0.5% agarose-2.5% acrylamide gel (b). The migration positions of the individual collagen VI subunits a1(VI), a2(VI), and a3(VI) are indicated on the right in a, and the 200- and 97-kDa molecular mass standards on the left. In addition to normally migrating collagen VI chains, the Bethlem myopathy cell layer contained a band that migrated faster than the normal a1(VI) and a2(VI) and was not secreted (arrowhead, lane 4). The collagen VI disulfide-bonded triple-helical monomers, dimers, and tetramers are labeled in b. Intracellular Bethlem myopathy collagen VI was present as disulfide-bonded monomers (lane 3).

The mRNA Deletion Results from a COL6A1 Splice Donor Site Mutation—Exon skipping is a relatively common finding in inherited diseases and is often caused by point mutations that alter the consensus splice donor or acceptor sequences within the flanking introns (20). To determine the precise nature of the Bethlem myopathy gene mutation, genomic DNA from the patient was PCR amplified using primers within COL6A1 exons 13 and 15 and directly sequenced. The patient was found to be heterozygous for a G → A transition at the +1 position of the intron 14 donor splice site that converts the obligatory GT of the recognition sequence to AT (Fig. 4). This mutation was confirmed by cloning and sequencing individual PCR products (data not shown) and would be predicted to prevent definition of exon 14 during pre-mRNA splicing and result in exon skipping (20).

**Fig. 3. Deletion of exon 14 sequences from the a1(VI) mRNA.** a, RT-PCR amplification of the a1(VI) triple helical domain produced two fragments of equal intensity in the Bethlem myopathy sample (BM), while only the larger fragment was seen in the control (C). The sizes of the normal (1153 base pairs) and deleted fragments (1097 base pairs) are indicated on the right, and the aX174 HaeIII molecular mass markers are shown on the left. Normal and mutant Bethlem myopathy RT-PCR products were cloned and sequenced (b), demonstrating that bases 1051–1104 corresponding to sequences coded by COL6A1 exon 14 were deleted from the mutant product. The cDNA and predicted amino acid sequences of the normal and mutant products are shown in c.

**An Engineered a3(VI) Triple Helical Deletion Also Prevents Collagen VI Dimer and Tetramer Assembly**—To further explore the biosynthetic consequences of collagen VI triple helical deletions and determine whether mutations in other subunits also affected intracellular multimer assembly, an a3(VI) cDNA expression construct encoding protein domains N9–C5 and
containing a 202-amino acid deletion within the triple helix was produced and transfected into SaOS-2 human bone cells. SaOS-2 cells produce α3(VI) and α2(VI) mRNAs at levels comparable with that of skin fibroblasts but are totally deficient in α3(VI) transcription and produce no stable collagen VI protein (9). Normal collagen VI biosynthesis can be restored in SaOS-2 cells by stable transfection with an α3(VI) cDNA expression construct (9), making these cells an ideal model system for expression of α3(VI) chains that have been modified by site-directed mutagenesis. Individual clones transfected with either a control construct (α3(VI) N9–C5) or the deleted mutant construct (α3(VI) N9–C5 Δh) were selected in medium containing G418 and then screened for expression of α3(VI) mRNA by Northern blot (data not shown). Cell lines expressing the highest levels of normal and mutant α3(VI) mRNA were metabolically labeled for 18 h with [35S]methionine, and the collagen VI was immunoprecipitated and analyzed by both SDS-polyacrylamide gel electrophoresis and composite acrylamide-agarose gel electrophoresis and composite acrylamide gel electrophoresis and composite acrylamide gel electrophoresis as before. As previously reported (9), no collagen VI was immunoprecipitated from either the cell or medium fraction of untransfected SaOS-2 cells (Fig. 5a, lanes 2 and 3). In contrast, the α3(VI) N9–C5 chain produced by cells transfected with the control construct associated with the endogenous α1(VI) and α2(VI), rescued them from intracellular degradation, and formed collagen VI assemblies that were efficiently secreted (Fig. 5a, lanes 4 and 5). Mutant α3(VI) N9–C5 Δh chains were also able to associate with endogenous α1(VI) and α2(VI), but these assemblies were almost entirely retained within the cell (Fig. 5a, lanes 6 and 7). Furthermore, analysis of the ability of the collagen VI to form multimeric assemblies (Fig. 5b), showed that although collagen VI tetramers were the major secreted form in control transfected cells (lane 2), the intracellular collagen VI in cells expressing the mutant α3(VI) chains had only assembled into disulfide-bonded monomers (lane 3). Thus deletions within the triple helical region of both the α1(VI) and α3(VI) chains can prevent intracellular dimer and tetramer assembly and secretion of the mutant-containing molecules into the extracellular matrix.

**DISCUSSION**

Analysis of naturally occurring and introduced collagen VI mutations promises to provide important new information about collagen VI molecular assembly, microfibril formation, and function in the extracellular matrix. Although three different collagen VI structural mutations have previously been reported in Bethlem myopathy patients, α1(VI) Gly<sup>256</sup> → Val<sup>2</sup>, α2(VI) Gly<sup>260</sup> → Ser<sup>3</sup>, and α3(VI) Gly<sup>1679</sup> → Glu<sup>4</sup>, the effect of these mutations on collagen VI biosynthesis, assembly, and structure is currently unknown. We have characterized a new Bethlem myopathy mutation in the donor splice site of COL6A1 intron 14 that results in exon skipping and deletion of α1(VI) amino acids 316–333 (residues 79–96 of the triple helical domain). In addition to this naturally occurring mutation, we also expressed α3(VI) chains with an engineered 202-amino acid deletion (residues 6–207 of the triple helix) in transfected cells to comprehensively assess the effect of helix deletions on collagen VI biosynthesis and assembly. Our data clearly show that both the α1(VI) and α3(VI) deletions produce identical biochemical phenotypes; the mutant chains were able to assemble with normal chains into disulfide-bonded monomers, but the mutant-containing molecules were unable to form disulfide-bonded dimers and tetramers and were not secreted. Because stable collagen VI helical monomers only form with the

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**FIG. 4.** Direct sequencing of COL6A1 genomic DNA. Bethlem myopathy genomic DNA was PCR amplified using primers located within exons 13 and 14 and directly sequenced using a primer within intron 14. The sequence of the noncoding strand is shown in a. The arrow indicates the heterozygous mutation at the +1 position of intron 14. No changes to intron 13 splice recognition sequences were present. The sequence of the coding strand is shown in b.

**FIG. 5.** Electrophoretic analysis of collagen VI immunoprecipitated from SaOS-2 osteosarcoma cells. Untransfected SaOS-2 cells and cells transfected with the α3(VI) N9–C5 and α3(VI) N9–C5 Δhelix constructs were biosynthetically labeled overnight with [35S]methionine and the collagen VI in the cell (C) and medium (M) fractions immunoprecipitated and analyzed under reducing conditions on 5% polyacrylamide gels (a) or without reduction on a composite 0.5% agarose-2.5% acrylamide gel (b). The migration positions of the α1(VI) and α2(VI) chains and the transfected α3(VI) N9–C5 (α3(VI)) and α3(VI) N9–C5 Δhelix (α3(VI)Δhelix) are indicated on the right of a. The 200-kDa molecular mass standard (lane 1) is shown on the left. The collagen VI disulfide-bonded triple-helical monomers, dimers, tetramers, and higher order structures (tetramer) are labeled in b.

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2 Amino acids are numbered from the presumed site of signal peptide cleavage.
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Fig. 6. Schematic drawing of a collagen VI dimer. Two collagen VI monomers (gray and black) are associated in an antiparallel fashion with a stagger of 30 nm. The lines represent the triple-helical domains, and the ovals represent globular domains at the C-terminal (COOH) and N-terminal (NH2) ends of the triple helix. The triple helical cysteines (C) important for dimer (α1(VI) or α2(VI)) Cys89 and tetramer (α3(VI) Cys89) stabilization are indicated. The approximate positions of the Bethlem myopathy α1(VI) deletion and the engineered α3(VI) deletion are shown at the bottom. The diagram was adapted from Engel et al. (37) and Chu et al. (22).

Exon-skipping mutations within triple helical domains are relatively common in other collagen diseases such as severe forms of the brittle bone disease osteogenesis imperfecta, which result from type I collagen mutations (23); the cartilage disease Kniest dysplasia; the consequence of type II collagen mutations (24, 25); and Ehlers-Danlos syndrome type IV, where type III collagen is affected (26). In these diseases mutant chains assemble with normal chains, disrupting the stability of the helix and leading to poor collagen secretion and increased intracellular breakdown. However, not all mutant molecules are degraded. A proportion are secreted and incorporated into the extracellular matrix where the presence of even a small number of abnormal molecules can exert a dominant negative effect, disturbing the entire matrix architecture and resulting in a severe disease (23, 25, 27). In contrast, protein haploinsufficiency, commonly because of the introduction of premature stop codons and mutant mRNA decay, leads to the milder diseases, osteogenesis imperfecta type I and Stickler syndrome (28–30).

The exon-skipping mutation characterized in this study clearly demonstrates that the biosynthetic effects of collagen VI structural mutations can be quite different from those seen in the fibrillar collagens. The requirement that collagen VI forms tetramers prior to secretion imposes an additional level of “quality control” that in this case of Bethlem myopathy prevents secretion of molecules containing mutant α1(VI) chains and leads to protein haploinsufficiency rather than a dominant negative effect because of the presence of structurally abnormal collagen VI in the extracellular matrix. As a result, both premature in-frame stop codons (5) and structural mutations that are incorporated into monomers but prevent intracellular dimer assembly have similar phenotypic consequences and produce clinically indistinguishable Bethlem myopathy. Single glycine substitutions in the α1(VI), α2(VI), and α3(VI) chains also cause Bethlem myopathy (3, 4). However, biosynthetic studies have not yet been performed on these cases to determine whether the mutations interfere with intracellular assembly and secretion and result in collagen VI haploinsufficiency or whether the disease results from the presence of structurally abnormal collagen VI in the extracellular matrix. These studies will be crucial to gain a comprehensive understanding of the molecular basis of Bethlem myopathy.

It is somewhat surprising that collagen VI mutations produce a muscle-specific disease rather than a more general phenotype consistent with its widespread distribution in virtually all connective tissues. Although collagen VI is closely associated with the basement membrane surrounding muscle cells where its function is disturbed in Bethlem myopathy, it is also abundant in skin and cornea and is found in cartilage and bone (18, 31, 32). However, these other tissues are not apparently affected by the mutations that have been characterized to date. Likewise, mice completely lacking collagen VI protein because of targeted inactivation of the col6a1 gene also showed histological features of myopathy but no other obvious differences to controls (33). These findings clearly identify collagen VI as a critical contributor to skeletal muscle function and suggest that a reduced collagen VI microfibrillar network can no longer adequately anchor the muscle cell to the surrounding connective tissue. Collagen VI α1(VI) and α2(VI) mRNAs are not expressed in cultured myoblasts but are induced during in vitro differentiation into myotubes (34, 35), raising the possibility that in addition to being an important structural component, collagen VI may also play a role in myotube formation and stability similar to that played by the muscle-specific basement membrane components laminin-2 and laminin-4 (36).

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