Collagen VI mutations in Bethlem myopathy

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Chapter 1

Review of the literature

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1.1 Clinical Features

Introduction

Myopathies with limb-girdle distribution of weakness and autosomal dominant inheritance constitute a disparate group of disorders. Due to the development of sophisticated diagnostic methods, a classification has emerged that distinguishes well-defined disorders such as congenital myopathies, metabolic myopathies and limb girdle muscular dystrophies. However, there still exists a wide variety of autosomal dominant myopathies that cannot be easily classified. Within this broad category, the autosomal dominant myopathy described by Bethlem and van Wijngaarden in three unrelated Dutch families in 1976 is considered a distinct nosological entity. This benign myopathy is characterized by the following salient clinical features: slow progression of limb-girdle weakness from childhood onward with periods of arrest for several decades, contractures of fingers, elbows and ankles, and absence of cardiac involvement. Subsequently, several other families with a similar condition have been identified, both by Bethlem and associates and by others. In 1988 the name "Bethlem myopathy" was proposed by Mohire et al.

Clinical findings

There exists some variation in the literature regarding the age of onset. In Bethlem's original article onset was around the fifth year of life, weakness being the first symptom. From subsequently published kindreds it has become apparent that onset can either be in infancy with slightly delayed developmental milestones or in early childhood. Somer et al. reported a Finnish family that displayed all the characteristics of Bethlem myopathy except for a wide variation in the age of onset ranging from early infancy to the fifth decade. Most patients of this kindred had an onset in adolescence. Some asymptomatic middle-aged patients appear to have weakness on examination. Combining the results of a retrospective analysis of Boers in five families (four previously published and one novel family) with observation of new cases in the course of time in the same kindreds and an additional one, Jöbsis et al. could obtain data about the mode of onset in 23 children. Nearly all children exhibited neuromuscular signs during the first two years of life. Diminished fetal movements were noted by two mothers in three pregnancies. Nine babies were retrospectively described by their mothers as being "floppy". A head lag phenomenon was noted in all floppy children and four non-floppy ones. Torticollis, congenital or ensuing in the course of several months, was present in nine children. During infancy and childhood, contractures had a strikingly dynamic nature. Congenital clubfeet with dorsiflexion contractures of the ankles (in ten out of 11 children from two families carrying the same COL6A2 mutation) spontaneously regressed in all but one child in several years. Other
apparent contractures during infancy included flexion contractures of the elbows, fingers and knees, of which the latter disappeared spontaneously during childhood. Hypermobility of the wrists and fingers, noted in five children, slowly evolved into flexion contractures. Eight children exhibited laxity of the hip joints with an increased range of endorotation. A Gowers' sign was present in 11 children at the age of 2 years.

During childhood there was difficulty in arising from a squat, clumsy or waddling gait, easy tripping, and a diminished inability to run. Typically insidious progression is observed in the first decade, later followed by a relative increase in muscle power. During adolescence the Gowers' sign disappeared. In some female patients muscle strength deteriorated during pregnancy.

Progression of weakness is reported to be slight with most patients remaining ambulant with help of a cane into old age and an unaffected life expectancy. In a recent study on the natural course, Jöbsis et al, also focussed on progression of weakness after middle age. Thirty-six patients from seven families were assessed for impairment by telephone interview. After the third decade muscle weakness tended to worsen gradually, hallmarked by the reappearance of the Gowers' sign. In most patients ability to work was preserved until old age. However, with advancing age, nearly all patients experienced sufficient weakness to warrant alterations at home, ranging from an elevated toilet seat to a fully automated system to assist the bed-wheelchair transfer. More than two-thirds of patients, aged 50 years and over, preferentially used a wheelchair for at least part of their ambulation, especially outdoors. Respiratory insufficiency necessitating artificial ventilation at night has been described in two patients.

Examination of a patient with Bethle myopathy will reveal generalized slight atrophy of the musculature and diffuse mild weakness, proximal more severe than distal. Extensors are weaker than flexors. The muscles innervated by the cranial nerves are preserved, except for slight facial weakness in a single patient from one family. In virtually all kindreds hypertrophic calves are absent, except for one individual from a Finnish pedigree and multiple sibs from two French families. Hypertrophy of the extensor digitorum brevis muscle has been reported in one pedigree.

Flexion contractures of the interphalangeal joints of the four last fingers (Figure 1), elbows (Figure 2), and ankles (pes equinovarus) are present in nearly all patients. The former, caused by a shortening of the flexor digitorum profundus muscle, is most striking at full wrist extension. Contractures of the metacarpophalangeal joints, wrists, knees, hips, and shoulders are encountered in many patients. Two reports mention contractures of the
Figure 1. Flexion contractures of the fingers in a patient with Bethlem myopathy (left) compared to a normal subject (right).

neck and spine. Contractures of the neck are present in one member from one pedigree with 19 examined patients and seven patients from two other families with 15 affected sibs, whereas limitation of the dorso-lumbar spine are present in one and three patients respectively.\textsuperscript{5,10} There is no relationship between the contractures and the severity of weakness.\textsuperscript{2,3,5} One author reports slow progression of contractures with age.\textsuperscript{10} Torticollis, either congenital or becoming apparent during the first two years of life, due to contracture of the sternocleidomastoideus muscle is reported in 11 patients from seven families out of a series of 19 published kindreds. Contractures can however be entirely absent. A recent report described a kindred with a collagen VI gene mutation (see below), in which only three patients out of 11 exhibited diminished joint movement.\textsuperscript{15}

Deep tendon reflexes are either preserved, decreased or absent, irrespective of the severity of weakness.

Details on the clinical features of Bethlem myopathy are mostly derived from articles describing one family only, thus making an objective comparison difficult. Interfamilial variability could mainly reflect interobserver variability. With the recent discovery of the genetic defect, some publications have appeared that hardly provide clinical details. Often it remains unclear whether the presence of contractures has been investigated systematically. However, certain features appear to be restricted to some families, e.g. congenital dorsiflexion contractures of the ankles present in two families that carry the same genetic defect.\textsuperscript{13} Neonatal hypotonia seems more prevalent in these kindreds as well. Muscle cramps occur preferentially in one kindred.\textsuperscript{13} Calf muscle hypertrophy has been recognized in two French families,\textsuperscript{6} and hypertrophy of the extensor digitorum brevis muscle in one Italian kindred.\textsuperscript{10} With regard to severity of weakness and contractures, interfamilial variability is not evident as indicated by equal distribution of the use of wheelchairs and other aids.\textsuperscript{13}
Within families clear differences exist, e.g. a 17 year old patient partially wheelchair dependent whose 68 year old uncle does not require any aid or facility.\textsuperscript{13}

\textbf{Figure 2.} Flexion contracture of the elbow in addition to mainly proximal muscle wasting.

\textbf{Additional investigations}

Serum creatine kinase (CK) activity is either normal or slightly elevated (2-5 times the upper limit). In one study a moderate increase in CK activity (up to 15 times the upper limit) was noted in three young individuals.\textsuperscript{10}

Generally, the electromyogram (EMG) shows on voluntary activity short-duration, small-amplitude, rapidly recruiting potentials with increased polyphasia. In most patients there is no spontaneous muscle activity at rest;\textsuperscript{2,5} however, in some patients fibrillation potentials, positive sharp waves, and high-frequency bizarre discharges are present.\textsuperscript{4,8} Satellite potentials and long-duration motor unit action potentials have been noted by some authors.\textsuperscript{4,8} In a minority of cases the EMG is entirely normal or shows a neurogenic picture.
with mildly prolonged insertional activity and reduced number of increased-amplitude polyphasic motor units.\(^4\)\(^5\)\(^6\)\(^7\)\(^8\)\(^9\) Motor conduction velocities are normal.

Computerized tomography scans of the musculature show diffuse replacement of muscle tissue by fat, with proximal preponderance and an increase with age.\(^4\)\(^11\) Unexpected early involvement of the lumbar paravertebral muscles was reported by Merlini et al.\(^10\) On magnetic resonance imaging, predominant involvement of the quadriceps femoris muscle was evident, whereas the gracilis and sartorius muscles and the hamstrings were relatively spared.\(^8\)\(^9\)

Cardiac involvement, assessed by medical history, physical examination, chest X-ray, electrocardiography, echocardiography, and Holter monitoring, is absent.\(^2\)\(^5\)\(^10\)\(^16\)

Muscle biopsy specimens show non-specific myopathic changes, including variation in muscle fibre diameter with a moderate increase in fibres with internal nuclei, a marked increase in fatty tissue, and a small increase in connective tissue. Lobulated or moth-eaten type 1 fibres have been noted in a few patients.\(^2\)\(^7\)\(^8\) Except for occasional necrosis and regeneration, structural abnormalities are absent (refs. 9-11 and unpublished observations). Staining for dystrophin and sarcoglycan reveals a normal pattern,\(^10\) as does staining for merosin.\(^13\) Electron microscopy reveals no specific changes in the muscle fibres.

Three autopsies have been reported showing no convincing abnormality of brain, spinal cord, or peripheral nerves.\(^2\)\(^5\) The distribution of pathological features in skeletal muscles is similar to that found clinically. Histopathological findings are comparable to those of muscle biopsies. Except for changes consistent with atherosclerotic coronary artery disease, microscopic examination of the heart is normal.\(^5\)

Management

No specific therapy is known. Patients should be counselled about the natural course of this myopathy. Many patients receive physical therapy to prevent worsening of contractures, its effectiveness has not been evaluated. Due to the dynamic nature of contractures in infancy, corrective surgery should be delayed unless hindrance interferes with development. Except for orthopaedic shoes no supportive measures are required in childhood and adolescence. From middle age onwards progressive weakness leads to sufficient impairment to necessitate additional aids like a cane, home alterations and wheelchairs, in the majority of cases.\(^13\)

The identification of the molecular defect has enabled presymptomatic diagnosis. Due to practical restraints and genetic heterogeneity (see below), prenatal diagnosis will be limited to those families with an identified mutation. Counselling is difficult and should emphasize clinical heterogeneity. Whether the severity of the disorder warrants an abortion depends on the couple involved. The fact that before the identification of collagen VI gene mutations in Bethlem myopathy some patients opted not to procreate because of the familial
affliction (unpublished observations), conveys sufficient weight to implement prenatal DNA testing in particular cases.

**Differential diagnosis**

Bethlem myopathy shares many features with Emery-Dreifuss muscular dystrophy (EDD) and the rigid spine syndrome (RSS); slowly progressive weakness of limb-girdle musculature, onset in infancy or early childhood, and widespread contractures. However, due to cardiac involvement both EDD and RSS run a less benign course. RSS usually occurs sporadically and mostly in males. EDD usually has an X-linked recessive inheritance, secondary to mutations in the gene for emerin, but autosomal dominant forms exist. Most but not all X-linked EDD patients have an emerin deficiency whereas emerin staining is normal in autosomal dominant cases. Autosomal dominant EDD kindreds link to the same locus on chromosome 1q as autosomal dominant limb girdle muscular dystrophy with atrio-ventricular conduction disturbances (LGMD1B), and carry mutations in the lamin A/C gene.

Several congenital myopathies have a limb-girdle distribution of weakness with slow or absent progression. Flexion contractures sometimes occur. Muscle biopsy should lead to the correct diagnosis.

Limb girdle muscular dystrophy with autosomal dominant inheritance (LGMD1) usually has its onset after adolescence, although exceptions to this rule occur. Progression is more rapid than in Bethlem myopathy, with the exception of LGMD1B. Early-onset contractures are usually absent.

Frijns et al. characterized a family with a dominant trait of congenital atrophy and weakness of mainly the lower limbs, tight heel cords and hyperlaxity of the elbows. Muscle biopsy disclosed evidence of a neurogenic disorder.

Taylor et al. reported on seven kindreds with a slowly progressive, early onset, autosomal dominant myopathy with proximal muscle weakness, calf hypertrophy, contractures, spinal rigidity and, in five adults a cardiac conduction defect. A deficiency of laminin β1 was restricted to the adult cases, most likely reflecting a secondary phenomenon. Two Bethlem myopathy patients, aged 11 and 40 years, have been reported to show normal laminin β1 staining, however clinical and molecular data have not been provided. Aspects of these laminin β1 deficient families atypical of Bethlem myopathy consist of spinal rigidity, calf hypertrophy and cardiac conduction defects. Contractures of the spine were present in four cases out of 19 in a Bethlem myopathy family with an identified COL6A3 mutation (see below), and in three out of eight sibs of an Italian family that maps to the COL6A1-COL6A2 Bethlem myopathy locus on chromosome 21q (see below and Chapter 2.2). Calf hypertrophy has been reported in three Bethlem myopathy families. Hypertrophic calves are present in a single case from a Finnish family with linkage to the
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COL6A1-COL6A2 locus (Chapter 2.2). No molecular data are available on two French kindreds with calf hypertrophy; in one family all affected sibs displayed this feature, in the other it was present in some patients.

Although Bethlem myopathy is apparently a distinct nosological entity, some reported kindreds share many, but not all, features with Bethlem myopathy, thus making a definite classification rather difficult. A family described by Bailey et al. showed onset of limb-girdle weakness in early infancy with delayed developmental milestones and very slow progression, contractures of elbows, heel cords, and knees, pes equinovarus, spine rigidity and thoracic scoliosis, absence of cardiac involvement, and a normal or moderately elevated CK. The findings of EMG and muscle biopsy were consistent with a myopathy. Inheritance was suggestive of an autosomal dominant trait with incomplete penetrance. A peculiar facies with frontal bossing and low-set ears in multiple cases and brisk tendon reflexes with ankle subclonus were features atypical of Bethlem myopathy.

Schmalbruch et al. described a family with benign limb-girdle weakness with onset in early childhood and autosomal dominant inheritance, Achilles tendon shortening, and torticollis in some individuals. Although not mentioned in the clinical description, contractures of the elbows can be observed from the photograph of one patient. However, the muscle biopsies showed features atypical of Bethlem myopathy, i.e., a necrotizing myopathy with pronounced regeneration and formation of aberrant myofibrils (ringbinden) and prominent fibrosis.

1.2 MOLECULAR BIOLOGY

Linkage analysis

From the published pedigrees it can be inferred that the mode of inheritance is autosomal dominant with complete penetrance. The clinical features and the aspecific myopathic changes seen on muscle biopsy provide no biochemical clue to the underlying defect. A genetic study with 17 protein polymorphisms failed to disclose significant linkage in one family. A genome-wide linkage analysis in six families using highly informative microsatellite markers established linkage with the telomeric region of chromosome 21q. A combined two-point lod score exceeded 6.00 for markers PFKL and COL6A1, the maximum two-point lod score obtained within one family was 3.76. Data of all families was in support of genetic linkage. The region contained a cluster of two genes (COL6A1 and COL6A2) that encode constituent peptides of an apparent candidate protein, collagen VI. One recombination with COL6A1, in a 67 year old patient with flexion contractures of the fingers but no weakness or wasting, placed the Bethlem myopathy locus telomeric of this gene. Whether COL6A2 was excluded depended on the orientation of the COL6A1-COL6A2 cluster. With various genetic maps showing different orientations, the results were
equivocal. The region telomeric of the cluster contained no other informative markers or interesting genes.

Three additional families, one from Finland and two from Italy were analysed for linkage to COL6A1 (Chapter 2.2). Confirming the absence of genetic heterogeneity of the first study, all families showed genetic linkage to the COL6A1-COL6A2 locus. One recombination was present. On reexamination it was deemed uncertain whether this person was affected. Linkage data in a family of French-Canadian descent, however, firmly excluded the telomeric region of 21q. Instead, this family showed linkage to 2q37. This region was investigated as it contained the COL6A3 gene, encoding the third peptide of collagen VI. The established linkage to either the COL6A1-COL6A2 region or to the COL6A3 region provided compelling support for collagen VI to be the protein at stake in Bethlem myopathy.

**Mutation analysis**

Sequence analysis of the entire coding region of COL6A1 and COL6A2 in four Dutch families revealed mutations in three. A COL6A1 mutation, c.962G>T (G286V) was present in one family whereas a COL6A2 mutation, c.898G>A (G250S), was shared by two other families. Both missense mutations resulted in disruption of the Gly-X-Y motif of the triple helical domain by substitution of glycine for another amino acid. Sequence analysis in a French-Canadian family identified a mutation in subdomain N2 of the N-terminal globular domain of COL6A3; c.5291G>A (G1679E).

Lamandé et al. reported a mutation in the splice acceptor site at the end of intron 11 of the triple helical domain of COL6A1. The genomic G to A substitution of the terminal nucleotide of the intron causes the first nucleotide of exon 12 to be spliced off, thus yielding an one nucleotide deletion in mRNA with a frame shift and premature stop 22 codons downstream. Subject to nonsense-mediated mRNA decay the mutant transcript is unstable and almost completely absent from fibroblasts and skeletal muscle. Sequence analysis (cDNA) in four Dutch COL6A1-COL6A2 linked families yielded a mutation in three. Apart from a mutation in a regulatory sequence, an intron nucleotide change leading to pre-mRNA instability might explain the absence of a cDNA variation in the fourth family, in which linkage to COL6A3 was excluded. A recent report described two novel mutations in two Italian families, one disrupting the Gly-X-Y motif of the triple helical domain of COL6A1 and one residing in the triple helical domain of COL6A3. These mutations have not yet been published in the peer-reviewed literature.

Six Bethlem myopathy families do not do not carry the COL6A1 c.962G>T or the COL6A2 c.898G>A mutation. Except for one family that is too small to contain linkage information, these families display genetic linkage to the COL6A1-COL6A2 locus on
chromosome 21q but not to COL6A3 on chromosome 2q (chapter 2.2). These kindreds remain to be genotyped.

1.3 PATHOPHYSIOLOGY

Collagen VI structure

Collagen VI is a member of the extensive collagen family that consists of 19 different types (collagen I-XIX), encoded by 33 genes. Collagen VI forms a microfibrillar network in the extracellular matrix of virtually all connective tissues, including skeletal muscle. In association with interstitial collagen fibres, the collagen VI meshwork is situated around cells and in contact with basement membranes. Collagen VI is comprised of three different polypeptide chains; α1(VI) encoded by COL6A1 on chromosome 21q, α2(VI) encoded by COL6A2 on chromosome 21q and α3(VI) encoded by COL6A3 on chromosome 2q. It is characterized by a short triple helical region with large N-terminal and C-terminal globular domains which make up greater than 70% of the mass (Figure 3). In the triple helical domain every third amino acid is a glycine, a motif designated as Gly-X-Y, enabling the tight turns of the peptides to form an α helix.

The globular domains are composed of several subdomains with homology to the von Willebrand factor A domain, the fibronectin III domain, the Kunitz protease inhibitor domain, and several other recognised protein motifs. Alpha1(VI) and α2(VI) each consist of one N-terminal von Willebrand factor A domain and two C-terminal von Willebrand factor A domains. The α3(VI) peptide is much larger, it has ten N-terminal von Willebrand factor A domains (N10-N1), two such C-terminal domains, one fibronectin type
III domain and one Kunitz type protease inhibitor domain. Alternative splicing at the 5'-end and 3'end of collagen α2(VI) and α3(VI) leads to heterogeneity at the protein level.\(^{48-50}\)

The three peptides α1-3(VI) associate intracellularly into a collagen VI monomer. These monomers align into antiparallel, overlapping dimers, held together by disulfide bonds.\(^{51}\) Prior to excretion, dimers form tetramers by covalent association with their ends in register.\(^{52,53}\) Extracellularly, tetramers aggregate into microfibrils in an end-to-end association by interaction of the globular domains with each other and with the triple helical domains.\(^{54,53}\)

**Collagen VI function**

Collagen VI has both cell adhesion properties and a range of extracellular matrix protein binding activities, suggesting a bridging role between cells and the pericellular matrix.\(^{46,55,56}\) The interaction spectrum of collagen VI is complex. Matrix components and membrane proteins that have been shown to interact with collagen VI include various proteoglycans, several collagens, hyaluronan, heparin, and numerous integrins. Conflicting results, partially depending on the kind of collagen VI substrate used, hamper interpretation of various studies.\(^{57,58}\) Table 1 summarizes various studies on the interaction spectrum of collagen VI. Furthermore, results of a yeast two-hybrid system indicate functional interactions with proteins that need yet to be defined.\(^{58}\) Relative affinities of native collagen VI tetramers to several collagens (I, II, IV, V, VI, XI) and fibronectin indicate that the major ligands for collagen VI appear to be collagen IV, collagen VI itself and fibronectin.\(^{58}\)

Extracellular matrix components are thought to be essential for morphogenesis of virtually all tissues, including skeletal muscle.\(^{59,60}\) By connecting matrix components to cells, collagen VI could serve a role in regulation of proliferation and differentiation.\(^{61-64}\) Several observations suggest such a role. In a myoblast cell line (C2C12) expression of COL6A2 mRNA is absent in exponentially growing cells and reaches peak levels just prior to myotube formation in an identical fashion as MyoD1 and myogenin.\(^{65}\) A similar pattern of expression of Col6a2 occurs during initial phases of myogenic differentiation in mice. Collagen VI has a stimulatory effect on mesenchymal cell growth and inhibits apoptosis.\(^{61,64}\)

Integrins, an extensive family of transmembrane proteins with cell signalling and regulatory properties, link the cytoskeleton to the extracellular matrix. Interaction of integrins with matrix components are critical in triggering metabolic events in myogenic differentiation.\(^{66}\) During myotube formation up-regulation of integrin β1D occurs together with a down-regulation of the integrin β1A and α5β1, αVβ3 forms.\(^{67,69}\) Beta1D associates with several alpha subunits, the switch from the β1A subunit to β1D during myogenic differentiation coincides with the expression of α7 subunit.\(^{70}\) Integrin α7β1, expressed in skeletal and cardiac muscle, binds laminin-1, with high affinity laminin-2/4, and fibronectin.\(^{71-73}\) Interaction of collagen VI with integrin α1β1 and α2β1 mediates attachment...
interaction of collagen VI with integrin α3β1 seems to play a role in fibroblast migration, development and matrix architecture of the cornea. By modulating laminin expression, insulin-like growth factor 1 and transforming growth factor beta have differential effects on mesenchymal cell adhesion to collagen VI. Alternative splicing events of COL6A3 mRNA leading to variation of the number of N-terminal von Willebrand factor A modules in the α3(VI) chain, occur in a tissue specific manner, and might thus modulate cell adhesion and interaction with other matrix components.

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Table 1. Proteins with affinity to collagen VI.

Pathophysiological model

Collagen VI is a component of virtually all connective tissues, patients with a collagen VI mutation exhibit however only muscle weakness and contractures. Mice that homozygously lack the α1(VI) chain, leading to an extracellular matrix completely deficient of collagen VI, merely show myopathic features. The mechanism by which collagen VI mutations give rise to Bethlem myopathy remains to be established. In view of the cell-extracellular matrix...
interaction of collagen VI, an analogous perturbation of the attachment of muscle cells to the surrounding matrix as hypothesised in various muscular dystrophies seems plausible. The difference in mode of inheritance must be accounted for; the muscular dystrophies caused by perturbation of the dystrophin-laminin axis are recessive in nature whereas the collagen VI mutations give rise to a dominantly inherited disorder.

The only collagen VI protein study in the Dutch kindreds involved staining of muscle biopsies with monoclonal antibodies, revealing a normal pattern of collagen VI distribution in the endo- and perimysium (unpublished observations). Except for exclusion of a massive reduction, no quantitative conclusion can be drawn from this. Although no collagen VI protein synthetic studies or structural analyses have been performed, the Gly-X-Y disrupting mutations of COL6A1 and COL6A2 are likely to be causative. This type of mutation is commonly encountered in other collagen disorders, like osteogenesis imperfecta, Ehlers-Danlos syndrome type IV and Alport syndrome. Gly-X-Y disruption leads to altered intracellular processing of procollagen, disturbed formation of monomers and higher order structures. Abnormal folding of the α helix has been visualised. With few exceptions, mainly transient fetal stages where α1(VI) and α2(VI) mRNAs can be more abundant than α3(VI) mRNA (without translation to collagen VI protein), nearly all tissues contain equimolar amounts of the constituent chains of collagen VI. If an allele of an α chain with a Gly-X-Y disrupting mutation is normally translated and intracellularly processed into monomers, dimers and tetramers, statistically one out of two monomers would be wild-type. For dimers this would be one out of four and for tetramers only one out of 16 would be wild-type. With such a dramatic reduction of normal tetramers, the building blocks of the microfibrillar network, it is easily envisaged that the described heterozygous mutations act in a dominant-negative fashion.

Fibroblasts harbouring the missense mutation in the N2 domain of α3(VI) express α3(VI) mRNA of normal length and in a normal α1(VI):α3(VI) ratio as analysed by Northern blot. Biosynthesis studies show that these fibroblasts produce collagen VI in normal amounts and with normal ratios of the constituent peptides. The α3(VI) chain differs from the other two chains by the presence of the N10-N2 domains and the C3-C5 domains (Figure 3). The function of these extended terminal domains is unclear. The N10-N7 domains are not required for assembly into monomers, dimers or tetramers. The N-terminal domain of α1(VI) binds with other extracellular matrix components like fibronectin and collagen IV. The tissue-specific distribution pattern of the various splice variants suggests the extended N-terminal domain mediates interaction with other matrix components. The established normal assembly of collagen VI protein by fibroblasts with the N2 mutation underscores the hypothesis about the function of the extended N-terminal domain. As described above, a heterozygous mutation will leave only one out of 16 tetramers unaffected, thus conveying a dominant-negative effect on the interaction with other matrix components.
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The intron 11-exon 12 redefining α1(VI) mutation yields an unstable mRNA molecule which is subject to nonsense-mediated decay. The resultant diminished availability of α1(VI) chains leads to intracellular formation of excess amounts of assembly intermediates composed of an α2(VI) and an α3(VI) chain. These assembly intermediates are degraded intracellularly. The extracellular matrix shows a greatly reduced amount of collagen VI. The reduction of collagen VI deposition has not been quantified, but was apparent on simple staining of fibroblasts culture. If α2(VI)-α3(VI) assembly intermediates partake in the formation of dimers and tetramers in competition with normal monomers, the reduction of excreted tetramers could fall below critical levels to maintain the structural function of muscle cell adhesion or matrix-matrix interaction. The clinical details of the kindred harbouring this mutation have not been published in extent, but contractures seem to be present in only three out of 11 patients. This relative scarcity of contractures could be a reflection of the difference of net effect of the mutation; reduction of normal collagen VI versus a normal amount of altered collagen VI in the extracellular matrix.

The pathogenicity of each of the described collagen VI mutation remains to be formally established. The type of mutation, e.g. the predicted disruption of the Gly-X-Y motif, or the effect, the greatly reduced amount of protein in case of the intronic mutation with haploinsufficiency, makes it very unlikely these sequence alterations are merely linked polymorphisms. Further evidence is provided by the recently created mouse model for Bethlem myopathy. Inactivation of Col6a1 by targeted gene disruption gives rise to a myopathy in homozygously deficient animals. Milder myopathic changes are present in heterozygous mutant mice.

Several muscular dystrophies are due to aberration of cell anchoring structures. The dystrophin-glycoprotein complex spans from the cytoskeletal protein actin to the extracellular matrix protein laminin. Duchenne and Becker muscular dystrophy are due to mutations of dystrophin. The autosomal recessive limb girdle muscular dystrophies LGMD2C, LGMD2D, LGMD2E, and LGMD2F have mutations in the sarcoglycans γ, α, β, and δ respectively. Mutations in laminin α2 give rise to several congenital muscular dystrophies including the merosin-negative form, and the dy2/dy2 mouse. Mice homozygously lacking dystroglycan have an embryonic lethality thought to arise from defects in extra-embryonic structures and their association with the extracellular matrix. For some cell anchoring structures that are mutated in muscular dystrophies, it is not yet clear whether they are part of the dystrophin-glycoprotein complex. Caveolin-3, a structural and regulatory integral membrane protein found at the sarcolemma, partially co-purifies with dystrophin but patients with a caveolin-3 mutation have no evident secondary deficiency of dystrophin or sarcoglycans. And, conversely, caveolin-3 expression is not reduced in patients with primary mutations in either dystrophin or the sarcoglycans. Likewise, the connection of plectin, a cytoskeleton-membrane anchorage protein, to the dystrophin-glycoprotein complex remains to be established. Mutations resulting in a deficiency of plectin cause autosomal recessive muscular dystrophy with epidermolysis...
Patients with a dystrophinopathy show some increased staining of plectin.\textsuperscript{102} Mutation of integrin $\alpha 7\beta 1$, a transmembrane protein that connects actin to laminin-$1$ and laminin-$2/4$ in skeletal and cardiac muscle, gives rise to progressive myopathic changes in mice with some dystrophic features like fibre necrosis and fibre regeneration.\textsuperscript{103} Skeletal muscle of animals homozygously lacking integrin $\alpha 7$ show no reduced amounts of dystrophin, dystroglycan, $\alpha$-sarcoglycan, laminin-$1$ ($\alpha 1\beta 1\gamma 1$), or laminin $\alpha 2$. The phenotype was mild and no overt weakness was noticed, taking into account however that observation was limited to 100 days after birth. So far, three patients with an integrin $\alpha 7$ mutation have been reported.\textsuperscript{104} All were sporadic cases with delayed motor development. Two patients had neonatal torticollis. Whether contractures were systemically sought for is not clear. In one patient with concomitant mental retardation, brain MRI scan was normal. Serum CK activity was mildly elevated (to 3 times upper limit of normal). Biopsied muscle showed non-specific myopathic changes.

It is very likely that mutated collagen VI leads to Bethlem myopathy through aberration of its cell anchorage function. It remains to be seen through which transmembrane protein, either directly or indirectly, collagen VI mediates its function. There are many candidates. A direct interaction with laminin is not apparent from the literature. A brain MRI scan in a Bethlem myopathy patient failed to show white matter changes that are observed in merosin-negative congenital muscular dystrophy (unpublished observation).\textsuperscript{105} Furthermore, merosin staining was normal in one patient.\textsuperscript{13} The similarities between Bethlem myopathy and the congenital myopathy observed in patients with an integrin $\alpha 7\beta 1$ mutation, are striking. There is evidence for direct binding of collagen VI to integrin $\beta 1$,\textsuperscript{57,61,74,75} and indirect binding through fibronectin.\textsuperscript{68,71-73} Other transmembrane proteins that are candidates include proteoglycan NG2 and plectin.\textsuperscript{106-109}

References


44. Chu M-L, Pan T-C, Conway D, et al. Sequence analysis of α1(VI) and α2(VI) chains of human type VI collagen reveals internal triplication of globular domains similar to the A domains of von Willebrand factor and two α2(VI) chain variants that differ in the carboxy terminus. EMBO J 1989;8:1939-1946.


Chapter 1


