Collagen VI related muscle disorders

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Mutations in the genes encoding collagen VI (*COL6A1*, *COL6A2*, and *COL6A3*) cause Bethlem myopathy (BM) and Ullrich congenital muscular dystrophy (UCMD), two conditions which were previously believed to be completely separate entities. BM is a relatively mild dominantly inherited disorder characterised by proximal weakness and distal joint contractures. UCMD was originally described as an autosomal recessive condition causing severe muscle weakness with proximal joint contractures and distal hyperlaxity. Here we review the clinical phenotypes of BM and UCMD and their diagnosis and management, and provide an overview of the current knowledge of the pathogenesis of collagen VI related disorders.

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The discovery of the underlying molecular basis of many muscular dystrophies and myopathies over recent years has highlighted the importance of the link between the cytoskeleton and the extracellular matrix in skeletal muscle. Extracellular matrix and basal lamina molecules are now known to be critical for skeletal muscle stability, regeneration, and muscle cell matrix adhesion.[1–4] Collagen VI is a ubiquitous extracellular matrix protein which forms a microfibrillar network in close association with the basement membrane around muscle cells and which interacts with several other matrix constituents.[5–7] Mutations in the genes encoding any of the three collagen VI chains have been demonstrated in Bethlem myopathy (MIM 158810) and Ullrich congenital muscular dystrophy (MIM 254090).

In this review we discuss the clinical phenotypes of Bethlem myopathy (BM) and Ullrich congenital muscular dystrophy (UCMD), the muscle pathology in these disorders, the role of collagen VI in their causation and provide a reappraisal of this important and under-recognised group of disorders.

BETHLEM MYOPATHY

Bethlem myopathy was first described in 1976 by Bethlem and van Wijngaarden as an autosomal dominantly inherited mild proximal myopathy with long finger flexion contractures occurring in 28 individuals of three Dutch pedigrees.[8] It has subsequently been reported worldwide[9–19] (for extensive review see De Visser *et al*[20]).

Although BM is classically described as a mild disorder with its major impact in adult life it is often possible in retrospect to elicit a history of neonatal hypotonia or torticollis, delayed motor milestones, or even decreased fetal movements.[21] Usually patients will become symptomatic within the first or second decade[21]; however, as some adult patients remain unaware of weakness, an age of onset cannot always be reliably established.[17] The development of contractures is a hallmark of this condition. The contractures may be of a strikingly dynamic nature during childhood,[21] appearing and disappearing in various joints, but nearly all patients eventually show flexion contractures of the fingers, wrists, elbows, and ankles (fig 1, panels A–D) and these, in addition to weakness, contribute to disability. Strikingly, hypermobility of distal interphalangeal joints can be present together with long finger flexion contractures. Where contractures are prominent, BM may resemble Emery–Dreifuss muscular dystrophy, particularly when a rigid spine is present.[11] [13] However, in other patients the contractures may be relatively subtle, leading to potential confusion in diagnosis with cases of limb girdle muscular dystrophy.[22] [23] Immunohistochemical testing (that is, western blotting and immunohistochemistry) carried out on the muscle biopsy or molecular genetic testing, or both, can help to exclude alternative diagnoses such as sarcoglycanopathy, calpainopathy, and dysferlinopathy[24] as well as X linked or autosomal dominant Emery–Dreifuss muscular dystrophy.[25]

BM patients typically have moderate predominantly proximal weakness and atrophy with the extensors more involved than the flexors.[21] Muscle magnetic resonance imaging (MRI) shows variable severity of muscle involvement paralleling the severity of motor function impairment, with a characteristic pattern of involvement of the peripheral region of the vastus lateralis and hamstring muscles but relative sparing of their central parts.[26] This peripheral predominance of pathology in BM has also been observed using muscle ultrasound and described as "central shadow sign."[27]

The condition is in most cases slowly progressive and more than two thirds of patients over 50 years of age will require aids for ambulation, especially outdoors. Whereas cardiac involvement is usually absent,[15] [28] respiratory muscle and especially diaphragmatic involvement necessitating nocturnal respiratory support is rarely reported in association with severe weakness in later life.[11] [29] In addition, unusual skin features may be present in some BM patients, including follicular hyperkeratosis and keloid formation or "cigarette paper" scarring[12] (see fig 1E).

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Abbreviations: BM, Bethlem myopathy; CMD, congenital muscular dystrophy; UCMD, Ullrich congenital muscular dystrophy; vWF, von Willebrand factor

Figure 1 Typical clinical features of Bethlem myopathy (panels A to E) and Ullrich congenital muscular dystrophy (panels F to I) (see text).

Electromyography typically shows a myopathic pattern of low amplitude, short duration motor unit action potentials[17] and the serum creatine kinase is normal or only mildly raised (usually less than five times the upper limit of normal).[15] The histopathological features of a BM muscle biopsy are non-specifically myopathic, usually consisting of a marked variation in muscle fibre diameter with possible increase of fatty tissue and occasional necrotic or dystrophic fibres.[8][17]

ULLRICH CONGENITAL MUSCULAR DYSTROPHY

UCMD, on the other hand, is classically described as an autosomal recessive condition. It is a clinically and genetically distinct entity within the congenital muscular dystrophies.[30]

UCMD was first described by Ullrich in 1930,[31][32] and subsequent publications confirmed a likely autosomal recessive inheritance and a recognisable pattern of disease.[33–42] The hallmarks of UCMD are muscle weakness of early onset with proximal joint contractures (fig 1, panel H) and striking hyperelasticity of the distal joints (fig 1, panels I to K). Posteriorly protruding calcanei are commonly seen (fig 1G). Weakness is profound and children typically either never achieve the ability to walk independently, or walk independently for short periods only.[43][44] Intelligence is normal. With progression of the disease, there is typically development of spinal rigidity and scoliosis and variable proximal contractures, while with time the distal hyperlaxity can give way to marked long finger flexion contractures and tight Achilles tendons.[45] Respiratory failure in the first or second decade is a common cause of death unless treated with nocturnal respiratory support, but cardiac involvement is not documented to date.[11][43][46] There is frequent failure to thrive. Other distinctive features observed in UCMD patients are congenital hip dislocations[42][43] and a transient kyphotic deformity at birth as well as follicular hyperkeratosis over the extensor surfaces of upper and lower limbs, soft velvety skin on the palms and soles, and the tendency to keloid or ''cigarette paper'' scar formation[30][45] (fig 1F).

Muscle MRI shows a characteristic pattern with diffuse involvement of the thigh muscles with relative sparing of sartorius, gracilis, adductor longus, and rectus.[47]

As for BM, the serum creatine kinase activity in UCMD patients is usually normal or mildly increased (rarely more than five times the upper limit of normal)[42][43] and electromyography reveals action potentials of low amplitude and short duration.[42] Variable pathology, ranging from non-specific mild myopathic changes to more dystrophic-like changes, can be observed in muscle biopsies from UCMD patients, the spectrum including variation in fibre size, type 1 fibre predominance, increased endomysial connective tissue, increased numbers of internal nuclei, and focal areas of necrosis, along with more indirect evidence of muscle fibre regeneration such as the presence of fibres containing fetal myosin.[38][43] In the neonatal period, the differential diagnosis includes other forms of congenital muscular dystrophy (CMD), congenital myopathies, spinal muscular atrophy, and forms of Ehlers–Danlos syndrome or Marfan syndrome. Other types of CMD do not generally present with the distal hyperlaxity characteristic of UCMD and are usually associated with serum creatine kinase levels higher than those observed in UCMD.[30][48] Biochemical testing (that is, western blotting and immunohistochemistry) carried out on the muscle biopsy, along with molecular genetic testing, can help to establish the diagnosis of some CMD subtypes such as MDC1A or MDC1C (*FKRP* mutations).[30][48] In addition, brain MRI may show structural abnormalities or white matter changes in some CMD subtypes such as MDC1A, Walker–Warburg syndrome, muscle-eye-brain disease, and Fukuyama CMD.[46] Spinal muscular atrophy can usually be diagnosed by demonstrating mutations in the *SMN* gene[49] and the muscle biopsy shows features of denervation rather than myopathic or dystrophic changes.[50] Forms of Ehlers–Danlos syndrome or Marfan syndrome are usually not associated with significant muscle weakness or an abnormal muscle biopsy, but may be confused with UCMD because of joint laxity.[51][52] Rigid spine syndromes, a proportion of which are caused by mutations in the *SEPN1* gene, may overlap with UCMD later as the phenotype develops.[30][48]

COLLAGEN VI

In 1996 linkage studies in BM demonstrated locus heterogeneity, with loci on chromosome 21q22.3[53] and 2q37,[54] respectively, and identified *COL6A1-3* as candidate genes. Mutations in all three genes have now been identified in both BM and UCMD. Collagen VI is a ubiquitous extracellular matrix protein that is present in the stroma but also forms a microfibrillar network in close association with the basement membrane of most tissues.[55–57] It is composed of three different peptide chains α1(VI) and α2(VI)—both 140 kDa in size—and α3(VI), which is much larger (260–300 kDa).

Genomic organisation

The α1(VI) and α2(VI) chains are encoded by two genes (*COL6A1* and *COL6A2* respectively) situated in a head to tail organisation on chromosome 21q22.3[58] (NT_011515), separated by 150 kb of genomic DNA. *COL6A3*, the gene for the α3(VI) chain, maps to chromosome 2q37[59] (NT_005120). All three chains contain a central short triple helical domain of 335 to 336 amino acids with repeating Gly-Xaa-Yaa sequences, flanked by two large N- and C-terminal globular domains made up of motifs of 200 amino acids each, which are homologous to von Willebrand factor (vWF) type A domains[60–64] (fig 2). *COL6A1*, which consists of 37 exons (35 coding), contains one promoter[65] and produces a single transcript (NM_001848) encoding a protein of 1021 amino acids (NP_001839) with two C-terminal and one N-terminal vWF type A-like domains. Two different promoters have been described for the 30 exons (29 coding) spanning *COL6A2*.[66] It has also been shown to produce multiple alternatively spliced mRNAs which differ in the 5′-untranslated region as well as the 3′-coding and non-coding sequences, producing at least three α2(VI) protein variants with distinct carboxyl termini[67][68] which similarly contain two C-terminal and one N-terminal vWF type A-like domains.

The *COL6A3* gene comprises 44 exons (43 coding) and encodes the α3(VI) chain, which can vary in size between 2970 and 3176 amino acids. The α3(VI) chain contains two C-terminal vWF type A-like domains, subdomains similar to type III fibronectin repeats, and Kunitz protease inhibitors as well as 6–10 N-terminal vWF type A-like domains, thus contributing most of the amino-terminal globular domain of the collagen VI heterotrimer. Various N-terminal exons of *COL6A3* are subject to alternative splicing and four variant transcripts encode proteins with variably sized N-terminal globular domains.[69–72]

Macromolecular structure

The assembly of collagen VI is a complex multistep process. Equimolar association of the three genetically distinct subunits—α1(VI), α2(VI), and α3(VI)—to form a triple helical monomer is followed by staggered assembly into disulphide bonded antiparallel dimers, which then align to form tetramers, also stabilised by disulphide bonds (fig 3).

Engineered mutation experiments indicate that, as with fibrillar collagens, the collagen VI triple helix folds from the C to the N terminus with folding being nucleated by C-terminal Gly-Xaa-Yaa triplets, with a high proportion of prolines in the Y-position, which have the potential of being hydroxylated by prolyl-4-hydroxylase.[73] Hydroxyproline was found to be the Y
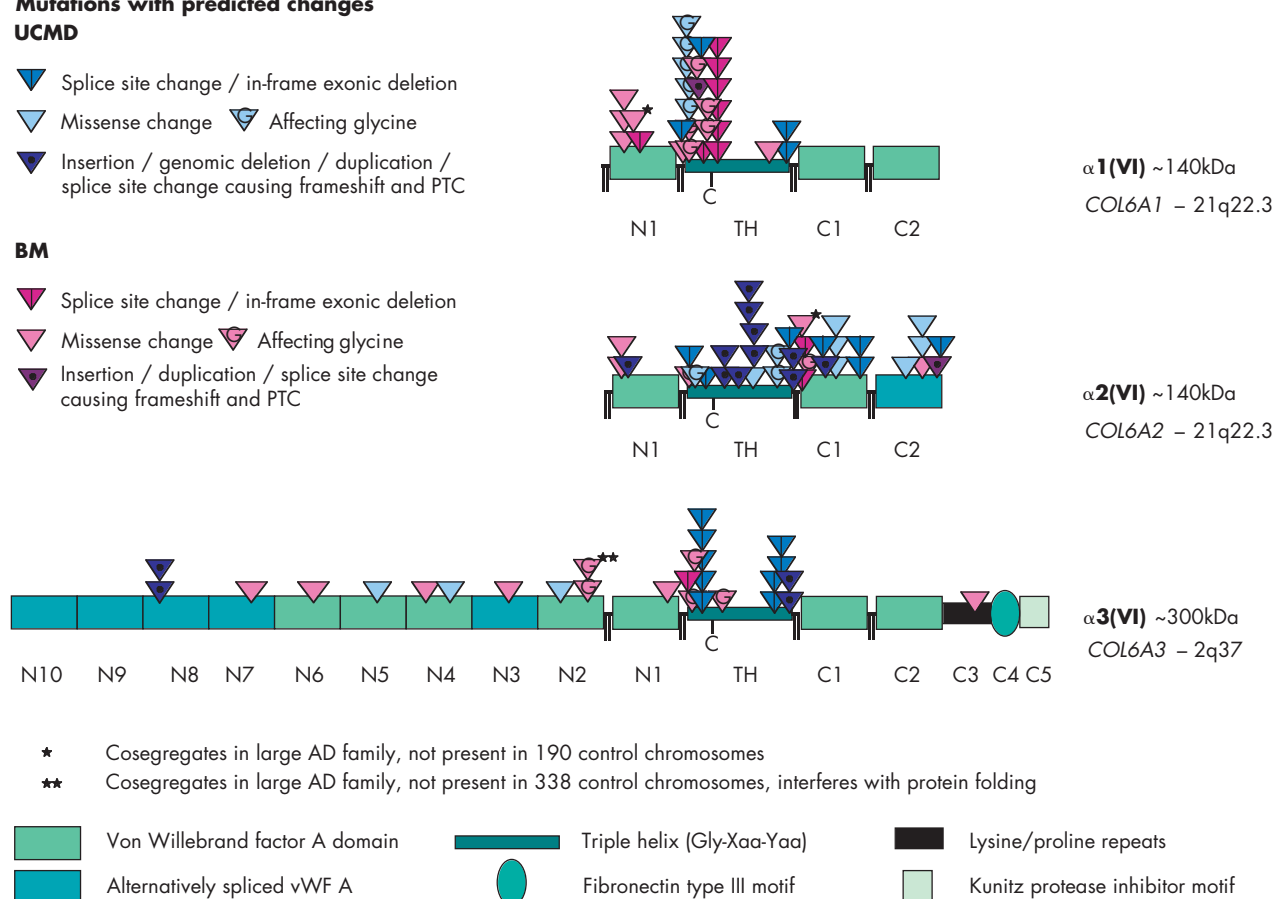
Figure 2 Genomic organisation of collagen VI and localisation of genomic changes reported for BM and UCMD to date; modification from Chu et al.[61] The triple helical domains contain a single cysteine residue (depicted as "C") which is important for dimer assembly. The localisation of the genomic changes reported for BM and UCMD to date is shown stratified by clinical phenotype.

position amino acid that confers the most stability to the collagen triple helix structure.[74] Whereas a single cysteine located in the triple helical domain of the α1(VI) or α2(VI) collagen chain, interacting with a cysteine residue in the C-globular domain, is thought to be responsible for the assembly/stability of dimers,[75–79] it is a cysteine residue in

Figure 3 Schematic model of collagen VI assembly; modification from Furthmayr et al[76] and Zhang et al.[84]

the triple helical domain of the α3(VI) chain that appears to be involved in tetramer formation and stability. An alternative explanation suggests that overall dimer formation is principally determined by α2(VI) interactions[80 81] between a metal ion dependent adhesion site motif in one α2C2 A domain and the Gly-Glu-Arg sequence in the helical sequence of another α2(VI) chain.[82] However, this is not wholly consistent with other studies[83 84] and remains unproven.

Further stabilisation of the dimers may be achieved by supercoiling of the overlapping triple helices. This has been observed by negative staining electron microscopy[76] and is further supported by the position of hydrophobic patches along and around the triple helix of the monomers, which—according to Fourier transform calculations—would be buried along the central line of contact of the molecules only in a supercoiled dimer with a pitch of 37.5 nm.[85] Transfection experiments have shown that *COL6A3* subdomains N10–N6 are not required for the molecular assembly of collagen VI.[83 86] However, immunoelectronmicroscopy studies of mutant fibroblast cultures indicate that the C-terminal globular domain of *COL6A2* is critical for the proper alignment of tetramers and for heterotopic interactions with other matrix molecules.[84]

Outside the cell, tetramers—the secreted form of collagen VI—associate end to end through overlapping N-terminal globular domains to form beaded microfibrils[87] with a distinctive 100 nm periodicity, comprising beaded globular domains separated by short triple helical regions.[76 88 89]
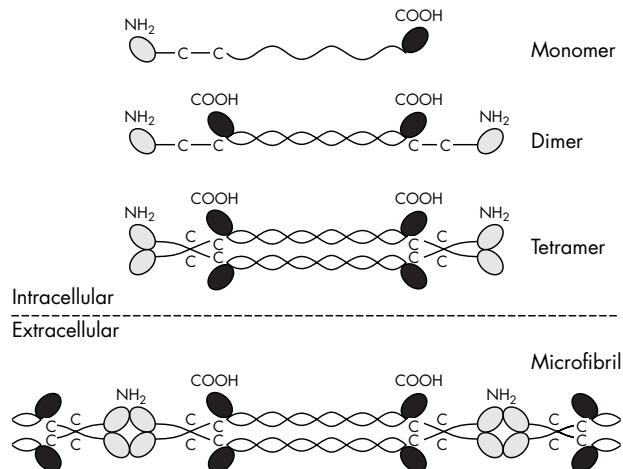
Table 1 Genetic changes published for Bethlem myopathy to date

Location and domain		Nucleotide change	Predicted consequence	Comments	Phenotype	Reference
COL6A1exon3	N1	c.347 G→A	p.Ser116Asn	Heterozygous change in 3/54 individuals, no confirmatory data	Mild BM	114
COL6A1exon3	N1	c.362A A→G	p.Lys121Arg	Heterozygous change in one large four generation family, not present in 190 control chromosomes	AD LGMD	23
COL6A1exon3	N1	c.428+1 G→A	p.Tyr122_Gly143del	Misplicing with partial loss of exon3; heterozygous change in one four generation family	Mild BM	118
COL6A1exon9	TH	c.821 C→T	p.Pro274Leu	Heterozygous change in 2/77 individuals (first degree relatives) who are also are compound heterozygous for Gly2047Asp in TH of COL6A3, no confirmatory data	BM	114
COL6A1exon10	TH	c.868 G→A	p.Gly290Arg	Heterozygous change in 2/77 individuals, one of them compound heterozygous for Arg677His in COL6A3	Severe BM	114
COL6A1exon11	TH	c.914 G→T	p.Gly305Val	Heterozygous change in one three generation family	BM	116
COL6A1intron11	TH	c.931-1 G→A	p.Gly311AlafsX22	Splice site moved by one base; heterozygous change in one family	BM	119
COL6A1intron13	TH	c.1003-3 C→A	p.Gly335_Asp352del	Exon14 skipping; heterozygous change in 1/78 individuals	BM	114 *
COL6A1exon14	TH	c.1022 G→T	p.Gly341Asp	Heterozygous change in one four generation family	AD LGMD	23
COL6A1exon14	TH	c.1022 G→T	p.Gly341Val	Heterozygous change in 1/78 individuals	BM	114
COL6A1exon14	TH	c.1056+1 G→A	p.Gly335_Asp352del	Exon14 skipping; heterozygous change in one family	BM	117
COL6A1exon14	TH	c.1056+1 G→A	p.Gly335_Asp352del	Exon14 skipping; heterozygous change in one individual (de novo)	BM	79
COL6A1intron14	TH	c.1056+1 G→A	p.Gly335_Asp352del	Exon14 skipping; heterozygous change in 3/77 individuals (two kindreds)	BM	114 *
COL6A1intron14	TH	c.1056+2 T→C	p.Gly335_Asp352del	Exon14 skipping; heterozygous change in one three generation family	BM	78
COL6A1exon26	TH	c.1712 A→C	p.Lys571Thr	Heterozygous change in 1/78 individuals who is also compound heterozygous for Val1985Met in COL6A3, no confirmatory data	BM	114
COL6A2exon3	N1	c.316 G→A	p.Glu106Lys	Heterozygous change in 2/76 individuals, no confirmatory data	BM	114
COL6A2exon6	TH	c.811 G→A	p.Gly271Ser	Heterozygous change in one three generation family	BM	116
COL6A2intron24	TH/C1	c.1817-3 C→G	p.Cys605_Asp606ins-GSEVSPVPPDDPATPR	Insertion of part of intron24; heterozygous change in 1/78 individuals	BM	114 *
COL6A2exon25	TH/C1	c.1861 G→A	p.Asp621Asn	Heterozygous change in one large three generation family, not present in 190 control chromosomes	AD LGMD	23
COL6A2intron25	C1	c.1970-3 C→A	p.Thr656_Ala698del	Misplicing with partial loss of exon26; heterozygous change in 1/77 individuals	BM	114 *
COL6A2exon26	C1	c.2098 G→A	p.Gly700Ser	Heterozygous change in 3/77 individuals (three first degree relatives), no confirmatory data	BM, 1/3 severe	114
COL6A2exon28	C2	c.2558 G→A	p.Arg853Gln	Heterozygous change in 1/75 individuals, no confirmatory data	BM	114
COL6A2exon28	C2	c.2663_2664dupAG	p.Gln889SerfsX6	Heterozygous change in 1/75 individuals	BM	114
COL6A3exon6	N7	c.2030 G→A	p.Arg677His	Heterozygous change in 1/79 individuals who is also compound heterozygous for Gly290Arg in TH of COL6A1	Severe BM	114
COL6A3exon7	N6	c.3040 A→G	p.Lys1014Glu	Heterozygous change in 1/79 individuals, no confirmatory data	BM	114
COL6A3exon9	N4	c.4156 G→A	p.Glu1386Lys	Heterozygous change in 1/79 individuals, no confirmatory data	BM	114
COL6A3exon10	N3	c.4399 A→G	p.Asn1467Asp	Heterozygous change in 1/79 individuals, no confirmatory data	BM	114
COL6A3exon11	N2	c.5036 G→A	p.Gly1679Glu	Heterozygous change in one large three generation family, not present in 338 control chromosomes, interferes with protein folding	BM	120
COL6A3exon11	N2	c.5036 G→A	p.Gly1679Glu	Heterozygous change in 1/79 individuals	BM	114
COL6A3exon14	N1	c.5953 G→A	p.Val1985Met	Heterozygous change in 1/79 individuals who is also compound heterozygous for Lys5711Thr in COL6A1, no confirmatory data	BM	114
COL6A3exon15	TH	c.6140 G→A	p.Gly2047Asp	Heterozygous change in 2/79 individuals (first degree relatives) who are also compound heterozygous for Pro274Leu in COL6A1	BM	114
COL6A3intron15	N1/TH	c.6156+1 G→A	p.Asp2022_Lys2052del	Exon15 skipping; heterozygous change in 1/79 individuals	Severe BM	114 *
COL6A3exon16	TH	c.6166 G→A	p.Gly2056Arg	Heterozygous change in one two generation family (de novo)	BM	115
COL6A3exon17	TH	c.6239 G→A	p.Gly2080Asp	Heterozygous change in 1/79 individuals	Severe BM	114
COL6A3exon40	C3	c.8822 C→T	p.Ala2941Val	Heterozygous change in 1/79 individuals, no confirmatory data	BM	114

All published data adjusted by authors to reference sequences NM_001848 for COL6A1, NM_001849 for COL6A2, and NM_004369 for COL6A3.
*Plus unpublished data.
AD LGMD, autosomal dominant limb girdle muscular dystrophy; BM, Bethlem myopathy.

Collagens undergo various steps of post-translational modification, such as hydroxylation of certain proline and lysine residues and glycosylation of certain hydroxylysine and asparagine residues.[70 90] The N-terminal end of α3(VI) has several potential glycosylation sites which could generate additional molecular heterogeneity by attachment of branched oligosaccharides.[70] Immunoelectronmicroscopy studies show that collagen VI forms a highly branched filamentous network in the extracellular matrix[56] which encircles interstitial collagen fibres and is particularly abundant close to the cells and in intimate contact with basement membranes surrounding muscle fibres.[7 91 92] In the murine myotendinous junction it has been found to associate with the external lamina of the muscle cells as well as with the inserting tendon collagen fibrils.[93] The amino-terminal domain of α1(VI) interacts with the carboxyl-terminal globular domain of collagen IV in vitro[7 92] and immunofluorescent labelling of skeletal muscle shows colocalisation of type IV with type VI collagen, thus suggesting that one of the major functions of collagen VI is to anchor the basement membrane to the underlying connective tissue.[7] Collagen VI also interacts with several other extracellular matrix constituents in vitro, including the fibrillar collagens I[94] and II,[95] fibril associated collagen XIV,[96] perlecan,[97] and microfibril associated glycoprotein MAGP1, which is found in association with elastin in elastic fibres.[98] A further possible role for collagen VI microfibril supramolecular assemblies is as scaffolds for the formation of the structurally critical fibrillar collagen networks through connection with the small proteoglycans decorin and biglycan,[6 95 99–101] and as fibrillogenesis modulators in proximity to growing fibrils. Apart from these mechanical functions, in vitro experiments also suggest that collagen VI is involved in cell cycle signalling[102–104] and contributes to the maintenance of tissue homeostasis. It mediates interactions of cells with the extracellular matrix,[105–112] and, through interconnection with the fibronectin microfibrillar system, is implicated in the development of the matrix supramolecular structure.[5 113] In this way the collagen VI network could have an important role in repair processes such as wound healing, as well as in tissue development and architecture.

COLLAGEN VI MUTATIONS AND MUSCLE DISEASE
Mutation detection techniques
Various mutation detection techniques have been applied to BM and UCMD patients, and the number of reported genetic changes is now in excess of 60 (summary shown in fig 2 and tables 1 and 2). As experience of mutation detection in these genes accumulates, it is clear that polymorphisms, even involving non-synonymous amino acid changes, are common.[79 114] Some particular ''hot spots'' for mutations are beginning to emerge, as is a high frequency of splice site mutations, leading to the conclusion that RNA sequencing is probably the most efficient method for mutation detection.

Mutations in BM
Single amino acid substitutions disrupting the Gly-Xaa-Yaa motif of the triple helical domain (fig 2) in COL6A1, COL6A2, or COL6A3[23 114–116] have been reported in nine different BM kindreds and thus constitute a frequent pathogenic mechanism. Their capacity to cause disease is supported by their absence in healthy controls and their segregation in large multigeneration BM families. More importantly the results of a recent study suggest that mutations towards the N-terminal region of the triple helix may cause kinking of the tetramers in the normally straight supercoiled triple helical region, thus reducing their ability to form microfibrils[73] and exerting a dominant negative effect.

Splice site mutations which cause skipping of COL6A1 exon 14 during pre-mRNA splicing and the consequent in-frame deletion of 18 amino acids from the triple helical domain of the α1(VI) chain form the second most frequent group of mutations in BM. Three different genomic mutations resulting in this missplicing have been identified in six kindreds.[78 79 114 117] The shortened α1(VI) chains allow intracellular monomer formation but lack a unique cysteine and prevent further assembly of the mutated chains into dimers and tetramers, resulting in production of half the normal amount of collagen VI.[78 79 117] The ensuing reduction of collagen VI in the extracellular matrix can be confirmed by immunohistochemical techniques in cultured fibroblasts. Other splice site mutations causing small in-frame deletions or insertions within domains flanking the triple helical domain, such as the N1 domain of COL6A1 or the C1 domain of COL6A2, have also been reported.[114 118] Ancillary data are only available for one of these[118] and it remains unclear whether their pathogenicity reflects a similar partial dominant negative effect or haploinsufficiency. The latter has been proposed as the pathogenic mechanism for a frameshifting splice site mutation which causes nonsense mediated mRNA decay.[119]

Missense mutations other than glycine substitutions within the triple helical domain have been reported for 19 kindreds.[23 114 120] However, given the highly polymorphic nature of the collagen VI genes (more than 25 non-synonymous polymorphic amino acid changes have been described to date[79 114]), it is difficult to be sure about their pathogenicity. As yet, two of these missense mutations (marked with an asterisk in fig 2) have been shown to cosegregate with the phenotype in large multigeneration families and to be absent in 190 healthy control chromosomes.[23 120] In addition, one of the latter (marked with a double asterisk) has been shown to interfere with protein folding both by homology modelling and radioimmunoassay data,[121] but additional confirmatory data are not available for the remainder of the missense changes; these therefore need to be interpreted with caution.

Mutations in UCMD
A large number of the mutations reported for patients with UCMD (10 mutations in 11 kindreds) appear to result in premature termination codons with consequent nonsense mediated mRNA decay and loss of the mutated chain. The premature termination codons occur either by direct introduction of a stop codon at the genomic level,[114 122] or through frameshift inducing genomic deletions,[114 123] insertions,[124] duplications,[114] and splice changes.[124 125]

In addition to five different missense mutations substituting glycine in the triple helical Gly-Xaa-Yaa motif which have been found in seven kindreds,[114] eight other missense changes have been reported within the triple helical and C-terminal domains of COL6A2 and the N-terminal domains of COL6A3.[114 126] As noted above, where additional confirmatory data are lacking for missense changes they need to be interpreted with caution.

Splice mutations leading to in-frame exonic deletions as well as in-frame genomic deletions form another very common mutation type in UCMD. To date 12 different mutations, located in the triple helical domains of COL6A1-3 as well as the C-terminal domains of COL6A2, have been reported in 16 kindreds.[79 114 122 126 127] A heterozygously occurring de novo large genomic deletion in COL6A1, which causes an in-frame deletion of 33 amino acids near the N-terminus of the triple helical domain, has been reported to result in a classical UCMD phenotype.[79] Its mutant α1(VI) chain preserves a unique cysteine important for dimer formation, allowing secretion of abnormal tetramers with a consequent dominant negative effect on microfibrillar assembly. A

Table 2 Genetic changes published for Ullrich congenital muscular dystrophy to date

Location and domain		Nucleotide change	Predicted consequence	Comment	Phenotype	Reference
COL6A1 intron8	TH	c.804+857_903+6del99	p.Gly269_Lys301del	Deletion exon9-10; heterozygous change in one individual (de novo)	UCMD	79
COL6A1 exon9	TH	c.841 G→A	p.Gly281Arg	Heterozygous change in 1/77 individuals who is also compound heterozygous for Arg498His in COL6A2	UCMD	114
COL6A1 exon9	TH	c.850 G→A	p.Gly284Arg	Heterozygous change in 2/77 individuals	UCMD	114
COL6A1 exon10	TH	c.868 G→A	p.Gly290Arg	Heterozygous change in 2/77 individuals, one of them compound heterozygous for Arg784His in COL6A2	Mild UCMD	114
COL6A1 intron12	TH	c.957+1 G→A	p.Gly311_Lys319del	Exon12 skipping; heterozygous change in one individual (de novo)	UCMD	126
COL6A1 intron27	TH	c.1776+1 G→A	p.Gly581_Asp592del	Exon27 skipping; homozygous change in 1/76 individuals (parents heterozygous)	UCMD	114*
COL6A2 exon3	N1	c.688_689 dupAC	p.Ile231ProfsX8	Heterozygous change in 1/77 individuals who is also compound heterozygous for complex insertion/deletion causing exon 27 skipping in COL6A2	UCMD	114*
COL6A2 intron5	TH	c.801+631_882del81	p.Gly268_Ile294del	Deletion exon6 and partial deletion of exon7; heterozygous change in one individual (mother mosaic carrier of genomic deletion)	UCMD	126
COL6A2 exon6	TH	c.847 G→A	p.Gly283Arg	Heterozygous change in 1/78 individuals	UCMD	114
COL6A2 intron9	TH	c.955-2 A→G	p.Gly319_Lys333del	Exon10 skipping; heterozygous change in 1/78 individuals	UCMD	114
COL6A2 exon13	TH	c.1147_1151 insC	p.Glu386ArgfsX64	Homozygous change in one individual (parents heterozygous)	UCMD	124
COL6A2 intron14	TH	c.1270-1 G→C	p.Gly424AlafsX138 and p.Gly424ProfsX108	Heterozygous change in one individual who is also compound heterozygous for splice change causing deletion of exon24 in COL6A2 with consequent frameshift and premature termination codon	UCMD	125
COL6A2 intron17	TH	c.1459-2 A→G	p.Gly487_Ala495delAspfsX48	Misplicing with partial loss of exon18; heterozygous change in 3 individuals; 2/3 are siblings and compound heterozygous for splice change causing deletion of exon24 in COL6A2 with consequent frameshift and premature termination codon (mother carrier); de novo in 1/3 individuals	UCMD	124
COL6A2 exon18	TH	c.1487_1512del26	p.Arg498_Pro506delGlnfsX58	Homozygous change in one individual	UCMD	123
COL6A2 exon18	TH	c.1493 G→A	p.Arg498His	Heterozygous change in 1/78 individuals who is also compound heterozygous for Gly281Arg in triple helix of COL6A1, no confirmatory data	UCMD	114
COL6A2 exon20	TH	c.1590 G→C	p.Gly531Arg	Homozygous change in 1/78 individuals	UCMD	114
COL6A2 intron23	TH	c.1770+5 G→A	p.Gly579_Thr590del	Exon23 skipping; heterozygous change in one individual who is also compound heterozygous for genomic 6bp deletion inCOL6A2 exon26 causing Ile759_Gly760del (mother carrier)	Mild UCMD	127
COL6A2 intron23	TH/C1	c.1771 C→G	p.Glu591_Cys605delThrfsX148	Deletion exon24 causing frameshift; heterozygous change in one individual who is also compound heterozygous for splice change causing partial insertion of intron14 and consequent frameshift with premature termination codon in COL6A2	UCMD	125
COL6A2 intron23	TH/C1	c.1771-1 G→A	p.Glu591_Cys605delThrfsX148	Deletion exon24 causing frameshift; heterozygous change in two individuals (siblings) who are also compound heterozygous for splice change causing partial loss of exon18 in COL6A2 with consequent frameshift and premature termination codon (father carrier)	UCMD	124
COL6A2 exon26	C1	c.2274_2279del6	p.Ile759_Gly760del	Genomic 6bp deletion; heterozygous change in one individual who is also compound heterozygous for splice change causing exon23 skipping in COL6A2 (father carrier)	Mild UCMD	127
COL6A2 exon26	C1	c.2319 C→G	p.Tyr773X	Heterozygous change in 1/78 individuals, who is also compound heterozygous for splice change predicted to cause exon27 skipping in COL6A2	UCMD	114
COL6A2 exon26	C1	c.2329 T→C	p.Cys777Arg	Homozygous change in 1/78 individuals, no confirmatory data	UCMD	114
COL6A2 exon26	C1	c.2351 G→A	p.Arg784His	Heterozygous change in 1/78 individuals, who is also compound heterozygous for Gly290Arg in triple helix of COL6A1	Mild UCMD	114
COL6A2 intron26	C1/C2	c.2423-2 A→G	p.Asp808_Thr820del	Exon27 skipping; heterozygous change in 1/78 individuals, who is also compound heterozygous for Tyr773X in COL6A2	UCMD	114
COL6A2 exon27	C1/C2	c.2455_2461+37 del46ins33	p.Asp808_Thr820del	Exon27 skipping; heterozygous change in 1/78 individuals, who is also compound heterozygous for frameshifting duplication in exon3 of COL6A2 causing premature termination codon (father carrier)	UCMD	114*
COL6A2 exon28	C2	c.2510 T→C	p.Leu837Pro	Heterozygous change in one individual also compound heterozygous for Asn897del (mother carrier)	UCMD	126
COL6A2 exon28	C2	c.2626 C→A	p.Arg876Ser	Homozygous change in 1/75 individuals, who is also compound heterozygous for Arg1395Gln and Asp1674Asn in COL6A3, no confirmatory data	UCMD	114
COL6A2 exon28	C2	c.2689_2691del3	p.Asn897del	Heterozygous change in one individual also compound heterozygous for Leu837Pro (father carrier)	UCMD	126

Table 2 Continued

Location and domain		Nucleotide change	Predicted consequence	Comment	Phenotype	Reference
COL6A3exon5	N8	c.1393 C→T	p.Arg465X	Homozygous change in one individual, N8 subject to alternative splicing (parents heterozygous)	Mild UCMD	122
COL6A3exon8	N5	c.3191 G→A	p.Arg1064Gln	Heterozygous change in 1/79 individuals, no confirmatory data	UCMD	114
COL6A3exon9	N4	c.4184 G→A	p.Arg1395Gln	Heterozygous change in 1/79 individuals, who is also compound heterozygous for Asp1674Asn in COL6A3 and homozygous for Arg876Ser in COL6A2, no confirmatory data	UCMD	114
COL6A3exon11	N2	c.5020 G→A	p.Asp1674Asn	Heterozygous change in 1/79 individuals, who is also compound heterozygous for Arg1395Gln in COL6A3 and homozygous for Arg876Ser in COL6A2, no confirmatory data	UCMD	114
COL6A3exon16	TH	c.6157-9_6177del30	p.Gly2053_Pro2070del	Exon16 skipping; heterozygous change in 1/78 individuals	UCMD	114
COL6A3intron16	TH	c.6210+1 G→A	p.Gly2053_Pro2070del	Exon16 skipping; heterozygous change in 3/78 individuals	UCMD	114
COL6A3intron16	TH	c.6210+1 G→A	p.Gly2053_Pro2070del	Exon16 skipping; heterozygous change in one individual (de novo)	UCMD	126
COL6A3exon27	TH	c.6816 G→A	p.Gly2252_Lys2272del	Exon27 skipping; homozygous change in 1/79 individuals (parents heterozygous)	UCMD	114*
COL6A3intron29	TH	c.6930+5 A→G	p.Gly2294_Lys2310del	Exon29 skipping; homozygous change in three individuals (siblings, parents heterozygous)	UCMD	122
COL6A3exon31	TH	c.7024 C→T	p.Arg2342X	Homozygous change in one individual (parents heterozygous)	UCMD	122

All published data adjusted by authors to reference sequences NM_001848 for COL6A1, NM_001849 for COL6A2 & NM_004369 for COL6A3; *plus unpublished data.
UCMD, Ullrich congenital muscular dystrophy.

similar mechanism has been documented for other heterozygously occurring N-terminal triple helical deletions.[126] However, for the vast majority of the in-frame deletions the exact nature of their pathogenic effect remains poorly documented.

UCMD is classically regarded as an autosomal recessive disease and for 21 of a total of 33 kindreds two mutations in any one of the three collagen VI genes have been described. As outlined above, there are two studies reporting a total of four UCMD cases caused by de novo dominant mutations,[79 126] and it is likely that there are other cases as well—for example, the three patients with single heterozygous splice site changes predicted to cause in-frame deletions of the triple helical domain of *COL6A3*.[114] For other patients in whom no second mutation has been identified other explanations appear more likely: Two patients with single triple helical glycine substitutions might represent a ''severe BM'' phenotype.[114] The premature termination codon within the triple helix of *COL6A2*, found as the only mutation in one patient,[124] in the same study was found to cause UCMD in another family only when present alongside another mutation, but not in heterozygous carriers.[124] It therefore seems likely that this patient might carry a second as yet undetected mutation.

Two other UCMD patients carry a sequence variation predicted to cause a glycine substitution within the triple helical domain of the α1(VI) chain alongside a separate unique putative missense mutation in *COL6A2*.[114] In addition to the above considerations, these raise the possibility that inheritance of alterations in two different *COL6* genes might cause or modify disease; however, it is important to note that as yet no confirmatory data are available for these individuals.

Pathogenicity of mutations and the UCMD carrier conundrum

It is beginning to be possible to draw some conclusions on genotype–phenotype correlations. Specific mutations tend to be strictly associated with either a BM or a UCMD phenotype (tables 1 and 2). The triple helical glycine substitution Gly290Arg in *COL6A1* forms the only exception to this observation: it was found as a heterozygous change in two patients with severe BM as well as two patients with a comparatively mild UCMD phenotype,[114] suggesting there might be a spectrum of collagen VI related disorders rather than two strictly separate entities.

Heterozygous triple helical glycine substitutions are encountered in other collagen disorders such as Ehlers–Danlos syndrome type IV (OMIM 120180) and osteogenesis imperfecta type I (OMIM166200), where their dominant negative effect has been explained by altered post-translational processing,[128] delayed triple helical monomer folding,[129] or disturbed formation of multimeric assemblies.[130] For collagen VI, glycine substitutions towards the N-terminus of the triple helix have been shown to cause kinking of the tetramers, which reduces their ability to form microfibrils.[73] Only one triple helical glycine mutation has been found in the C-terminal part and is present homozygously in a UCMD patient.[114] Artificially introduced mutations toward the triple helical C-terminus severely impair triple helical monomer association, presumably by interfering with the nucleation of triple helix folding.[73] Mutated chains would not be effectively incorporated into monomers, which should lead to virtual absence of collagen VI in the extracellular matrix when homozygously present, or secretion of half the normal amount of collagen VI if present in a heterozygous state, similar to functional haploinsufficiency. In this context it is interesting to note that virtually no BM mutations (which would be assumed to be dominantly acting) have been documented in the C-terminal part of the triple helix.
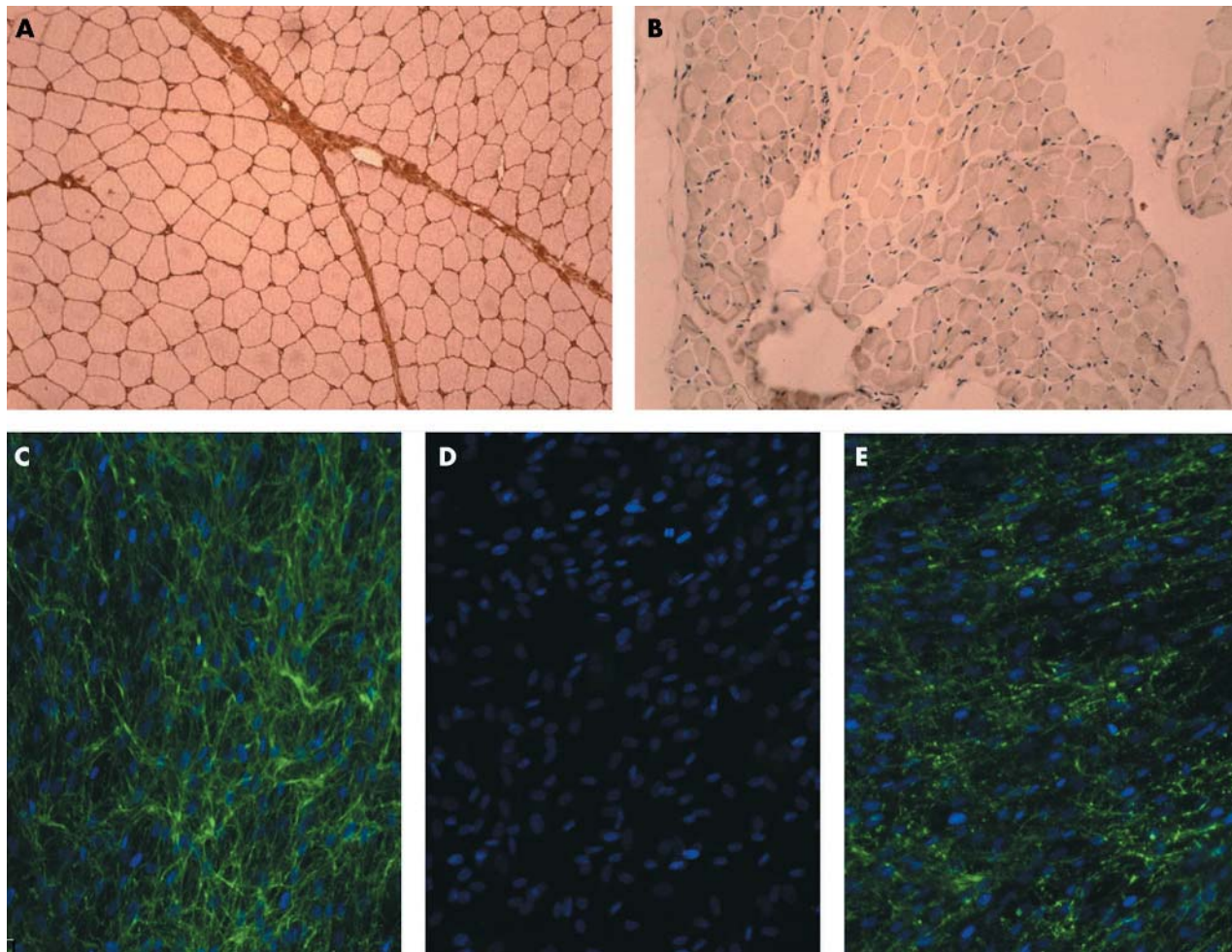
Figure 4 Immunolabelling of collagen VI in skeletal muscle (panels A and B) and cultured dermal fibroblasts (panels C to E). Collagen VI is virtually absent in basement membrane surrounding muscle fibres of a UCMD patient with a homozygous splice mutation causing in-frame deletion in N-terminal triple helical domain of α3(VI) (B) when compared with a normal control (A). Fibroblasts from an unaffected control individual (C), the UCMD patient described above (D), and a BM patient with a heterozygous missense mutation in the N2 domain of α3(VI) (E) were grown in the presence of 50 µg/ml L-ascorbic acid phosphate for five days after confluency. When stained with a collagen VI specific antibody (MAB3303, Chemicon) collagen VI is not detectable in the extracellular matrix of the UCMD patient and appears reduced and less organised in the BM patient (E) when compared with the normal control (C).

Conversely, a large number of recessively acting in-frame deletions in UCMD patients appear to cluster in the C-terminal triple helix which might interfere with monomer formation in a similar way.

It remains intriguing that the heterozygous parents of UCMD patients, in particular parents who carry a single copy of a mutation known to cause nonsense mediated mRNA decay, are clinically unaffected. A study showed that mRNA levels in fibroblasts with a single *COL6A2* nonsense mutation can be reduced to 57–73% of normal controls, but long term collagen VI extracellular matrix deposition is virtually normal.[84] If haploinsufficiency does not lead to myopathic symptoms in parents in UCMD patients, it is difficult to understand how it could cause a BM phenotype.[119] It is tempting to speculate whether phenotypic variation may arise from alternative splicing, modifying polymorphisms, or the influence of other genes yet to be identified but up to now this conundrum has remained unresolved. Alternative splicing is used to explain the surprisingly mild phenotype in a patient with a homozygous nonsense mutation in the N8 domain of *COL6A3*.[122] For a large number of the in-frame deletions and missense changes the exact pathogenic mechanism has not yet been fully elucidated, but site specific effects impairing particular steps in the complex intracellular and extracellular assembly process of collagen VI are likely to explain the degree of the negative effect of individual mutations.[86][121]

COLLAGEN VI PROTEIN EXPRESSION

Collagen VI immunohistochemistry in BM and UCMD is variable and can show anything from a mild reduction of endomysial/basal lamina collagen VI staining to a complete absence of collagen VI in the extracellular matrix[11][45][131] (fig 4B). In BM patients, immunostaining with various collagen VI antibodies in muscle biopsies is usually normal and cannot therefore be used for diagnostic purposes. Deficiency of the basal lamina component laminin β1 in muscle biopsies of adult patients with BM has been reported but is not specific.[23][132] This phenomenon has not been commonly reported in UCMD, which might in part be related to the patient's age at muscle biopsy, as secondary laminin β1deficiency may only become apparent in adulthood.[133][134] If laminin β1 is secondarily deficient, staining is characteristically reduced in the muscle fibre basal lamina but not in the basal lamina of capillaries, suggesting that collagen VI mutations specifically affect the myofibre basal lamina.[23]

Muscle biopsies from UCMD patients do often show complete or partial absence of collagen VI immunolabelling, so this can be very useful diagnostically (fig 4B). One report from Japan, however, found collagen VI to be present in the interstitium but absent from the sarcolemma in a group of patients with a clinical diagnosis of UCMD by using double immunostaining for collagen VI and IV.[127] Electron microscopic findings described a lack of connection between microfibrils in the interstitium and the basal lamina but intriguingly, these investigators were unable to identify mutations in seven of the eight patients they studied. An earlier electronmicroscopic study of collagen VI negative UCMD muscle biopsies described a space between muscle fibres and connective tissue, with folding of the plasma membrane and thickening of the basal lamina. The basal lamina appeared intact in itself, even in degenerating muscle fibres, with disorganised myofibrils, indicating a loose connection between the basal lamina and other extracellular matrix collagens.[135] Capillaries in muscle biopsy specimens from UCMD patients have been described as being devoid of collagen VI, with associated capillary fenestration on electronmicroscopy[136]; however, in skin biopsies of UCMD patients the papillary dermis and the hair follicles show a reduced amount of collagen VI protein while vessels, peripheral nerves, smooth muscle, and sweat glands show labelling comparable with normal controls.[11]

DIAGNOSIS OF COLLAGEN VI RELATED DISORDERS AND THERAPEUTIC CONSIDERATIONS

Detection of mutations in the three collagen VI genes remains the gold standard for diagnosis. Mutation detection rates in one high throughput genomic sequencing study of 79 patients reached 66% for BM and 79% for UCMD,[114] but seemed much lower in a study of a Japanese cohort of patients.[127 137] Owing to the large size of the genes involved, diagnostic mutation analysis is currently problematic. The diagnosis of BM and UCMD depends on the typical clinical features, with the serum creatine kinase concentration usually being normal or only mildly increased. Muscle biopsy shows myopathic or dystrophic changes, with collagen VI immunolabelling of the endomysium and basal lamina usually normal in BM (with the exception of a few cases showing patchy minor abnormalities) and ranging from absent to moderately or markedly reduced in UCMD (fig 4, panel B). Collagen VI immunolabelling studies on dermal fibroblast cultures appear to be more sensitive than muscle immunohistochemistry and can be a useful adjunct (fig 4, panels C to E). In the past, linkage analysis has also been used for UCMD families,[138] but the recent description of de novo dominant mutations causing UCMD[79 114] means this can no longer be regarded as a reliable method to exclude collagen VI related disease.

Genetic counselling

In fully characterised families genetic counselling is straightforward. BM is inherited in an autosomal dominant manner and UCMD is classically described as an autosomal recessive disorder. However, some caveats have emerged from our better understanding of these disorders: First, distinguishing between BM and UCMD in a neonate may not be easy, as BM can occur de novo[115] and may present at birth. Further follow up should allow the recognition of a milder phenotype; in fact, it is becoming clear that BM and UCMD represent opposite end points of a clinical continuum in which individuals presenting with intermediate phenotypes could be considered to have either ''mild UCMD'' or ''severe BM.'' In addition, autosomal dominant inheritance now needs to be considered when counselling families for UCMD. Delineation of a specific mutation which has previously been

consistently recognised as being associated with either BM or UCMD may help to resolve some of these counselling issues.

Prenatal diagnosis

Prenatal diagnosis for UCMD has been undertaken in a consanguineous family where the disease was consistent with linkage to COL6A3, and collagen VI immunolabelling in the proband's skeletal muscle was severely reduced.[139] Haplotype analysis together with the immunohistochemical absence of collagen VI in chorionic villus samples were used to determine that the fetus was affected, and later on this was confirmed by examining fetal skeletal muscle. For families where the muscle biopsy of the proband shows absence of collagen VI immunolabelling, examination of a chorionic villus biopsy—preferably in conjunction with linkage analysis—thus does offer a potential route for prenatal diagnosis even if the causative mutation in the family is not known.

Specific therapeutic issues

Treatment for patients with BM and UCMD is supportive and follows identical principles, but depends in its intensity on the severity of symptoms and the age of onset.

Children with a severe UCMD phenotype require active management as soon as the diagnosis is established, to promote mobility and independence. Early mobilisation in a standing frame is important to achieve upright posture and protect against the development of scoliosis and other contractures. Regular stretching and splinting are used to keep contractures at bay and maintain mobility both for UCMD and BM patients, but there have been no formal studies on the efficacy of these measures. The contractures of UCMD patients in particular tend to be aggressive and may require surgical release on more than one occasion in order to maintain or achieve a certain level of mobility. In BM the contractures may be of a strikingly dynamic nature during early childhood, which makes it advisable to try to delay possible orthopaedic management until established contractures emerge. Scoliosis often develops in UCMD patients in the first or second decade of life and may require active management including spinal surgery to prevent progression.

Respiratory failure is a common complication of UCMD, whereas in BM it appears to be rare and related to more severe weakness in later life. Follow up with regular assessments of respiratory function, including spirometry and overnight pulse oximetry studies, is important for all patients with collagen VI related disorders to detect asymptomatic decline.[140] Respiratory support with nocturnal ventilation usually becomes necessary in the first or second decade for UCMD patients and can be effective in reducing symptoms, promoting quality of life, and allowing normal schooling.[140 141] In BM, respiratory failure with diaphragmatic involvement may supervene even before loss of ambulation, and symptoms of nocturnal hypoventilation respond well to non-invasive respiratory support such as mask ventilation.[140] Prophylaxis of chest infections with influenza and pneumococcal vaccination and physiotherapy, as well as early and aggressive use of antibiotics, may prevent further respiratory problems in both BM and UCMD.[140]

In addition, feeding difficulties in UCMD patients can manifest as failure to thrive or excessive time taken to finish eating a meal. Consultation with a nutrition specialist may be needed to boost energy intake; for serious problems, feeding by gastrostomy may be the best solution to promote a normal weight gain.

Potential future therapeutic options

A collagen VI deficient ($Col6a1^{-/-}$) mouse model with a muscle histology phenotype resembling BM has been generated.[142] Recently, mitochondrial dysfunction was shown to be implicated in the pathogenesis of its myopathic phenotype, with the

muscle ultrastructural defects appearing to be at least partially reversible by treatment with cyclosporin A.[143] These findings clearly have interesting implications with regard to pharmacological treatment options for collagen VI related disorders. However, before embarking on treatment trials based on this single study it will be important to demonstrate that a similar cyclosporin A responsive mitochondrial dysfunction can be demonstrated in humans patients affected by BM or UCMD, and to check whether the mitochondrial dysfunction found in collagen VI deficient mice is specific for this disorder or might be present in mice models of other muscular dystrophies and thus represent a common pathogenic end point.

CONCLUSIONS

The genetics of the collagen VI related muscular dystrophies are complex. Independent description of the two distinct clinical phenotypes, Bethlem myopathy and Ullrich congenital muscular dystrophy, was followed by the discovery that both of these disorders are caused by mutations in collagen VI. Subsequent reports of clinical phenotypes and associated mutations have led to a blurring of the boundaries between these disorders with classical BM and UCMD now forming opposite end points of a phenotypic spectrum. Collagen VI is composed of three different peptide chains which are encoded by three large genes, and its assembly involves several different stages. Different mutations have been shown to have variable effects on protein assembly, secretion, and its ability to form a functioning extracellular network. This multitude of possible mutational mechanisms and modes of inheritance has important consequences for the genetic counselling of patients and their families. Currently no curative therapeutic approaches have reached a clinical level, but supportive treatment can dramatically improve longevity and quality of life.

DATA ACCESS

Accession numbers and URLs for data presented herein are as follows:

● GenBank, http://www.ncbi.nlm.nih.gov/GenBank/ (for sequences of the *COL6A1* transcript [accession number NM_001848], the *COL6A2* isoforms 2c2 [accession number NM_001849], 2c2a [accession number NM_058174] and 2c2a' [NM_058175] as well as the full length isoform of *COL6A3* [accession number NM_004369] and its 4 variant transcripts [NM_057164, NM_057165, NM_057166 and NM_057167])

● Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/(for BM [MIM 158810] and UCMD [MIM 254090])

● http://www.genome.utah.edu/DMD/collagensnps/(for collagen VI sequence variations)

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REFERENCES

1 **Sewry CA**, Muntoni F. Inherited disorders of the extracellular matrix. *Curr Opin Neurol* 1999;**12**:519–26.

2 **Helbling-Leclerc A**, Zhang X, Topaloglu H, Cruaud C, Tesson F, Weissenbach J, Tome FM, Schwartz K, Fardeau M, Tryggvason K. Mutations in the laminin alpha 2-chain gene (LAMA2) cause merosin-deficient congenital muscular dystrophy. *Nat Genet* 1995;**11**:216–18.

3 **Timpl R**, Brown JC. Supramolecular assembly of basement membranes. *BioEssays* 1996;**18**:123–32.

4 **Sanes JR**. The basement membrane/basal lamina of skeletal muscle. *J Biol Chem* 2003;**278**:12601–4.

5 **Sabatelli P**, Bonaldo P, Lattanzi G, Braghetta P, Bergamin N, Capanni C, Mattioli E, Columbaro M, Ognibene A, Pepe G, Bertini E, Merlini L, Maraldi NM, Squarzoni S. Collagen VI deficiency affects the organization of fibronectin in the extracellular matrix of cultured fibroblasts. *Matrix Biol* 2001;**20**:475–86.

6 **Wiberg C**, Klatt AR, Wagener R, Paulsson M, Bateman JF, Heinegard D, Morgelin M. Complexes of matrilin-1 and biglycan or decorin connect collagen VI microfibrils to both collagen II and aggrecan. *J Biol Chem* 2003;**278**:37698–704.

7 **Kuo HJ**, Maslen CL, Keene DR, Glanville RW. Type VI collagen anchors endothelial basement membranes by interacting with type IV collagen. *J Biol Chem* 1997;**272**:26522–9.

8 **Bethlem J**, Van Wijngaarden GK. Benign myopathy with autosomal dominant inheritance: a report of three pedigrees. *Brain* 1976;**99**:91–100.

9 **Arts WF**, Bethlem J, Volkers WS. Further investigations on benign myopathy with autosomal dominant inheritance. *J Neurol* 1978;**217**:201–6.

10 **Somer H**, Laulamaa V, Paljarvi L, Partenen J, Lamminen A, Pihko H, Sariola H, Haltia M. Benign muscular dystrophy with autosomal dominant inheritance. *Neuromusc Disord* 1991;**1**:267–74.

11 **Pepe G**, Bertini E, Bonaldo P, Bushby K, Giusti B, de Visser M, Guicheney P, Lattanzi G, Merlini L, Muntoni F, Nishino I, Nonaka I, Yaou RB, Sabatelli P, Sewry C, Topaloglu H, van der Kooi A. Bethlem myopathy (BETHLEM) and Ullrich scleroatonic muscular dystrophy: 100th ENMC international workshop, 23–24 November 2001, Naarden, The Netherlands. *Neuromuscul Disord* 2002;**12**:984–93.

12 **Pepe G**, de Visser M, Bertini E, Bushby K, Camacho Vanegas O, Chu ML, Lattanzi G, Merlini L, Muntoni F, Urtizberea A. Bethlem myopathy (BETHLEM) 86th ENMC international workshop, 10–11 November 2000, Naarden, The Netherlands. *Neuromuscul Disord* 2002;**12**:296–305.

13 **Bailey RO**, Dentinger MP, Toms ME, Hans MB. Benign muscular dystrophy with contractures: a new syndrome? *Acta Neurol Scand* 1986;**73**:439–43.

14 **Schmalbruch H**, Kamieniecka Z, Fuglsang-Frederiksen A, Trojaborg W. Benign congenital muscular dystrophy with autosomal dominant heredity: problems of classification. *J Neurol* 1987;**234**:146–51.

15 **Mohire MD**, Tandan R, Fries TJ, Little BW, Pendlebury WW, Bradley WG. Early-onset benign autosomal dominant limb-girdle myopathy with contractures (Bethlem myopathy). *Neurology* 1988;**38**:573–80.

16 **Tachi N**, Tachi M, Sasaki K, Imamura S. Early-onset benign autosomal dominant limb-girdle myopathy with contractures (Bethlem myopathy). *Pediatr Neurol* 1989;**5**:232–6.

17 **Merlini L**, Morandi L, Granata C, Ballestrazzi A. Bethlem myopathy: early-onset benign autosomal dominant myopathy with contractures. Description of two new families. *Neuromuscul Disord* 1994;**4**:503–11.

18 **Nielsen JF**, Jakobsen J. A Danish family with limb-girdle muscular dystrophy with autosomal dominant inheritance. *Neuromuscul Disord* 1994;**4**:139–142.

19 **Lien LM**, Yang CC, Chen WH, Chiu HC. Bethlem myopathy in a Taiwanese family. *J Formos Med Assoc* 2001;**100**:416–19.

20 **De Visser M**, van der Kooi A, Jobsis GJ. Bethlem Myopathy. In: Engel AG, Franzini-Armstrong C, eds. *Myology*. New York: McGraw-Hill, 2004:1135–46.

21 **Jobsis GJ**, Boers JM, Barth PG, de Visser M. Bethlem myopathy: a slowly progressive congenital muscular dystrophy with contractures. *Brain* 1999;**122**:649–55.

22 **Gordon ES**, Hoffman EP. The ABC's of limb-girdle muscular dystrophy: alpha-sarcoglycanopathy, Bethlem myopathy, calpainopathy and more. *Curr Opin Neurol* 2001;**14**:567–73.

23 **Scacheri PC**, Gillanders EM, Subramony SH, Vedanarayanan V, Crowe CA, Thakore N, Bingler M, Hoffman EP. Novel mutations in collagen VI genes: expansion of the Bethlem myopathy phenotype. *Neurology* 2002;**58**:593–602.

24 **Bushby KMD**. The limb-girdle muscular dystrophies. In: Emery AEH, ed. *The muscular dystrophies*. Oxford: Oxford University Press, 2001:109–36.

25 **Toniolo D**. Emery-Dreifuss muscular dystrophy. In: Emery AEH, ed. *The muscular dystrophies*. Oxford: Oxford University Press, 2001:95–108.

26 **Mercuri E**, Cini C, Counsell S, Allsop J, Zolkipli Z, Jungbluth H, Sewry C, Brown SC, Pepe G, Muntoni F. Muscle MRI findings in a three-generation family affected by Bethlem myopathy. *Eur J Paediatr Neurol* 2002;**6**:309–14.

27 **Bonnemann CG**, Brockmann K, Hanefeld F. Muscle ultrasound in Bethlem myopathy. *Neuropediatrics* 2003;**34**:335–6.

28 **de Visser M**, de Voogt WG, la Riviere GV. The heart in Becker muscular dystrophy, facioscapulohumeral dystrophy, and Bethlem myopathy. *Muscle Nerve* 1992;**15**:591–6.

29 **Haq RU**, Speer MC, Chu M-L, Tandan R. Respiratory muscle involvement in Bethlem myopathy. *Neurology* 1999;**52**:174–6.

30 **Muntoni F**, Voit T. The congenital muscular dystrophies in 2004: a century of exciting progress. *Neuromuscul Disord* 2004;**14**:635–49.

31 **Ullrich O**. Kongenitale, atonisch-sklerotische Muskeldystrophie. *Monatsschr Kinderheilkd* 1930;**47**:502–510.

32 **Ullrich O**. Kongenitale, atonisch-sklerotische Muskeldystrophie, ein weiterer Typus der heredodegenerativen Erkrankungen des neuromuskulaeren Systems. *Z Ges Neurol Psychiatr* 1930;**126**:171–201.

33 **Stoeber E**. Ueber atonisch-sklerotische Muskeldystrophie (Typ Ullrich). *Z Kinderheilkd* 1939;**60**:279–84.

34 **Nihei K**, Kamoshita S, Atsumi T. A case of Ullrich's disease (Kongenitale, Atonisch-Sklerotische Muskeldystrophie). *Brain Dev* 1979;**1**:61–7.

35 **Goett H**, Josten EA. Beitrag zur kongenitalen atonisch-sklerotischen Muskeldystrophie (Typ Ullrich). *Z Kinderheilkd* 1954;**75**:105–18.

36 **Rotthauwe HW**, Kowalewski S, Mumenthaler M. Kongenitale Muskeldystrophie. *Z Kinderheilkd* 1969;**106**:131–62.

37 **Schneider H**. Die atonisch-sklerotische Muskeldystrophie (Ullrich) im Rahmen mesodermaler Dysplasien. *Z Orthop Grenzgeb* 1957;**88**:387–404.

38 **Nonaka I**, Une Y, Ishihara T, Miyoshino S, Nakashima T, Sugita H. A clinical and histological study of Ullrich's disease (congenital atonic-sclerotic muscular dystrophy). *Neuropediatrics* 1981;**12**:197–208.

39 **Serratrice G**, Pellissier JF. [A forgotten muscular dystrophy: Ullrich's disease.] *Rev Neurol (Paris)* 1983;**139**:523–5.

40 **De Paillette L**, Aicardi J, Goutieres F. Ullrich's congenital atonic sclerotic muscular dystrophy. A case report. *J Neurol* 1989;**236**:108–10.

41 **Santoro L**, Marmo C, Gasparo-Rippa P, Toscano A, Sadile F, Barbieri F. A new case of Ullrich's disease. *Clin Neuropathol* 1989;**8**:69–71.

42 **Furukawa T**, Toyokura Y. Congenital, hypotonic-sclerotic muscular dystrophy. *J Med Genet* 1977;**14**:426–9.

43 **Mercuri E**, Yuva Y, Brown SC, Brockington M, Kinali M, Jungbluth H, Feng L, Sewry CA, Muntoni F. Collagen VI involvement in Ullrich syndrome: a clinical, genetic, and immunohistochemical study. *Neurology* 2002;**58**:1354–9.

44 **Voit T**. Congenital muscular dystrophies: 1997 update. *Brain Dev* 1998;**20**:65–74.

45 **Muntoni F**, Bertini E, Bonnemann C, Brockington M, Brown S, Bushby K, Fiszman M, Korner C, Mercuri E, Merlini L, Hewitt J, Quijano-Roy S, Romero N, Squarzoni S, Sewry CA, Straub V, Topaloglu H, Haliloglu G, Voit T, Wewer U, Guicheney P. 98th ENMC International Workshop on Congenital Muscular Dystrophy (CMD), 7th Workshop of the International Consortium on CMD, 2nd Workshop of the MYO CLUSTER project GENRE. 26–28th October, 2001, Naarden, The Netherlands. *Neuromuscul Disord* 2002;**12**:889–96.

46 **Mercuri E**, Muntoni F. Congenital muscular dystrophies. In: Emery AEH, ed. *The muscular dystrophies*. Oxford: Oxford University Press, 2001:10–38.

47 **Mercuri E**, Cini C, Pichiecchio A, Allsop J, Counsell S, Zolkipli Z, Messina S, Kinali M, Brown SC, Jimenez C, Brockington M, Yuva Y, Sewry CA, Muntoni F. Muscle magnetic resonance imaging in patients with congenital muscular dystrophy and Ullrich phenotype. *Neuromuscul Disord* 2003;**13**:554–8.

48 **Voit T**, Tome FMS. The congenital muscular dystrophies. In: Engel AE, Franzini-Armstrong C, eds. *Myology*. New York: McGraw-Hill, 2004:1203–38.

49 **Biros I**, Forrest S. Spinal muscular atrophy: untangling the knot? *J Med Genet* 1999;**36**:1–8.

50 **Rudnik-Schoneborn S**, De Visser M, Zerres K. Spinal muscular atrophies. In: Engel AE, Franzini-Armstrong C, eds. *Myology*. New York: McGraw-Hill, 2004:1845–64.

51 **Pyeritz RE**, Dietz HC. Marfan syndrome and other microfibrillar disorders. In: Royce PM, Steinmann B, eds. *Connective tissue and its heritable disorders*. New York: Wiley-Liss, 2002:585–626.

52 **Steinmann B**, Royce PM, Superti-Furga A. The Ehlers-Danlos syndrome. In: Royce PM, Steinmann B, eds. *Connective tissue and its heritable disorders*. New York: Wiley-Liss, 2002:431–523.

53 **Jobsis GJ**, Bolhuis PA, Boers JM, Baas F, Wolterman RA, Hensels GW, de Visser M. Genetic localization of Bethlem myopathy. *Neurology* 1996;**46**:779–82.

54 **Speer MC**, Tandan R, Rao PN, Fries T, Stajich JM, Bolhuis PA, Jobsis GJ, Vance JM, Viles KD, Sheffield K, James C, Kahler SG, Pettenati M, Gilbert JR, Denton PH, Yamaoka LH, Pericak-Vance MA. Evidence for locus heterogeneity in the Bethlem myopathy and linkage to 2q37. *Hum Mol Genet* 1996;**5**:1043–6.

55 **Hessle H**, Engvall E. Type VI collagen. Studies on its localization, structure, and biosynthetic form with monoclonal antibodies. *J Biol Chem* 1984;**259**:3955–61.

56 **von der Mark H**, Aumailley M, Wick G, Fleischmajer R, Timpl R. Immunochemistry, genuine size and tissue localization of collagen VI. *Eur J Biochem* 1984;**142**:493–502.

57 **Bruns RR**. Beaded filaments and long-spacing fibrils: relation to type VI collagen. *J Ultrastruct Res* 1984;**89**:136–45.

58 **Heiskanen M**, Saitta B, Palotie A, Chu ML. Head to tail organization of the human COL6A1 and COL6A2 genes by fiber-FISH. *Genomics* 1995;**29**:801–3.

59 **Weil D**, Mattei MG, Passage E, N'Guyen VC, Pribula-Conway D, Mann K, Deutzmann R, Timpl R, Chu ML. Cloning and chromosomal localization of human genes encoding the three chains of type VI collagen. *Am J Hum Genet* 1988;**42**:435–45.

60 **Chu ML**, Mann K, Deutzmann R, Pribula-Conway D, Hsu-Chen CC, Bernard MP, Timpl R. Characterization of three constituent chains of collagen type VI by peptide sequences and cDNA clones. *Eur J Biochem* 1987;**168**:309–17.

61 **Chu ML**, Zhang RZ, Pan TC, Stokes D, Conway D, Kuo HJ, Glanville R, Mayer U, Mann K, Deutzmann R. Mosaic structure of globular domains in the human type VI collagen alpha 3 chain: similarity to von Willebrand factor, fibronectin, actin, salivary proteins and aprotinin type protease inhibitors. *EMBO J* 1990;**9**:385–93.

62 **Chu ML**, Pan TC, Conway D, Saitta B, Stokes D, Kuo HJ, Glanville RW, Timpl R, Mann K, Deutzmann R. The structure of type VI collagen. *Ann NY Acad Sci* 1990;**580**:55–63.

63 **Trikka D**, Davis T, Lapenta V, Brahe C, Kessling AM. Human COL6A1: genomic characterization of the globular domains, structural and evolutionary comparison with COL6A2. *Mamm Genome* 1997;**8**:342–5.

64 **Saitta B**, Wang YM, Renkart L, Zhang RZ, Pan TC, Timpl R, Chu ML. The exon organization of the triple-helical coding regions of the human alpha 1(VI) and alpha 2(VI) collagen genes is highly similar. *Genomics* 1991;**11**:145–53.

65 **Saitta B**, Chu ML. Characterization of the human alpha 1(VI) collagen promoter and its comparison with human alpha 2(VI) promoters. *Eur J Biochem* 1995;**234**:542–9.

66 **Saitta B**, Chu ML. Two promoters control the transcription of the human alpha 2(VI) collagen gene. *Eur J Biochem* 1994;**223**:675–82.

67 **Saitta B**, Timpl R, Chu ML. Human alpha 2(VI) collagen gene. Heterogeneity at the 5′-untranslated region generated by an alternate exon. *J Biol Chem* 1992;**267**:6188–96.

68 **Saitta B**, Stokes DG, Vissing H, Timpl R, Chu ML. Alternative splicing of the human alpha 2(VI) collagen gene generates multiple mRNA transcripts which predict three protein variants with distinct carboxyl termini. *J Biol Chem* 1990;**265**:6473–80.

69 **Stokes DG**, Saitta B, Timpl R, Chu ML. Human alpha 3(VI) collagen gene. Characterization of exons coding for the amino-terminal globular domain and alternative splicing in normal and tumor cells. *J Biol Chem* 1991;**266**:8626–33.

70 **Zanussi S**, Doliana R, Segat D, Bonaldo P, Colombatti A. The human type VI collagen gene. mRNA and protein variants of the alpha 3 chain generated by alternative splicing of an additional 5-end exon. *J Biol Chem* 1992;**267**:24082–9.

71 **Dziadek M**, Kazenwadel JS, Hendrey JA, Pan TC, Zhang RZ, Chu ML. Alternative splicing of transcripts for the alpha 3 chain of mouse collagen VI: identification of an abundant isoform lacking domains N7-N10 in mouse and human. *Matrix Biol* 2002;**21**:227–41.

72 **Arnoux B**, Ducruix A, Prange T. Anisotropic behaviour of the C-terminal Kunitz-type domain of the alpha3 chain of human type VI collagen at atomic resolution (0.9 A). *Acta Crystallogr D Biol Crystallogr* 2002;**58**:1252–4.

73 **Lamande SR**, Morgelin M, Selan C, Jobsis GJ, Baas F, Bateman JF. Kinked collagen VI tetramers and reduced microfibril formation as a result of Bethlem myopathy and introduced triple helical glycine mutations. *J Biol Chem* 2002;**277**:1949–56.

74 **Persikov AV**, Ramshaw JA, Kirkpatrick A, Brodsky B. Amino acid propensities for the collagen triple-helix. *Biochemistry* 2000;**39**:14960–7.

75 **Colombatti A**, Mucignat MT, Bonaldo P. Secretion and matrix assembly of recombinant type VI collagen. *J Biol Chem* 1995;**270**:13105–11.

76 **Furthmayr H**, Wiedemann H, Timpl R, Odermatt E, Engel J. Electron-microscopical approach to a structural model of intima collagen. *Biochem J* 1983;**211**:303–11.

77 **Chu ML**, Conway D, Pan TC, Baldwin C, Mann K, Deutzmann R, Timpl R. Amino acid sequence of the triple-helical domain of human collagen type VI. *J Biol Chem* 1988;**263**:18601–6.

78 **Pepe G**, Giusti B, Bertini E, Brunelli T, Saitta B, Comeglio P, Bolognese A, Merlini L, Federici G, Abbate R, Chu ML. A heterozygous splice site mutation in COL6A1 leading to an in-frame deletion of the alpha1(VI) collagen chain in an Italian family affected by Bethlem myopathy. *Biochem Biophys Res Commun* 1999;**258**:802–7.

79 **Pan TC**, Zhang RZ, Sudano DG, Marie SK, Bonnemann CG, Chu ML. New molecular mechanism for Ullrich congenital muscular dystrophy: a heterozygous in-frame deletion in the COL6A1 gene causes a severe phenotype. *Am J Hum Genet* 2003;**73**:355–69.

80 **Shuttleworth A**, Ball S, Baldock C, Fakhoury H, Kielty CM. Functional role of A-domains in type VI collagen. *Biochem Soc Trans* 1999;**27**:821–3.

81 **Ball SG**, Baldock C, Kielty CM, Shuttleworth CA. The role of the C1 and C2 a-domains in type VI collagen assembly. *J Biol Chem* 2001;**276**:7422–30.

82 **Ball S**, Bella J, Kielty C, Shuttleworth A. Structural basis of type VI collagen dimer formation. *J Biol Chem* 2003;**278**:15326–32.

83 **Lamande SR**, Sigalas E, Pan TC, Chu ML, Dziadek M, Timpl R, Bateman JF. The role of the alpha3(VI) chain in collagen VI assembly. Expression of an alpha3(VI) chain lacking N-terminal modules N10-N7 restores collagen VI assembly, secretion, and matrix deposition in an alpha3(VI)-deficient cell line. *J Biol Chem* 1998;**273**:7423–30.

84 **Zhang RZ**, Sabatelli P, Pan TC, Squarzoni S, Mattioli E, Bertini E, Pepe G, Chu ML. Effects on collagen VI mRNA stability and microfibrillar assembly of three COL6A2 mutations in two families with Ullrich congenital muscular dystrophy. *J Biol Chem* 2002;**277**:43557–64.

85 **Knupp C**, Squire JM. A new twist in the collagen story – the type VI segmented supercoil. *EMBO J* 2001;**20**:372–6.

86 **Fitzgerald J**, Morgelin M, Selan C, Wiberg C, Keene DR, Lamande SR, Bateman JF. The N-terminal N5 subdomain of the alpha 3(VI) chain is important for collagen VI microfibril formation. *J Biol Chem* 2001;**276**:187–93.

87 **Engvall E**, Hessle H, Klier G. Molecular assembly, secretion, and matrix deposition of type VI collagen. *J Cell Biol* 1986;**102**:703–10.

88 **Baldock C**, Sherratt MJ, Shuttleworth CA, Kielty CM. The supramolecular organization of collagen VI microfibrils. *J Mol Biol* 2003;**330**:297–307.

89 **Engel J**, Furthmayr H, Odermatt E, von der Mark H, Aumailley M, Fleischmajer R, Timpl R. Structure and macromolecular organization of type VI collagen. *Ann NY Acad Sci* 1985;**460**:25–37.

90 **Myllyharju J**, Kivirikko KI. Collagens and collagen-related diseases. *Ann Med* 2001;**33**:7–21.

91 **Bruns RR**, Press W, Engvall E, Timpl R, Gross J. Type VI collagen in extracellular, 100-nm periodic filaments and fibrils: identification by immunoelectron microscopy. *J Cell Biol* 1986;**103**:393–404.

92 **Keene DR**, Engvall E, Glanville RW. Ultrastructure of type VI collagen in human skin and cartilage suggests an anchoring function for this filamentous network. *J Cell Biol* 1988;**107**:1995–2006.

93 **Senga K**, Kobayashi M, Hattori H, Yasue K, Mizutani H, Ueda M, Hoshino T. Type VI collagen in mouse masseter tendon, from osseous attachment to myotendinous junction. *Anat Rec* 1995;**243**:294–302.

94 **Bonaldo P**, Russo V, Bucciotti F, Doliana R, Colombatti A. Structural and functional features of the alpha 3 chain indicate a bridging role for chicken collagen VI in connective tissues. *Biochemistry* 1990;**29**:1245–54.

95 **Bidanset DJ**, Guidry C, Rosenberg LC, Choi HU, Timpl R, Hook M. Binding of the proteoglycan decorin to collagen type VI. *J Biol Chem* 1992;**267**:5250–6.

96 **Brown JC**, Mann K, Wiedemann H, Timpl R. Structure and binding properties of collagen type XIV isolated from human placenta. *J Cell Biol* 1993;**120**:557–67.

97 **Tillet E**, Wiedemann H, Golbik R, Pan TC, Zhang RZ, Mann K, Chu ML, Timpl R. Recombinant expression and structural and binding properties of alpha 1(VI) and alpha 2(VI) chains of human collagen type VI. *Eur J Biochem* 1994;**221**:177–85.

98 **Finnis ML**, Gibson MA. Microfibril-associated glycoprotein-1 (MAGP-1) binds to the pepsin-resistant domain of the alpha3(VI) chain of type VI collagen. *J Biol Chem* 1997;**272**:22817–23.

99 **Specks U**, Mayer U, Nischt R, Spissinger T, Mann K, Timpl R, Engel J, Chu ML. Structure of recombinant N-terminal globule of type VI collagen alpha 3 chain and its binding to heparin and hyaluronan. *EMBO J* 1992;**11**:4281–90.

100 **Wiberg C**, Hedbom E, Khairullina A, Lamande SR, Oldberg A, Timpl R, Morgelin M, Heinegard D. Biglycan and decorin bind close to the n-terminal region of the collagen VI triple helix. *J Biol Chem* 2001;**276**:18947–52.

101 **Wiberg C**, Heinegard D, Wenglen C, Timpl R, Morgelin M. Biglycan organizes collagen VI into hexagonal-like networks resembling tissue structures. *J Biol Chem* 2002;**277**:49120–6.

102 **Howell SJ**, Doane KJ. Type VI collagen increases cell survival and prevents anti-beta 1 integrin-mediated apoptosis. *Exp Cell Res* 1998;**241**:230–41.

103 **Ruhl M**, Sahin E, Johannsen M, Somasundaram R, Manski D, Riecken EO, Schuppan D. Soluble collagen VI drives serum-starved fibroblasts through S phase and prevents apoptosis via down-regulation of Bax. *J Biol Chem* 1999;**274**:34361–8.

104 **Ruhl M**, Johannsen M, Atkinson J, Manski D, Sahin E, Somasundaram R, Riecken EO, Schuppan D. Soluble collagen VI induces tyrosine phosphorylation of paxillin and focal adhesion kinase and activates the MAP kinase erk2 in fibroblasts. *Exp Cell Res* 1999;**250**:548–57.

105 **Pfaff M**, Aumailley M, Specks U, Knolle J, Zerwes HG, Timpl R. Integrin and Arg-Gly-Asp dependence of cell adhesion to the native and unfolded triple helix of collagen type VI. *Exp Cell Res* 1993;**206**:167–76.

106 **Atkinson JC**, Ruhl M, Becker J, Ackermann R, Schuppan D. Collagen VI regulates normal and transformed mesenchymal cell proliferation in vitro. *Exp Cell Res* 1996;**228**:283–91.

107 **Somasundaram R**, Ruehl M, Schaefer B, Schmid M, Ackermann R, Riecken EO, Zeitz M, Schuppan D. Interstitial collagens I, III, and VI sequester and modulate the multifunctional cytokine oncostatin M. *J Biol Chem* 2002;**277**:3242–6.

108 **Tillet E**, Ruggiero F, Nishiyama A, Stallcup WB. The membrane-spanning proteoglycan NG2 binds to collagens V and VI through the central nonglobular domain of its core protein. *J Biol Chem* 1997;**272**:10769–76.

109 **Nishiyama A**, Stallcup WB. Expression of NG2 proteoglycan causes retention of type VI collagen on the cell surface. *Mol Biol Cell* 1993;**4**:1097–108.

110 **Stallcup WB**, Dahlin K, Healy P. Interaction of the NG2 chondroitin sulfate proteoglycan with type VI collagen. *J Cell Biol* 1990;**111**:3177–88.

111 **Burg MA**, Tillet E, Timpl R, Stallcup WB. Binding of the NG2 proteoglycan to type VI collagen and other extracellular matrix molecules. *J Biol Chem* 1996;**271**:26110–16.

112 **Tillet E**, Gential B, Garrone R, Stallcup WB. NG2 proteoglycan mediates beta1 integrin-independent cell adhesion and spreading on collagen VI. *J Cell Biochem* 2002;**86**:726–36.

113 **Hu J**, Higuchi I, Shiraishi T, Suehara M, Niiyama T, Horikiri T, Uchida Y, Saito A, Osame M. Fibronectin receptor reduction in skin and fibroblasts of patients with Ullrich's disease. *Muscle Nerve* 2002;**26**:696–701.

114 **Lampe AK**, Dunn DM, von Niederhausern AC, Hamil C, Aoyagi A, Laval SH, Marie SK, Chu M-L, Swoboda K, Muntoni F, Bonnemann CG, Flanigan KM, Bushby KMD, Weiss RB. Automated genomic sequence analysis of the three collagen VI genes: applications to Ullrich congenital muscular dystrophy and Bethlem myopathy. *J Med Genet* 2005;**42**:108–20.

115 **Pepe G**, Bertini E, Giusti B, Brunelli T, Comeglio P, Saitta B, Merlini L, Chu ML, Federici G, Abbate R. A novel de novo mutation in the triple helix of the COL6A3 gene in a two-generation Italian family affected by Bethlem myopathy. A diagnostic approach in the mutations' screening of type VI collagen. *Neuromuscul Disord* 1999;**9**:264–71.

116 **Jobsis GJ**, Keizers H, Vreijling JP, de Visser M, Speer MC, Wolterman RA, Baas F, Bolhuis PA. Type VI collagen mutations in Bethlem myopathy, an autosomal dominant myopathy with contractures. *Nat Genet* 1996;**14**:113–15.

117 **Lamande SR**, Shields KA, Kornberg AJ, Shield LK, Bateman JF. Bethlem myopathy and engineered collagen VI triple helical deletions prevent intracellular multimer assembly and protein secretion. *J Biol Chem* 1999;**274**:21817–22.

118 **Camacho Vanegas O**, Zhang RZ, Sabatelli P, Lattanzi G, Bencivenga P, Giusti B, Columbaro M, Chu ML, Merlini L, Pepe G. Novel COL6A1 splicing mutation in a family affected by mild Bethlem myopathy. *Muscle Nerve* 2002;**25**:513–19.

119 **Lamande SR**, Bateman JF, Hutchison W, McKinlay Gardner RJ, Bower SP, Byrne E, Dahl HH. Reduced collagen VI causes Bethlem myopathy: a heterozygous COL6A1 nonsense mutation results in mRNA decay and functional haploinsufficiency. *Hum Mol Genet* 1998;**7**:981–9.

120 **Pan TC**, Zhang RZ, Pericak-Vance MA, Tandan R, Fries T, Stajich JM, Viles K, Vance JM, Chu ML, Speer MC. Missense mutation in a von Willebrand factor type A domain of the alpha 3(VI) collagen gene (COL6A3) in a family with Bethlem myopathy. *Hum Mol Genet* 1998;**7**:807–12.

121 **Sasaki T**, Hohenester E, Zhang RZ, Gotta S, Speer MC, Tandan R, Timpl R, Chu ML. A Bethlem myopathy Gly to Glu mutation in the von Willebrand factor A domain N2 of the collagen alpha3(VI) chain interferes with protein folding. *FASEB J* 2000;**14**:761–8.

122 **Demir E**, Sabatelli P, Allamand V, Ferreiro A, Moghadaszadeh B, Makrelouf M, Topaloglu H, Echenne B, Merlini L, Guicheney P. Mutations in COL6A3 cause severe and mild phenotypes of Ullrich congenital muscular dystrophy. *Am J Hum Genet* 2002;**70**:1446–58.

123 **Higuchi I**, Shiraishi T, Hashiguchi T, Suehara M, Niiyama T, Nakagawa M, Arimura K, Maruyama I, Osame M. Frameshift mutation in the collagen VI gene causes Ullrich's disease. *Ann Neurol* 2001;**50**:261–5.

124 **Camacho Vanegas O**, Bertini E, Zhang RZ, Petrini S, Minosse C, Sabatelli P, Giusti B, Chu ML, Pepe G. Ullrich scleroatonic muscular dystrophy is caused by recessive mutations in collagen type VI. *Proc Natl Acad Sci U S A* 2001;**98**:7516–21.

125 **Ishikawa H**, Sugie K, Murayama K, Ito M, Minami N, Nishino I, Nonaka I. Ullrich disease: Collagen VI deficiency: EM suggests a new basis for muscular weakness. *Neurology* 2002;**59**:920–3.

126 **Baker NL**, Morgelin M, Peat R, Goemans N, North KN, Bateman JF, Lamande SR. Dominant collagen VI mutations are a common cause of Ullrich congenital muscular dystrophy. *Hum Mol Genet* 2005;**14**:279–93.

127 **Ishikawa H**, Sugie K, Murayama K, Awaya A, Suzuki Y, Noguchi S, Hayashi YK, Nonaka I, Nishino I. Ullrich disease due to deficiency of collagen VI in the sarcolemma. *Neurology* 2004;**62**:620–3.

128 **Vogel BE**, Doelz R, Kadler KE, Hojima Y, Engel J, Prockop DJ. A substitution of cysteine for glycine 748 of the alpha 1 chain produces a kink at this site in the procollagen I molecule and an altered N-proteinase cleavage site over 225 nm away. *J Biol Chem* 1988;**263**:19249–55.

129 **Steinmann B**, Raghunath M. Delayed helix formation of mutant collagen. *Science* 1995;**267**:258.

130 **Romanic AM**, Spotila LD, Adachi E, Engel J, Hojima Y, Prockop DJ. Self-assembly of collagen I from a proband homozygous for a mutation that substituted serine for glycine at position 661 in the alpha 2(I) chain. Possible relationship between the effects of mutations on critical concentration and the severity of the phenotype. *J Biol Chem* 1994;**269**:11614–19.

131 **Higuchi I**, Suehara M, Iwaki H, Nakagawa M, Arimura K, Osame M. Collagen VI deficiency in Ullrich's disease. *Ann Neurol* 2001;**49**:544.

132 **Merlini L**, Villanova M, Sabatelli P, Malandrini A, Maraldi NM. Decreased expression of laminin beta 1 in chromosome 21-linked Bethlem myopathy. *Neuromuscul Disord* 1999;**9**:326–9.

133 **Taylor J**, Muntoni F, Robb S, Dubowitz V, Sewry C. Early onset autosomal dominant myopathy with rigidity of the spine: a possible role for laminin beta 1? *Neuromuscul Disord* 1997;**7**:211–16.

134 **Johnson MA**. Immunocytochemical Analysis. In: Bushby KMD, Anderson LVB, eds. *Muscular dystrophy: methods and protocols*. Totowa, New Jersey: Humana Press, 2001:339–67.

135 **Niiyama T**, Higuchi I, Suehara M, Hashiguchi T, Shiraishi T, Nakagawa M, Arimura K, Maruyama I, Osame M. Electron microscopic abnormalities of skeletal muscle in patients with collagen VI deficiency in Ullrich's disease. *Acta Neuropathol (Berl)* 2002;**104**:67–71.

136 **Niiyama T**, Higuchi I, Hashiguchi T, Suehara M, Uchida Y, Horikiri T, Shiraishi T, Saitou A, Hu J, Nakagawa M, Arimura K, Osame M. Capillary changes in skeletal muscle of patients with Ullrich's disease with collagen VI deficiency. *Acta Neuropathol (Berl)* 2003;**106**:137–42.

137 **Sugie K**, Ishikawa H, Driss A, Noguchi S, Hayashi YK, Nonaka I, Nishino I. Collagen VI deficiency is a common cause of congenital muscular dystrophy [abstract]. *Neuromuscul Disord* 2004;**14**:614.

138 **Demir E**, Ferreiro A, Sabatelli P, Allamand V, Makri S, Echenne B, Maraldi M, Merlini L, Topaloglu H, Guicheney P. Collagen VI status and clinical severity in Ullrich congenital muscular dystrophy: phenotype analysis of 11 families linked to the COL6 loci. *Neuropediatrics* 2004;**35**:103–12.

139 **Brockington M**, Brown SC, Lampe A, Yuva Y, Feng L, Jimenez-Mallebrera C, Sewry CA, Flanigan KM, Bushby K, Muntoni F. Prenatal diagnosis of Ullrich congenital muscular dystrophy using haplotype analysis and collagen VI immunocytochemistry. *Prenat Diagn* 2004;**24**:440–4.

140 **Wallgren-Pettersson C**, Bushby K, Mellies U, Simonds A. 117th ENMC workshop: ventilatory support in congenital neuromuscular disorders – congenital myopathies, congenital muscular dystrophies, congenital myotonic dystrophy and SMA (II) 4–6 April 2003, Naarden, The Netherlands. *Neuromuscul Disord* 2004;**14**:56–69.

141 **Mellies U**, Ragette R, Dohna SC, Boehm H, Voit T, Teschler H. Long-term noninvasive ventilation in children and adolescents with neuromuscular disorders. *Eur Respir J* 2003;**22**:631–6.

142 **Bonaldo P**, Braghetta P, Zanetti M, Piccolo S, Volpin D, Bressan GM. Collagen VI deficiency induces early onset myopathy in the mouse: an animal model for Bethlem myopathy. *Hum Mol Genet* 1998;**7**:2135–40.

143 **Irwin WA**, Bergamin N, Sabatelli P, Reggiani C, Megighian A, Merlini L, Braghetta P, Columbaro M, Volpin D, Bressan GM, Bernardi P, Bonaldo P. Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency. *Nat Genet* 2003;**35**:367–71.