

# 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.

milestones, or even decreased fetal movements.<sup>21</sup> Usually patients will become symptomatic within the first or second decade<sup>21</sup>; however, as some adult patients remain unaware of weakness, an age of onset cannot always be reliably established.<sup>17</sup> The development of contractures is a hallmark of this condition. The contractures may be of a strikingly dynamic nature during childhood,<sup>21</sup> 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.<sup>11–13</sup> However, in other patients the contractures may be relatively subtle, leading to potential confusion in diagnosis with cases of limb girdle muscular dystrophy.<sup>22–23</sup> 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<sup>24</sup> as well as X linked or autosomal dominant Emery–Dreifuss muscular dystrophy.<sup>25</sup>

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.<sup>1–4</sup> 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.<sup>5–7</sup> 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).

BM patients typically have moderate predominantly proximal weakness and atrophy with the extensors more involved than the flexors.<sup>21</sup> 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.<sup>26</sup> This peripheral predominance of pathology in BM has also been observed using muscle ultrasound and described as “central shadow sign.”<sup>27</sup>

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.

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,<sup>15–28</sup> respiratory muscle and especially diaphragmatic involvement necessitating nocturnal respiratory support is rarely reported in association with severe weakness in later life.<sup>11–29</sup> In addition, unusual skin features may be present in some BM patients, including follicular hyperkeratosis and keloid formation or “cigarette paper” scarring<sup>12</sup> (see fig 1E).

## 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.<sup>8</sup> It has subsequently been reported worldwide<sup>9–19</sup> (for extensive review see De Visser *et al*<sup>20</sup>).

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

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

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**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<sup>17</sup> and the serum creatine kinase is normal or only mildly raised (usually less than five times the upper limit of normal).<sup>15</sup> 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.<sup>8, 17</sup>

### 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.<sup>30</sup>

UCMD was first described by Ullrich in 1930,<sup>31, 32</sup> and subsequent publications confirmed a likely autosomal recessive inheritance and a recognisable pattern of disease.<sup>33–42</sup> 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.<sup>43–44</sup> 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.<sup>45</sup> 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.<sup>11, 43, 46</sup> There is frequent failure to thrive. Other distinctive features observed in UCMD patients are congenital hip dislocations<sup>42, 43</sup> 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<sup>30, 45</sup> (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.<sup>47</sup>

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)<sup>42, 43</sup> and electromyography reveals action potentials of low amplitude and short duration.<sup>42</sup> 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 I 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.<sup>38, 43</sup> 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.<sup>30, 48</sup> 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).<sup>30, 48</sup> 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.<sup>46</sup> Spinal muscular atrophy can usually be

diagnosed by demonstrating mutations in the *SMN* gene<sup>49</sup> and the muscle biopsy shows features of denervation rather than myopathic or dystrophic changes.<sup>50</sup> 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.<sup>51, 52</sup> Rigid spine syndromes, a proportion of which are caused by mutations in the *SEPN1* gene, may overlap with UCMD later as the phenotype develops.<sup>30, 48</sup>

### COLLAGEN VI

In 1996 linkage studies in BM demonstrated locus heterogeneity, with loci on chromosome 21q22.3<sup>33</sup> and 2q37,<sup>54</sup> 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.<sup>55–57</sup> It is composed of three different peptide chains  $\alpha 1(VI)$  and  $\alpha 2(VI)$ —both 140 kDa in size—and  $\alpha 3(VI)$ , which is much larger (260–300 kDa).

#### Genomic organisation

The  $\alpha 1(VI)$  and  $\alpha 2(VI)$  chains are encoded by two genes (*COL6A1* and *COL6A2* respectively) situated in a head to tail organisation on chromosome 21q22.3<sup>38</sup> (NT\_011515), separated by 150 kb of genomic DNA. *COL6A3*, the gene for the  $\alpha 3(VI)$  chain, maps to chromosome 2q37<sup>59</sup> (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<sup>60–64</sup> (fig 2). *COL6A1*, which consists of 37 exons (35 coding), contains one promoter<sup>65</sup> 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*.<sup>66</sup> 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  $\alpha 2(VI)$  protein variants with distinct carboxyl termini<sup>67, 68</sup> 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  $\alpha 3(VI)$  chain, which can vary in size between 2970 and 3176 amino acids. The  $\alpha 3(VI)$  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.<sup>69–72</sup>

#### Macromolecular structure

The assembly of collagen VI is a complex multistep process. Equimolar association of the three genetically distinct subunits— $\alpha 1(VI)$ ,  $\alpha 2(VI)$ , and  $\alpha 3(VI)$ —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.<sup>73</sup> Hydroxyproline was found to be the Y

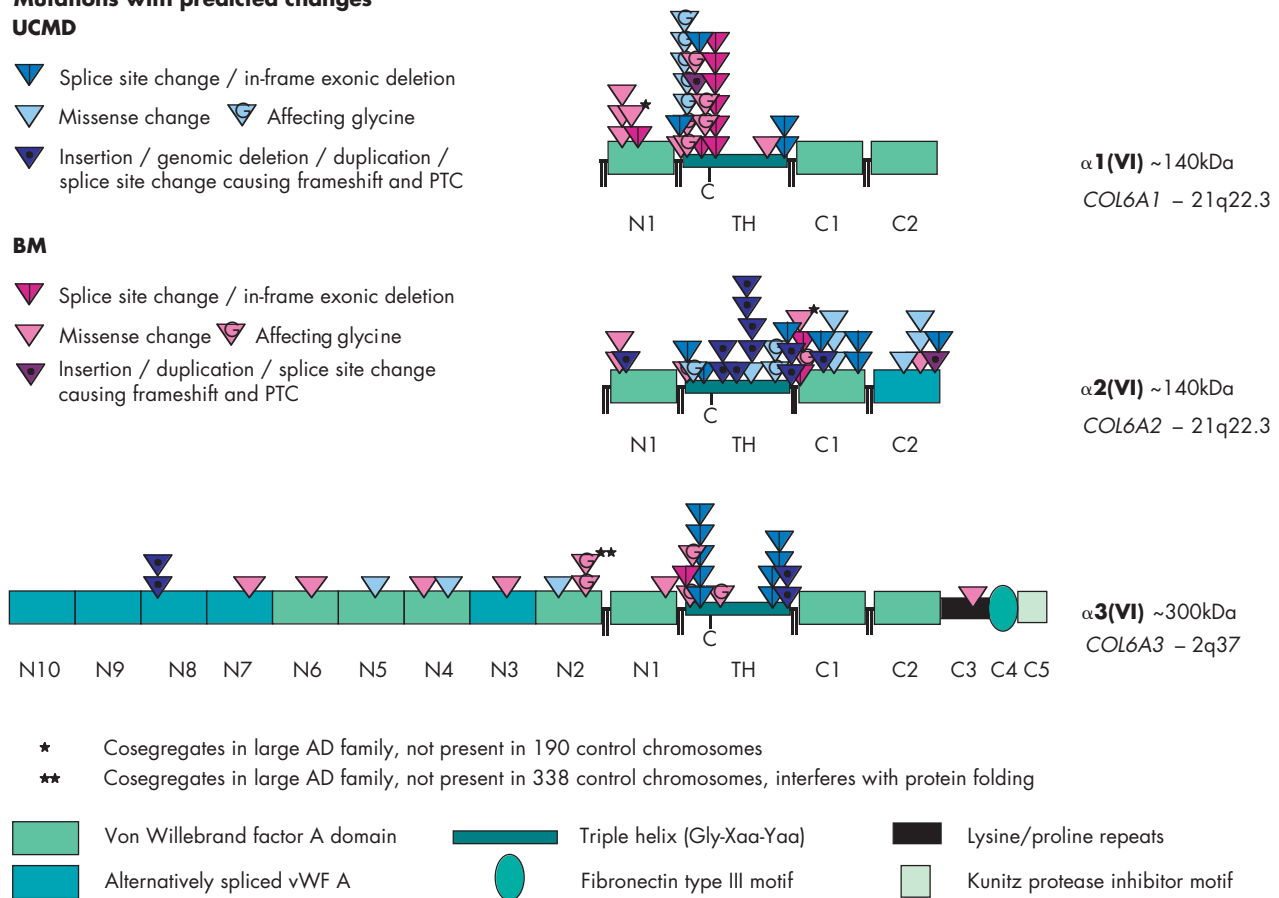
**Mutations with predicted changes**

**UCMD**

- ▼ Splice site change / in-frame exonic deletion
- ◀ Missense change    ▶ Affecting glycine
- ▼ Insertion / genomic deletion / duplication / splice site change causing frameshift and PTC

**BM**

- ▼ Splice site change / in-frame exonic deletion
- ◀ Missense change    ▶ Affecting glycine
- ▼ Insertion / duplication / splice site change causing frameshift and PTC

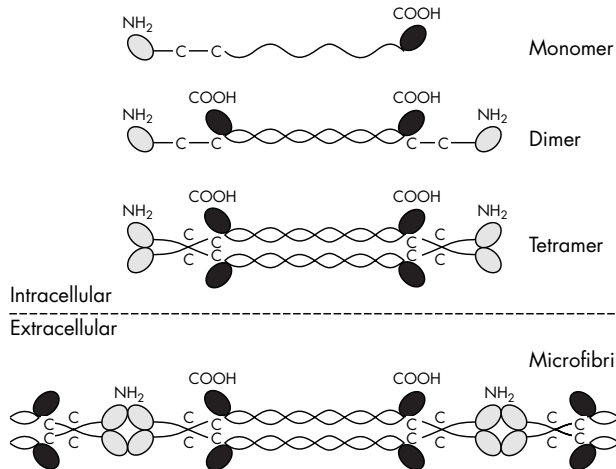


- \* Cosegregates in large AD family, not present in 190 control chromosomes
- \*\* Cosegregates in large AD family, not present in 338 control chromosomes, interferes with protein folding

**Figure 2** Genomic organisation of collagen VI and localisation of genomic changes reported for BM and UCMD to date; modification from Chu *et al.*<sup>61</sup> 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.<sup>74</sup> Whereas a single cysteine located in the triple helical domain of the  $\alpha 1(\text{VI})$  or  $\alpha 2(\text{VI})$  collagen chain, interacting with a cysteine residue in the C-globular domain, is thought to be responsible for the assembly/stability of dimers,<sup>75–79</sup> it is a cysteine residue in

the triple helical domain of the  $\alpha 3(\text{VI})$  chain that appears to be involved in tetramer formation and stability. An alternative explanation suggests that overall dimer formation is principally determined by  $\alpha 2(\text{VI})$  interactions<sup>80–81</sup> between a metal ion dependent adhesion site motif in one  $\alpha 2\text{C}2$  A domain and the Gly-Glu-Arg sequence in the helical sequence of another  $\alpha 2(\text{VI})$  chain.<sup>82</sup> However, this is not wholly consistent with other studies<sup>83–84</sup> and remains unproven.



**Figure 3** Schematic model of collagen VI assembly; modification from Furthmayr *et al.*<sup>6</sup> and Zhang *et al.*<sup>84</sup>

Further stabilisation of the dimers may be achieved by supercoiling of the overlapping triple helices. This has been observed by negative staining electron microscopy<sup>76</sup> 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.<sup>85</sup> Transfection experiments have shown that *COL6A3* subdomains N10–N6 are not required for the molecular assembly of collagen VI.<sup>83–86</sup> 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.<sup>84</sup>

Outside the cell, tetramers—the secreted form of collagen VI—associate end to end through overlapping N-terminal globular domains to form beaded microfibrils<sup>87</sup> with a distinctive 100 nm periodicity, comprising beaded globular domains separated by short triple helical regions.<sup>76–88–89</sup>

**Table 1** Genetic changes published for Bethlem myopathy to date

Location and domain	Nucleotide change	Predicted consequence	Comments	Phenotype	Reference
COL6A1 exon3	c.347 G→A	p.Ser116Asn	Heterozygous change in 3/54 individuals, no confirmatory data	Mild BM	114
COL6A1 exon3	c.362A A→G	p.Lys121Arg	Heterozygous change in one large four generation family, not present in 190 control chromosomes	AD LGMD	23
COL6A1 exon3	c.428+1 G→A	p.Tyr122_Gly143del	Missplicing with partial loss of exon3; heterozygous change in one four generation family	Mild BM	118
COL6A1 exon9	c.821 C→T	p.Pro274Leu	Heterozygous change in 2/77 individuals (first degree relatives) who are also compound	BM	114
COL6A1 exon10	c.868 G→A	p.Gly290Arg	Heterozygous for Gly204/Asp in TH of COL6A3, no confirmatory data Heterozygous change in 2/77 individuals, one of them compound heterozygous for Arg677His in COL6A3	Severe BM	114
COL6A1 exon11	c.914 G→T	p.Gly305Val	Heterozygous change in one three generation family	BM	116
COL6A1 intron11	c.931-1 G→A	p.Gly311AlafsX22	Splice site moved by one base; heterozygous change in one family	BM	119
COL6A1 intron13	c.1003-3 C→A	p.Gly335_Asp352del	Exon 14 skipping; heterozygous change in 1/78 individuals	BM	114 *
COL6A1 exon14	c.1022 G→A	p.Gly341Asp	Heterozygous change in one four generation family	AD LGMD	23
COL6A1 exon14	c.1022 G→T	p.Gly341Val	Heterozygous change in 1/78 individuals	BM	114
COL6A1 exon14	c.1056+1 G→A	p.Gly335_Asp352del	Exon 14 skipping; heterozygous change in one family	BM	117
COL6A1 exon14	c.1056+1 G→A	p.Gly335_Asp352del	Exon 14 skipping; heterozygous change in one individual (de novo)	BM	79
COL6A1 intron14	c.1056+1 G→A	p.Gly335_Asp352del	Exon 14 skipping; heterozygous change in 3/77 individuals (two kindreds)	BM	114 *
COL6A1 intron14	c.1056+2 T→C	p.Gly335_Asp352del	Exon 14 skipping; heterozygous change in one three generation family	BM	78
COL6A1 exon26	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
COL6A2 exon3	c.316 G→A	p.Glu106Lys	Heterozygous change in 2/76 individuals, no confirmatory data	BM	114
COL6A2 exon6	c.811 G→A	p.Gly271Ser	Heterozygous change in one three generation family	BM	116
COL6A2 intron24	c.1817-3 C→G	p.Cys605_Asp606ins- GSEYSPVPPDDPAITPR	Insertion of part of intron24; heterozygous change in 1/78 individuals	BM	114 *
COL6A2 exon25	c.1861 G→A	p.Asp621Asn	Heterozygous change in one large three generation family, not present in 190 control chromosomes	AD LGMD	23
COL6A2 intron25	c.1970-3 C→A	p.Thr656_Ala698del	Missplicing with partial loss of exon26; heterozygous change in 1/77 individuals	BM	114 *
COL6A2 exon26	c.2098 G→A	p.Gly700Ser	Heterozygous change in 3/77 individuals (three first degree relatives), no confirmatory data	BM, 1/3 severe	114
COL6A2 exon28	c.2558 G→A	p.Arg853Gln	Heterozygous change in 1/75 individuals, no confirmatory data	BM	114
COL6A2 exon28	c.2663_2664dupAG	p.Gly889SerfsX6	Heterozygous change in 1/75 individuals	BM	114
COL6A3 exon6	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
COL6A3 exon7	c.3040 A→G	p.Lys1014Glu	Heterozygous change in 1/79 individuals, no confirmatory data	BM	114
COL6A3 exon9	c.4156 G→A	p.Glu1386Lys	Heterozygous change in 1/79 individuals, no confirmatory data	BM	114
COL6A3 exon10	c.4399 A→G	p.Asn1467Asp	Heterozygous change in 1/79 individuals, no confirmatory data	BM	114
COL6A3 exon11	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
COL6A3 exon11	c.5036 G→A	p.Gly1679Glu	Heterozygous change in 1/79 individuals	BM	114
COL6A3 exon14	c.5953 G→A	p.Val1985Met	Heterozygous change in 1/79 individuals who is also compound heterozygous for Lys571Thr in COL6A1, no confirmatory data	BM	114
COL6A3 exon15	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
COL6A3 intron15	c.6156+1 G→A	p.Asp2022_Lys2052del	Exon 15 skipping; heterozygous change in 1/79 individuals	Severe BM	114 *
COL6A3 exon16	c.6166 G→A	p.Gly2056Arg	Heterozygous change in one two generation family (de novo)	BM	115
COL6A3 exon17	c.6239 G→A	p.Gly2080Asp	Heterozygous change in 1/79 individuals	Severe BM	114
COL6A3 exon40	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.<sup>70-90</sup> The N-terminal end of  $\alpha 3(VI)$  has several potential glycosylation sites which could generate additional molecular heterogeneity by attachment of branched oligosaccharides.<sup>70</sup> Immunoelectronmicroscopy studies show that collagen VI forms a highly branched filamentous network in the extracellular matrix<sup>56</sup> which encircles interstitial collagen fibres and is particularly abundant close to the cells and in intimate contact with basement membranes surrounding muscle fibres.<sup>7-91-92</sup> 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.<sup>93</sup> The amino-terminal domain of  $\alpha 1(VI)$  interacts with the carboxyl-terminal globular domain of collagen IV in vitro<sup>7-92</sup> 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.<sup>7</sup> Collagen VI also interacts with several other extracellular matrix constituents in vitro, including the fibrillar collagens I<sup>94</sup> and II,<sup>95</sup> fibril associated collagen XIV,<sup>96</sup> perlecan,<sup>97</sup> and microfibril associated glycoprotein MAGP1, which is found in association with elastin in elastic fibres.<sup>98</sup> 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,<sup>6-95-99-101</sup> 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<sup>102-104</sup> and contributes to the maintenance of tissue homeostasis. It mediates interactions of cells with the extracellular matrix,<sup>105-112</sup> and, through interconnection with the fibronectin microfibrillar system, is implicated in the development of the matrix supramolecular structure.<sup>5-113</sup> 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.<sup>79-114</sup> 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*<sup>23-114-116</sup> 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<sup>73</sup> 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  $\alpha 1(VI)$  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.<sup>78-79-114-117</sup> The shortened  $\alpha 1(VI)$  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.<sup>78-79-117</sup> 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.<sup>114-118</sup> Ancillary data are only available for one of these<sup>118</sup> 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.<sup>119</sup>

Missense mutations other than glycine substitutions within the triple helical domain have been reported for 19 kindreds.<sup>23-114-120</sup> 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<sup>79-114</sup>), 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.<sup>23-120</sup> 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,<sup>121</sup> 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,<sup>114-122</sup> or through frameshift inducing genomic deletions,<sup>114-123</sup> insertions,<sup>124</sup> duplications,<sup>114</sup> and splice changes.<sup>124-125</sup>

In addition to five different missense mutations substituting glycine in the triple helical Gly-Xaa-Yaa motif which have been found in seven kindreds,<sup>114</sup> eight other missense changes have been reported within the triple helical and C-terminal domains of *COL6A2* and the N-terminal domains of *COL6A3*.<sup>114-126</sup> 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.<sup>79-114-122-126-127</sup> 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.<sup>79</sup> Its mutant  $\alpha 1(VI)$  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	c.804+857_903+6del199	p.Gly269_Lys301 del	Deletion exon9-10; heterozygous change in one individual (de novo)	UCMD	79
COL6A1 exon9	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	c.850 G→A	p.Gly284Arg	Heterozygous change in 2/77 individuals	UCMD	114
COL6A1 exon10	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	c.957+1 G→A	p.Gly311_Lys319del	Exon12 skipping; heterozygous change in one individual (de novo)	UCMD	126
COL6A1 intron27	c.1776+1 G→A	p.Gly581_Asp592del	Exon27 skipping; homozygous change in 1/76 individuals (parents heterozygous)	UCMD	114*
COL6A2 exon3	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	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	c.847 G→A	p.Gly283Arg	Heterozygous change in 1/78 individuals	UCMD	114
COL6A2 intron9	c.955-2 A→G	p.Gly319_Lys333del	Exon10 skipping; heterozygous change in 1/78 individuals	UCMD	114
COL6A2 exon13	c.1147_1151 insC	p.Glu386ArgfsX64	Homozygous change in one individual (parents heterozygous)	UCMD	124
COL6A2 intron14	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	c.1459-2 A→G	p.Gly487_Ala495delAspfsX48	Missplicing 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	c.1487_1512del26	p.Arg498_Pro506delGlnfsX58	Homozygous change in one individual	UCMD	123
COL6A2 exon18	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	c.1590 G→C	p.Gly531Arg	Homozygous change in 1/78 individuals	UCMD	114
COL6A2 intron23	c.1770+5 G→A	p.Gly579_Thr590del	Exon23 skipping; heterozygous change in one individual who is also compound heterozygous for genomic dbp deletion in COL6A2 exon26 causing Ile759_Gly760del (mother carrier)	Mild UCMD	127
COL6A2 intron23	c.1771-3 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	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	c.2274_2279del6	p.Ile759_Gly760del	Genomic dbp 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	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	c.2329 T→C	p.Cys777Arg	Homozygous change in 1/78 individuals, no confirmatory data	UCMD	114
COL6A2 exon26	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	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	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	c.2510 T→C	p.Leu837Pro	Heterozygous change in one individual also compound heterozygous for Asn897del (mother carrier)	UCMD	126
COL6A2 exon28	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	c.2689_2691 del3	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, NB 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.<sup>126</sup> 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,<sup>79, 126</sup> 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.<sup>114</sup> 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.<sup>114</sup> The premature termination codon within the triple helix of COL6A2, found as the only mutation in one patient,<sup>124</sup> in the same study was found to cause UCMD in another family only when present alongside another mutation, but not in heterozygous carriers.<sup>124</sup> It therefore seems likely that this patient might carry a second as yet undetected mutation.

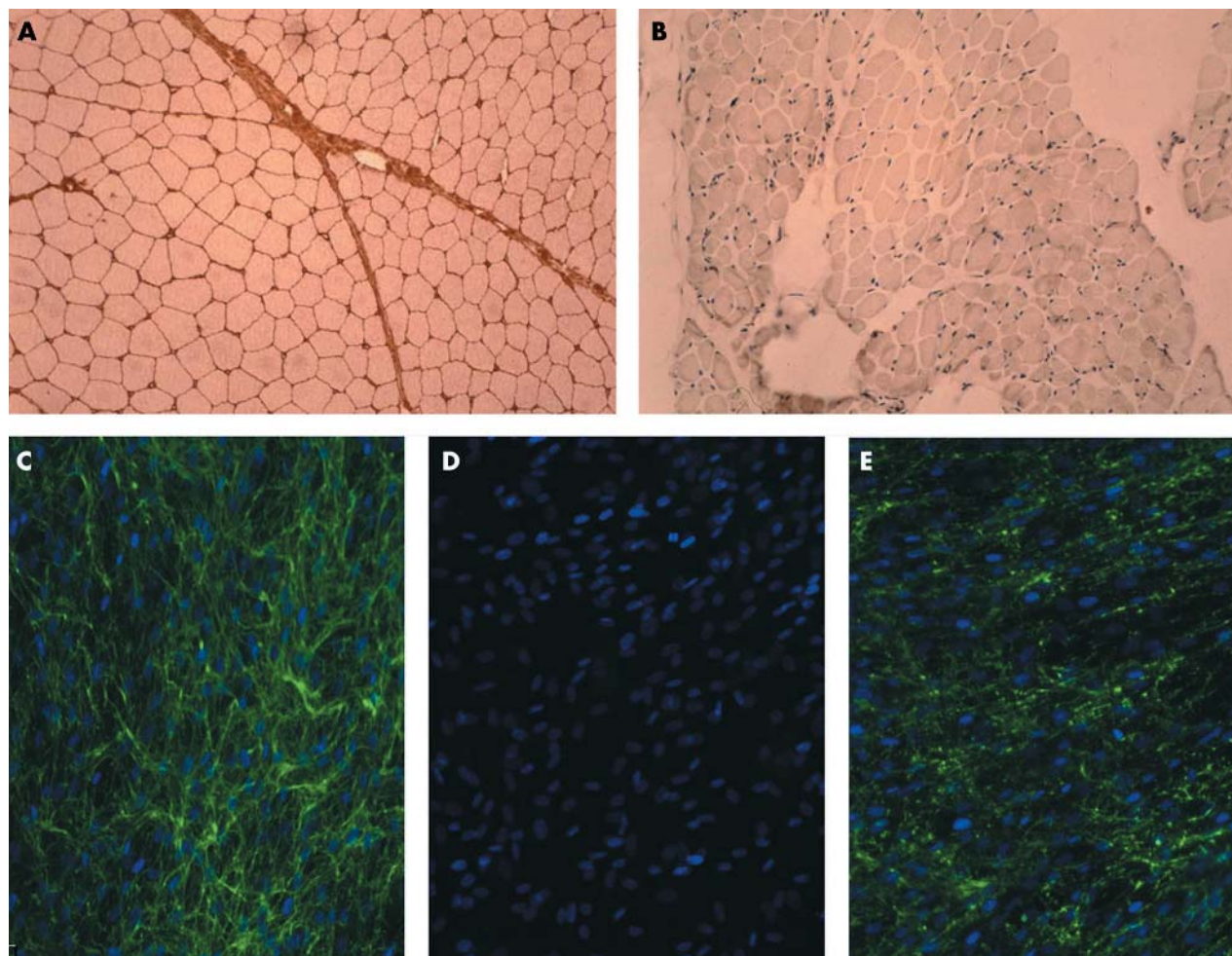
Two other UCMD patients carry a sequence variation predicted to cause a glycine substitution within the triple helical domain of the  $\alpha 1(\text{VI})$  chain alongside a separate unique putative missense mutation in COL6A2.<sup>114</sup> In addition to the above considerations, these raise the possibility 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,<sup>114</sup> 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,<sup>128</sup> delayed triple helical monomer folding,<sup>129</sup> or disturbed formation of multimeric assemblies.<sup>130</sup> 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.<sup>73</sup> Only one triple helical glycine mutation has been found in the C-terminal part and is present homozygously in a UCMD patient.<sup>114</sup> 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.<sup>73</sup> 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  $\alpha 3(VI)$  (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  $\alpha 3(VI)$  (E) were grown in the presence of 50  $\mu\text{g}/\text{ml}$  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.<sup>84</sup> 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.<sup>119</sup> 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*.<sup>122</sup> 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.<sup>86 121</sup>

#### **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<sup>11 45 131</sup> (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  $\beta 1$  in muscle biopsies of adult patients with BM has been reported but is not specific.<sup>23 132</sup> 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  $\beta 1$  deficiency may only become apparent in adulthood.<sup>133 134</sup> If laminin  $\beta 1$  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.<sup>23</sup>

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.<sup>127</sup> 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.<sup>135</sup> Capillaries in muscle biopsy specimens from UCMD patients have been described as being devoid of collagen VI, with associated capillary fenestration on electronmicroscopy<sup>136</sup>; 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.<sup>11</sup>

## 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,<sup>114</sup> but seemed much lower in a study of a Japanese cohort of patients.<sup>127–137</sup> 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,<sup>138</sup> but the recent description of de novo dominant mutations causing UCMD<sup>79–114</sup> 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<sup>115</sup> 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.<sup>139</sup> 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.<sup>140</sup> 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.<sup>140–141</sup> 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.<sup>140</sup> 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.<sup>140</sup>

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*<sup>-/-</sup>) mouse model with a muscle histology phenotype resembling BM has been generated.<sup>142</sup> 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.<sup>143</sup> 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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