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REVIEW

GNE myopathy: current update and future therapy

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ABSTRACT

GNE myopathy is an autosomal recessive muscle disease caused by biallelic mutations in *GNE*, a gene encoding for a single protein with key enzymatic activities, UDP-N-acetylglucosamine 2-epimerase and N-acetylmannosamine kinase, in sialic acid biosynthetic pathway. The diagnosis should be considered primarily in patients presenting with distal weakness (foot drop) in early adulthood (other onset symptoms are possible too). The disease slowly progresses to involve other lower and upper extremities' muscles, with marked sparing of the quadriceps. Characteristic findings on biopsies of affected muscles include 'rimmed' (autophagic) vacuoles, aggregation of various proteins and fibre size variation. The diagnosis is confirmed by sequencing of the *GNE* gene. Note that we use a new mutation nomenclature based on the longest transcript (GenBank: NM_001128227), which encodes a 31-amino acid longer protein than the originally described one (GenBank: NM_005476), which has been used previously in most papers. Based upon the pathophysiology of the disease, recent clinical trials as well as early gene therapy trials have evaluated the use of sialic acid or *N*-acetylmannosamine (a precursor of sialic acid) in patients with GNE myopathy. Now that therapies are under investigation, it is critical that a timely and accurate diagnosis is made in patients with GNE myopathy.

INTRODUCTION

GNE myopathy is a progressive muscle disease caused by mutations in the *GNE* gene, which encodes for a key enzyme in the sialic acid biosynthesis pathway (figure 1). In 2001, the gene defect associated with hereditary inclusion body myopathy (HIBM) was identified in Iranian Jews and other ethnicities.[1] Several mutations in the gene encoding sialic acid synthesis, called *GNE*, were identified. Soon afterwards, it became clear that distal myopathy with rimmed vacuoles (DMRV), first described in Japan by Nonaka and collegues,[2] is also caused by defects in the same gene. More than a decade afterwards, numerous patients with GNE defects were described worldwide. Other names such as inclusion body myopathy type 2 and quadriceps-sparing myopathy have been used to describe this disease. To avoid confusion, a group of international experts working in the field of GNE myopathy recently met and decided to unify the nomenclature to GNE myopathy (name of disease and its mutations).[3]

In the passing decade, much progress has been achieved in clarifying some biochemical, genetic and phenotypic variations of this myopathy, but enigmas still persist about its pathogenesis.[4] Importantly, formal therapeutic trials have been initiated in the past 2 years. This timely review of the current knowledge about this unique myopathy also contains information presented at the recent third meeting of the GNE Consortium (San Francisco, September 2013).

CLINICAL FEATURES

GNE myopathy is a relatively rare muscle disease with some typical clinical and pathological characteristics that may be very important for its correct identification. This is especially true in regions where the disease is probably less prevalent or under-recognised (see Demographics section). GNE myopathy is an adult onset muscle disorder with signs typically appearing in the third decade of life. However, onset at teenage has been reported, the earliest probably around 12 years of age. The commonest presentation is weakness of the distal muscle of the leg (foot drop), thus GNE myopathy is still classified in the group of distal myopathies. Less common presentations include asymmetric foot drop or manifestations initially appearing in upper extremities and in the proximal leg musculature. The disease does not remain limited to the distal musculature but slowly progresses to involve more proximal leg muscles and the upper limbs. A very unique feature of this myopathy is the relative or full sparing of the quadriceps, even in advanced stages of the disease. This pattern, when recognised in a patient, is probably diagnostic and can be visualised by muscle imaging, which will also help differential diagnosis and selecting the biopsy site (see Diagnosis section). However, the unique pattern of involvement becomes evident only after the proximal leg musculature becomes affected. It is of note that about 5%[5] of patients may have marked early quadriceps involvement making diagnosis more difficult. The pattern of muscle weakness in the upper limbs is more variable and can include scapular weakness (mimicking scapuloperoneal syndrome) or distal weakness of the hands with varying degrees of involvement. There are patients with onset in proximal leg muscles only mimicking an unusual pattern of limb girdle muscular dystrophy[6]; such onset may delay diagnosis, but in retrospect, clinical and imaging features show that the posterior thigh muscles become markedly affected while the quadriceps is spared.

Cardiac involvement is not a classical feature of GNE myopathy. However, some patients with histological or electrophysiological evidence for heart disease have been reported.[7] Although its association with GNE myopathy needs to be further defined, ECG may need to be performed every few years. Respiratory muscles are usually

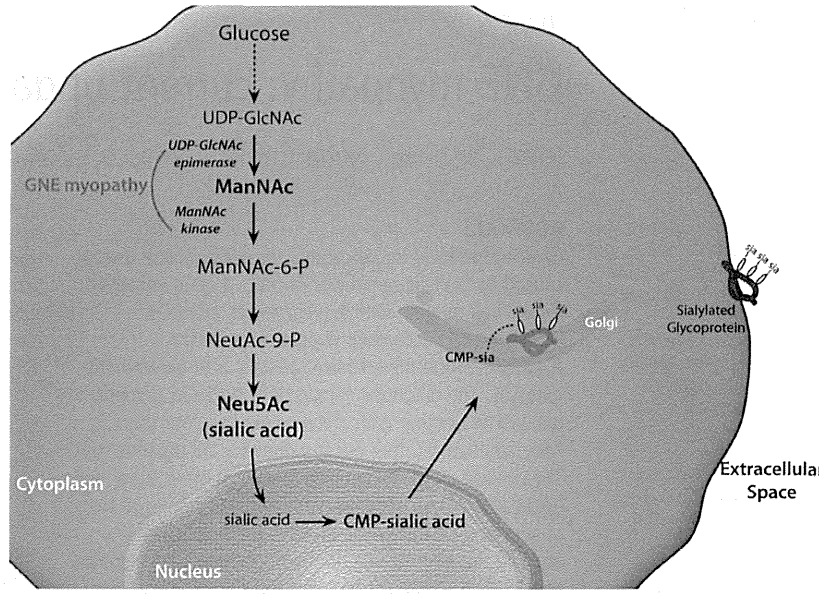

Figure 1 Sialic acid biosynthesis pathway. The biosynthesis of sialic acid (5-*N*-acetylneuraminic acid (Neu5Ac)) occurs in the cytoplasm. The initial substrate for this pathway (UDP-*N*-acetylglucosamine (GlcNAc)) is derived from glucose. In the rate-limiting step of the pathway, UDP-GlcNAc is epimerised into *N*-acetylmannosamine kinase (ManNAc) by GlcNAc 2-epimerase, encoded by the epimerase domain of *GNE*. ManNAc is phosphorylated by ManNAc kinase encoded by 'kinase' domain of *GNE*. Once Neu5Ac acid is synthesised, it becomes 'activated' by the effect of cytidine monophosphate (CMP)-sialic acid synthetase in the nucleus. CMP-sialic acid, the active form of Neu5Ac is used as a donor of sialic acid to nascent proteins in the golgi for the generation of glycoproteins. CMP-sialic acid also acts as a feedback inhibitor of the UDP-GlcNAc 2-epimerase enzyme by binding to its allosteric site.

not clinically affected during the course of the disease until the later stages when a proportion of wheelchair users have reduced respiratory function.[8] It is very rare to have a patient with a need of respiratory support even in the final stage of the disease, but this may occur.[9]

The course is slowly progressive with variable pace. In many patients, especially those of Persian Jewish ancestry, walking is still maintained (at least on flat ground) for 15–20 years (and even more) after the onset of the disease.[5] However, a study from a large cohort of patients in Japan noted an average 10 years until the need to use wheelchair. In this cohort, there was a suggestion that patients with a homozygous kinase mutation do better than those with a compound heterozygous mutation for such GNE mutation.[10] The progression of GNE myopathy and the contribution of genetic and environmental factors to its variability need to be further delineated.

PATHOLOGICAL FEATURES

Pathological features of GNE myopathy include 'rimmed' vacuoles, aggregation of various proteins and fibre size variation. 'Rimmed' vacuoles are recognised as small empty spaces surrounded by tiny red granules in the cytoplasm of muscle fibres typically on modified Gomori trichrome (mGT) staining. Although this empty space is called 'vacuole', this is a space artificially produced during staining procedures. The area was originally occupied mostly by red-coloured granules, but they become detached from the slide glass. On electron microscopy (EM), clusters of autophagic vacuoles are seen and each autophagic vacuole corresponds to a red-coloured granule on mGT.

Rimmed vacuoles are probably the most prominent finding on routine muscle histochemistry as protein aggregates are often hardly visualised without immunohistochemical staining. Aggregated proteins include β-amyloid, phosphorylated τ, TAR DNA-binding protein 43 kDa (TDP-43) and α-synuclein. β-Amyloid is supposed to be detected on Congo red stain but in reality often needs immunostaining for visualisation.

Most of the aggregated proteins are ubiquitinated and are believed to be targeted for autophagy clearance through p62-dependent aggresome formation, which is sometimes termed 'aggrephagy'.[11] However, these proteins cannot be digested; thereby autophagy buildup occurs, which is detected as rimmed vacuoles on histochemistry. Therefore, protein aggregation should be upstream in the pathological cascade that produces rimmed vacuoles. In support of this notion, aggregation of β-amyloid is observed prior to the development of rimmed vacuoles in GNE myopathy model mouse. On EM, autophagic vacuoles are often present next to the filamentous inclusions, also suggesting a close relationship between autophagy and protein aggregation. In the nucleus as well as the cytoplasm, tubulofilamentous inclusions 18–21 nm in diameter are observed. Of note, this protein aggregation-rimmed vacuole pathology is not an exclusively specific feature of GNE myopathy but is rather commonly seen in other hereditary and acquired myopathies, including sporadic inclusion body myositis (IBM).

Fibre size variation is mainly due to the presence of atrophied fibres, which are often angular in shape. For unknown reasons, atrophic fibres tend to cluster in GNE myopathy, sometimes giving a false impression of neurogenic atrophy. In the mouse model, muscle fibre atrophy starts earlier than protein aggregation and rimmed vacuole formation, indicating that, at least in part, the mechanism of muscle fibre atrophy is independent from that of aggrephagy-related degenerative pathway.

Although inflammatory change is usually not a feature of GNE myopathy, there are reports of rare cases with lymphocyte infiltration into the endomysium[5 12 13] that could potentially mislead to a diagnosis of sporadic IBM. Nevertheless, the pattern of muscle involvement and the age of disease onset are different. Of note, a recent study showed upregulation of proinflammatory cell stress response with overexpression of αB-crystallin and inducible nitric oxide synthase (iNOS), which seems to precede muscle degeneration with accumulation of β-amyloid, suggesting that inflammation may play a role in the early stages of the pathological cascade of GNE myopathy although cellular response is absent.[14]

Another pitfall is the selection of biopsy site. As mentioned earlier, one of the most characteristic clinical features is

quadriceps sparing. Therefore, biopsy of quadriceps muscle, which is one of the most frequently biopsied muscles, often gives a minimal or even completely normal histology. A significant number of cases may thus be undiagnosed or misdiagnosed because of quadriceps biopsy. When available, muscle imaging is highly recommended for choosing an appropriate biopsy site.

GENETIC CAUSE AND POSSIBLE MOLECULAR MECHANISM

GNE myopathy is an autosomal recessive disease caused by biallelic *GNE* gene mutations[1 2 15] (figure 2). Missense mutations account for the majority of alleles and no patient with biallelic null mutations has ever been found, suggesting that probably only 'mildly deleterious' mutations that are not associated with complete loss of GNE protein are necessary to cause this adult-onset myopathy. In fact, knocking out the *Gne* gene in mice results in embryonic lethality.[16] It is possible that in humans biallelic null mutations are either lethal too or associated with a different, currently unrecognised disorder.

In humans, at least six different *GNE* transcripts have been described.[3 17] The originally described transcript (GenBank: NM_005476; Ensembl: ENST00000377902; UCSC: uc010mlh.3) encodes 722 amino acids, while the longest transcript (GenBank: NM_001128227; Ensembl: ENST00000396594; UCSC: uc010mli.3) encodes 753 amino acids. Both transcripts are encoded in 12 exons and the difference between the two transcripts is in alternative first exons. NM_005476 has a non-coding first exon and initial codon starts in the 43rd nucleotide in the second exon. In contrast, the longer NM_001128227 uses a different, 17-amino acid coding exon 1. The second exon is the same as NM_005476 but the first 42 nucleotides before NM_005476's initial codon are also transcribed in NM_001128227, making the NM_001128227 transcript 31 amino acid longer than NM_005476. As this 31-amino acid coding sequence is added in the 5′ part of NM_005476, description of the mutation position will be changed depending on which transcript is used as the standard sequence. Since so far no pathogenic mutation has been found in NM_001128227 specific region, it is still unknown which transcript is crucial for causing GNE myopathy. We adopt the mutation nomenclature based on NM_001128227 throughout this manuscript, following the guidelines of the Human Genome Variation Society (http://www.hgvs.org). Furthermore, as the NM_001128227's first exon resides before the NM_005476's first exon, now the former is named exon 1 and the latter exon 2, and the remaining exons are labelled exons 3–13 (figure 2).

GNE encodes a single protein with two enzymatic activities in the biosynthetic pathway of 5-*N*-acetylneuraminic acid (Neu5Ac): UDP-*N*-acetylglucosamine 2-epimerase (GlcNAc 2-epimerase) and *N*-acetylmannosamine kinase (ManNAc) (figure 1). Sialic acids are monosaccharides and Neu5Ac is the most abundant sialic acid in mammals. Neu5Ac is usually present in the terminal portion of sugar chains in glycoproteins and glycolipids where they mediate several biological processes.[18]

Owing to recessive mutations in the *GNE* gene, sialic acid production is decreased and consequently, sialylation, that is, incorporation of sialic acid to glycoproteins and glycolipids, is also decreased.[19 20] Hyposialylation appears to be a major cause of this myopathy as administration of sialic acid or its precursor ManNAc prevents or arrests the development of disease in the mouse models of GNE myopathy.[21] This is the rationale behind current therapeutic trials (see below). However, the exact mechanism by which GNE defects lead to the human disease is still not fully understood and additional processes may contribute to it.

DIAGNOSIS

Currently, the diagnosis of GNE myopathy relies on identifying characteristic clinical manifestations and histopathological findings on muscle biopsy and is confirmed by the identification of biallelic *GNE* mutations.[22]

The diagnosis should be considered in patients presenting in young adulthood with foot drop, although the identification of the disease may be done at more advanced stages of the disease, when more proximal lower extremity or upper extremity muscles are affected. Clinically, the diagnosis may be confused with other conditions, such as other distal myopathies, limb girdle muscular dystrophy,[23] spinal muscular atrophy or Charcot-Marie-Tooth disease. The reliability of muscle biopsy for the diagnosis of GNE myopathy appears to depend on the technical skill and diagnostic expertise of those handling and evaluating the specimen (see above). GNE protein is present in the diseased muscle; thus, immunohistology may not identify the defect and furthermore no specific GNE antibody that could be used for diagnostics has yet been synthesised.

The use of muscle imaging can guide the choice of muscle for biopsy and can help establish disease severity. Muscle MRI of the affected muscles initially shows increased hyperintensity on T2 STIR sequences followed by fatty-fibrous replacement evident on T1-weighted images.[24]

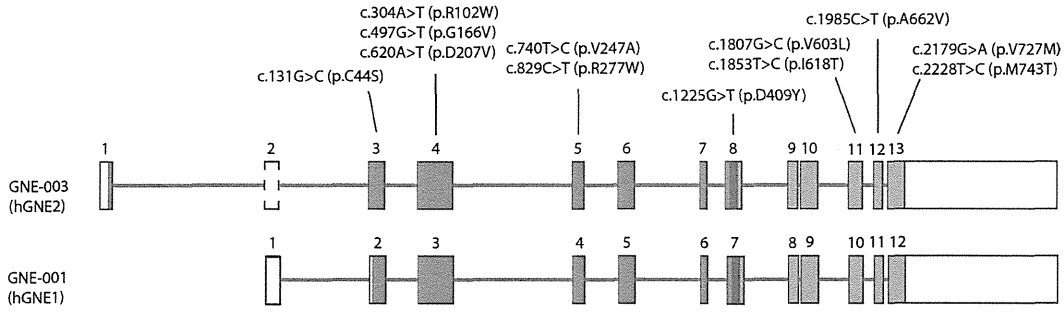

Figure 2 Schematic illustration of GNE gene structure. Gene structure for the two most representative transcripts is shown. The longest transcript (NM_001128227) encodes 753 amino acids, including 17 amino acid encoded by exon 1. The originally described transcript shown at the bottom (GenBank: NM_005476) uses an alternative first exon which is non-coding and the initial codon resides in the 43rd–45th nucleotides in the second exon, which makes the protein shorter by 31 amino acids. Note exon 8 encodes the last part of epimerase domain, junctional region and initial part of kinase domain. The size of exons is to scale but that of introns is not. Boxes indicate exons. Open box means non-coding region. Blue and pink, respectively, indicate epimerase and kinase encoding regions. Mutations mentioned in the text are included for reference.

The identification of biallelic mutations in *GNE* is the only definite diagnostic tool. As there are 147 known *GNE* mutations associated with GNE myopathy to date (based on HGMD Professional V.2013.4), sequencing of *GNE* is necessary when considering the diagnosis. In regions where one mutation is very prevalent (eg, p.M743T in the Middle East), testing for it may suffice. Patients with typical clinical and histological manifestations and only one heterozygous *GNE* mutation identified by sequencing have been encountered. Such patients may have deletions[25] not identified by sequencing or mutations in non-coding regions of *GNE* on the other allele. Alternatively, they may have a genetically different disorder. In such cases, next generation sequencing could be considered in the further diagnostic effort. Heterozygous carriers have no phenotype, although heterozygous mice have decreased sialylation.[19]

Owing to the rarity of this disease and the diagnostic difficulties aforementioned, patients may remain undiagnosed for a long period of time. In one cohort of patients followed at the National Institutes of Health (NIH), the diagnosis was delayed by an average of 10 years (NCC, unpublished).

DEMOGRAPHICS

GNE myopathy is a disorder found worldwide; however, until recently, it was mostly recognised in patients of Japanese and Persian Jewish ethnicity, where founder mutations are prevalent and different names, namely DMRV and HIBM, are used. However, after the identification of the genetic defect,[1] it is now clear that this is a worldwide disorder with an estimated prevalence of about 1/1 000 000 (higher prevalence is seen in Middle-Eastern Jews and Japanese; figure 3). In the past decade, there have been a plethora of reports from Europe, many Asian countries and North America. Interestingly, no patients were reported from South America, apart from two families of Persian Jewish ancestry residing in Argentina (ZA's personal observations). The lack of report from South America may be due to a decreased recognition of the condition.

Japan and Asian Oceanian region

Among all patients whose muscle biopsy was examined at the National Center of Neurology and Psychiatry (NCNP) in Tokyo between 1978 and 2005, 42 had GNE myopathy. During the same period of time, 502 had Duchenne muscular dystrophy (DMD), suggesting that the prevalence of GNE myopathy is roughly one log lower than that of DMD. In Japan, the prevalence of DMD ranges roughly from 1500 to 4000, indicating that 150–400 patients may be present in Japan. The cumulative number of Japanese patients who have been diagnosed to have biallelic GNE mutations at NCNP since 1978 is 237 at the time of writing. Although some patients may not be alive by now, it is of note that the estimated number of patients and actual number of genetically diagnosed patients are in a similar range.

Among all mutations identified, 95% are missense, as aforementioned. Three most frequent mutations are p.V603L, p.D207V and p.C44S, with allele frequency of 46.8%, 21.9% and 3.2%, respectively.[2 26 27] The p.V603L and p.C44S mutations were also identified in Korea and northern part of China, probably being compatible with a hypothesis of historical migration of people from the continent to Japan through Korean peninsula.[28 29]

In other parts of Asia, much fewer patients have been reported. Nevertheless, p.A662V and p.V727M seem to be common in the South-East Asian region: the former in Vietnam and Malaysia while the latter in Thailand and Malaysia, in addition to India.[29–31] The former has also been found in the USA and Australia. However, ethnically, they appear to originate from Vietnam.

Israel and Middle East

The largest cluster of GNE myopathy is that of Jews originating from Iran and neighbouring countries (Uzbekistan, Afghanistan, Iraq and Syria). They are all homozygous for the kinase mutation p.M743T, which is the commonest *GNE* mutation worldwide. About 150 such patients were identified in Israel over the years, and the estimated carrier frequency is 1 in 20 in this ethnic group.[1] A survey in the large Persian (Iranian) Jewish community residing in southern California suggested an even higher carrier rate of 1 in 11.[32]

Interestingly, the p.M743T mutation has been identified not only in Middle-Eastern Jews but also in Muslim Arabs in Israel (of Bedouin and Palestinian origins) who all (five families) carry it in a homozygous genotype. Furthermore, this homozygous mutation has been reported in Muslim patients from North

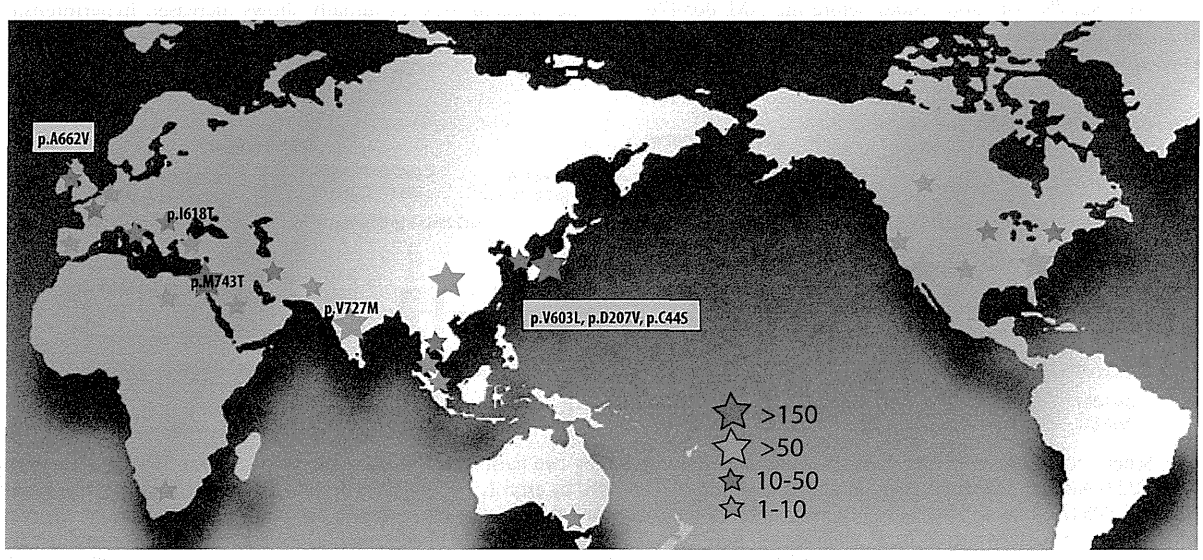

Figure 3 The worldwide prevalence of GNE myopathy is estimated at 1/1 000 000.

Africa (Egypt and Tunisia).[33] Thus, a regional founder mutation is strongly suggested and unpublished data suggest this mutation to be about 2500 years old. The origin of this high-frequency p.M743T GNE mutation in Persian Jews coming from various regions of Iran is unclear, as no data on general population testing in Iran are available. However, a cluster of patients with GNE myopathy due to p.M743T mutation were identified in a small town (Sangesar) in northern Iran. They all belonged to the Bahai religion (a relatively new religion originating in Persia during the 19th century), and a carrier rate of 1 in 25 was estimated.[34] It is unclear if this cluster is due to 'spread' of mutation from neighbouring Jewish residents.

Knowledge about this common mutation is important for easy diagnosis in patients originating from the Middle East residing outside this region. However, one should be cautious since although for more than a decade no patient with GNE myopathy having other mutations was identified in Israel, three families with different mutations were identified in 2013. One of those is a Jewish family from Mumbai, India. Both patients were homozygous to a mutation not reported in patients from other regions of India. This fact emphasises the need for pattern recognition of the clinical features of GNE myopathy in order not to delay correct diagnosis.

North America

Many patients in North America have been identified as having GNE myopathy, mostly in the USA and Canada. A significant portion of these patients are homozygous for the p.M743T mutation and are of Middle-Eastern background. The remainder is comprised mostly by patients who are compound heterozygotes for private mutations of *GNE*, reflecting the mixed ethnic background in the USA. Mutations in these patients have been traced to various ethnic backgrounds such as German p.V247A, p.D409Y and p.F559C; British p.G166V and p.R277W; Irish p.A662V and p.D409Y; Indian p.V727M and Cajun p.I618T.[1 35–37] Other mutations, such as p.R102W, have only been described in America.[37]

The only description of GNE myopathy in Hispanics is of a compound heterozygote patient (p.A555V/Y706H) whose ethnic background included Mexico.[38]

Europe

Since the identification of the causative gene, patients with novel *GNE* mutations were identified in numerous European countries (eg, Italy, Germany, the Netherlands, France and Belgium). However, because many European countries have large immigrant communities, including Asian, the recognition of the clinical pattern of GNE myopathy is critical for neuromuscular practice in this continent. Special attention should be given to mutations with possible founder effect. One such cluster was identified in Gypsies/Roma patients who are all homozygous for the kinase mutation p.I618T.[39] The mutation was not new when identified, however, at least 27 patients shared it. Two unusual features were mentioned: atrophy of thenar muscles and cardiac arrhythmias. Another region with relatively high GNE myopathy prevalence was recently identified in northern UK and Ireland. Point prevalence was estimated to be 0.19–0.44 in 100 000 for Scotland and northern Ireland. Two mutations were the most frequent: p.A662V, which is a mutation described in other regions of the world, and p.A409T, which seemed to be of northern British origin.[40]

NATURAL HISTORY AND PATIENT REGISTRY

Patient monitoring programme (Ultragenyx/TREAT-NMD)

The rate of progression of GNE myopathy has been variable over a few decades. There is a need for more accurate assessment of the clinical variability as well as identifying markers of progression that will optimise the design and interpretation of therapeutic trials. In addition, there is a need for patients' registry that will identify patients worldwide and serve as a source for patients' information. Such a programme was developed by TREAT-NMD and Ultragenyx (HIBM patient monitoring programme). There are two components of this programme: the first is patients' registry that will be open to all patients worldwide based on their willingness to add their data. This programme will combine the physician's reported information with the patient's personal report and will be conducted under the auspice of TREAD-NMD complying with Good Clinical Practice guidelines. This module has already been initiated (http://gnem-dmp.com/). The second part of this programme looking at the natural history of GNE myopathy will be conducted in several sites with large cohorts of patients. These will be different from the sites running therapeutic trials and will have larger distribution in Europe and North America. This second module of the programme is currently in progress as a sponsored clinical trial.

NIH study

In 2011, a longitudinal, prospective, single-centre natural history study of patients with GNE myopathy was initiated at the NIH (NIH study 11-HG-0218; ClinicalTrials.gov: NCT01417533). The objectives of the study are to delineate the natural history of GNE myopathy in a genetically diverse cohort by characterising the pattern and rate of progression of muscle weakness, its effect on patients' function and their quality of life and its correlation with genotype and environmental factors; to identify ideal outcome measures to be used in clinical trials and to discover blood biomarkers that would allow for diagnosis and monitoring of patients. Patients are evaluated every 6–12 months during an inpatient visit that lasts 3–4 days at the NIH Clinical Center. Evaluations include confirmation of *GNE* mutations, blood and urine laboratory tests, ECG, echocardiogram, pulmonary function tests, muscle MRI and measures of strength, function and quality of life.

Remudy (Japanese registry)

Remudy (Registry of Muscular Dystrophy) is a national patient registry for muscle diseases in Japan that was originally established for dystrophinopathy[41] (http://remudy.jp). GNE myopathy patient registration began in June 2012. By the end of 2013, 146 patients with GNE myopathy had been registered. Registered items include personal information, family history, diagnostic information and current clinical status. The registration form is filled and signed by patients themselves and their physicians. This registry will be harmonised with the international registry, which is run by TREAT-NMD and Ultragenyx as part of the patient monitoring programme (see above).

MOUSE MODEL AND THERAPEUTIC DEVELOPMENT

As mentioned, the *Gne* knock-out mouse model is embryonic lethal.[16]

The NIH-USA group established a mouse model by knocking-in the p.M743T mutation. However, most mice died with 72 h after birth due to renal disease and showed no myopathic phenotype; ManNAc administration rescued the neonatal

lethal phenotype in these mice.[42] Similar results were obtained in other laboratories.[43] Interestingly, the *Gne* M712T knock-in model developed by the Jerusalem group had a different phenotype. In some animals, no renal disease was observed and animals survived more than 1 year without any therapy.[43] Those that died at a later age did not show muscle abnormalities. The explanation for these variations in the model remains unclear but may be due to genetic background differences. A group in Kanazawa University in Japan developed *Gne* V603L knock-in model mouse. Their mice also showed a renal phenotype with shorter lifespan but without myopathy, which was rescued by the administration of NeuAc.[44]

The Tokyo group cross-mated heterozygote mice with a transgenic mouse model expressing human p.D207V mutant *GNE*, eventually obtaining mice overexpressing human mutant GNE protein and disrupting the production of their own *Gne*. This transgenic mouse model recapitulated the phenotype GNE myopathy clinically, pathologically and biochemically. Mice developed muscle atrophy and weakness after 20 weeks of age, β-amyloid after 30 weeks and rimmed vacuoles after 40 weeks while their sialic acid level was persistently low.[45] NeuAc, ManNAc and sialyllactose were administered presymptomatically to these mice and continued for 54–57 weeks, when all the clinicopathological features are supposed to have already developed. Treated mice showed improved survival, body weight, muscle pathology and muscle mass and strength comparable to that of their unaffected littermates.[21] Sialic acid content in muscle was increased but was still considerably lower than in littermates, indicating that even mild increase of muscle sialic acid level is efficacious at least in mice, and that we could expect even better efficacy if sialic acid level could be further increased. Overall, these results provided a proof-of-concept evidence supportive of initiating clinical trials in humans.

CLINICAL TRIALS

Metabolic supplementation with ManNAc, sialic acid and intravenous immunoglobulin (IVIG; as a source of sialic acid) has been evaluated (Table 1). It is not clear the extent to which metabolic supplementation can correct the defect or modify the course of the disease. Given the slow progression in GNE myopathy, significant changes in muscle strength may not be observed after a relatively short-term metabolic treatment. As muscle is replaced by fibrofatty tissue over time in GNE myopathy, stopping or slowing the progression of the disease is realistic, and can have a considerable impact in patients with this chronic debilitating myopathy.

IVIG trial (NIH)

In 2005, IVIG was used to investigate the effects of sialic acid (Neu5Ac) in four patients with GNE myopathy at the NIH (ClinicalTrials.gov: NCT00195637), since IgG contains 8 μmol of Neu5Ac/g. IVIG was infused as a loading dose of 1 g/kg on two consecutive days followed by three doses of 400 mg/kg at weekly intervals, providing a total of 1.8 mmol (0.55 g) of Neu5Ac for an average participant weighing 70 kg, that is, roughly 6 days worth of normal Neu5Ac production (0.3 mmol/24 h). IVIG administration improved objective measures of muscle strength (by 35% in the quadriceps and 46% in the shoulders), as well as function in patients with GNE myopathy.[46] Patients lost the benefit of IVIG and its sialic acid contribution about 2 weeks after stopping its administration. The clinical improvements were not accompanied by demonstrable histological changes or increased sialylation of target glycoproteins (using available methods at that time), possibly because such changes require longer term treatment or muscle regeneration. However, the finding of definitive improvements after IVIG treatment suggests that provision of sialic acid holds therapeutic promise.

NeuAc (Japan)

Phase 1 clinical trial was conducted at Tohoku University from November 2010 to June 2011 (ClinicalTrials.gov: NCT01236898). Three genetically confirmed patients were recruited and were given 800 mg of NeuAc three times a day up to five consecutive days. No significant adverse effects were observed.

SA-ER (Ultragenyx)

Since regular sialic acid is rapidly excreted after oral administration, a slow release product (sialic acid extended release (SA-ER)) was developed by Ultragenyx, a company involved in developing metabolic treatments for rare diseases. A trial of 47 recruited patients for oral supplementation using this investigational new drug was started in 2012 (ClinicalTrials.gov: NCT01517880). Baseline serum sialic acid levels were reduced in patients and this highly correlated to their performance in several muscle functional measurements.[47] The trial design was 24 weeks of double-blind administration of two doses of SA-ER at a dose of 3 or 6 g/day and a placebo-control group. This was followed by continued administration of either the high or the low dose for an additional 24 weeks. Results of the first phase of the trial gave a modest positive sign in the upper limb functional measurements, compared with a decline in the placebo group (unpublished data presented at the GNE myopathy Consortium meeting, September 2013). Patients with greater walking ability at baseline had a

Table 1 Clinical trials for the development of therapy in GNE myopathy

Clinical trial ID	Sponsor	Drug	Phase	Number of Patients	Status	Outcomes
NCT00195637	NHGRI	Immune globulin	1	4	Completed	
NCT01236898	Tohoku University	NeuAc	1	6	Completed	Safe, no ADE
UMIN000011532	Tohoku University	SA-ER tablet	1	9	Active	
NCT01359319	Ultragenyx Pharmaceutical Inc	SA-ER tablet	1	46	Completed	
NCT01517880	Ultragenyx Pharmaceutical Inc	SA-ER tablet	2	46	Completed	
NCT01830972	Ultragenyx Pharmaceutical Inc	SA-ER/SA-IR capsule	2	56	Active, not recruiting	
NCT01634750	TRND/NHGRI	ManNAc	1	22	Completed	Safe

ADE, adverse drug event; ManNAc, *N*-acetylmannosamine kinase; NeuAc, *N*-acetylneuraminic acid; NHGRI, National Human Genome Research Institute; SA-ER, sialic acid extended release; SA-IR, sialic acid immediate release; TRND, Therapeutics for Rare and Neglected Diseases.

better effect, suggesting that the degree of advancement of this myopathy may be a factor in the observed response. As expected, the serum sialic acid levels rose significantly. There were no serious side effects, and minimal adverse events were not dose related. Results of phase 2 are pending. All 46 of the continuing patients are now on an open-label, high-dose SA-ER for additional 48 weeks.

ManNAc (NIH)

ManNAc is a naturally occurring uncharged monosaccharide and is the first committed precursor for the biosynthesis of Neu5Ac and a substrate of the GNE enzyme. Oral administration of ManNAc in two independent GNE myopathy mouse models improved muscle pathology and hyposialylation.[21 42]

There is an anecdotal evidence of patients with GNE myopathy using ManNAc from a non-pharmaceutical source and without medical supervision in doses up to approximately 12 g/day and ranging from a period of 2 months to several years. The most common reported symptoms are gastrointestinal symptoms, such as abdominal cramps and diarrhoea.

A first-in-human phase 1a, randomised, placebo-controlled, double-blind, single-dose study (ClinicalTrials.gov NCT01634750; IND No.78 091) was conducted at the NIH in 2012–2013. The purpose of this study was to evaluate the safety, pharmacokinetics and pharmacodynamics of ManNAc in participants with GNE myopathy. A total of 22 participants were enrolled in three cohorts. Cohort A included six participants who were randomly assigned in a 2:1 ratio to receive ManNAc (n=4) or placebo (n=2) orally as a liquid solution. Cohorts B and C included eight participants randomly assigned in a 3:1 ratio to receive ManNAc (n=6) or placebo (n=2). The dose levels investigated were 3000, 6000 and 10 000 mg. ManNAc was safe and well tolerated in all participants who participated in this study.

A phase 1b escalating multiple-dose study and a phase 2 efficacy study of ManNAc in participants with GNE myopathy are being planned.

Liposomal systemic GNE delivery

A single patient with GNE myopathy due to two missense mutations (one in the kinase and one in the epimerase domains) was given seven intravenous injections of incremental doses of wild-type GNE over a period of 13 months.[48] The DNA vector was coupled to a human cytomegalovirus immediate early enhancer and promoter (CMV promoter) and delivered systemically in a liposomal package (lipoplex). The effect on muscle function was minimal, but the patient was in an advanced phase of the disease and much strength recovery could not be expected. However, 72 h after the highest dose, expression of wild-type *GNE* and increased sialylation in muscle could be demonstrated. This single-patient trial for compassionate use showed proof-of-principle for this delivery method, although it is expected that infusions will have to be intermittently repeated, as the delivered gene is not expected to persist in the cell cytoplasm.

Future therapeutic development

While metabolic supplementation as therapy for GNE myopathy seems promising, there are still other strategies including developments of: (1) better GNE metabolites or sialic acid compounds,[49] (2) drugs to block or modify degenerative process and (3) gene-based or cell-based therapy. These may be combined with supplementation therapy in the future. Approaches should be explored as they may better correct all deleterious effects of decreased *GNE* function, although safety and feasibility will need to be established. The GNE research laboratory in Jerusalem (under S Mitrani Rosenbaum) with collaboration of other laboratories is trying to develop an AAV-mediated gene vector for systemic administration of *GNE*. Initial results of this approach in animals are promising,[50] but the final proof-of-principle of this approach will be only when human trials are started.

CONCLUSIVE REMARK

Much progress towards understanding and treating GNE myopathy has been achieved, but the final target of developing an efficacious therapy is still underway. However, this is one of the first human hereditary myopathies where a logical metabolic therapy is currently being evaluated and a gene therapy is actively developed.

As clinical trials for potential therapies for GNE myopathy are underway, it is necessary to provide a timely diagnosis for patients with GNE myopathy. An early diagnosis has the potential of maximising the effect of such therapies and reducing anxiety and unnecessary testing in these patients.

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GNE myopathy: current update and future therapy

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REVIEW

Ullrich congenital muscular dystrophy: clinicopathological features, natural history and pathomechanism(s)

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ABSTRACT

Collagen VI is widely distributed throughout extracellular matrices (ECMs) in various tissues. In skeletal muscle, collagen VI is particularly concentrated in and adjacent to basement membranes of myofibers. Ullrich congenital muscular dystrophy (UCMD) is caused by mutations in either *COL6A1*, *COL6A2* or *COL6A3* gene, thereby leading to collagen VI deficiency in the ECM. It is known to occur through either recessive or dominant genetic mechanism, the latter most typically by *de novo* mutations. UCMD is well defined by the clinicopathological hallmarks including distal hyperlaxity, proximal joint contractures, protruding calcanei, scoliosis and respiratory insufficiency. Recent reports have depicted the robust natural history of UCMD; that is, loss of ambulation by early teenage years, rapid decline in respiratory function by 10 years of age and early-onset, rapidly progressive scoliosis. Muscle pathology is characterised by prominent interstitial fibrosis disproportionate to the relative paucity of necrotic and regenerating fibres. To date, treatment for patients is supportive for symptoms such as joint contractures, respiratory failure and scoliosis. There have been clinical trials based on the theory of mitochondrion-mediated myofiber apoptosis or impaired autophagy. Furthermore, the fact that collagen VI producing cells in skeletal muscle are interstitial mesenchymal cells can support proof of concept for stem cell-based therapy.

INTRODUCTION

Collagen VI is an important component of the ECM of skeletal muscle and is involved in maintaining tissue integrity by providing a structural link between different ECM molecules and in promoting adhesion,[1 2] proliferation,[3] migration[4] and survival[5] of various cell types. Collagen VI-related myopathies are the hereditary myopathies caused by mutations in either *COL6A1*, *COL6A2* or *COL6A3* gene, each encoding a subunit of collagen VI. Patients have the clinicopathological features of a muscle disorder as well as of a connective tissue disorder, although the link between this defect of ECM and phenotype remains to be fully elucidated.

Recent advance in molecular biology has evolved the aetiological definition of collagen VI-related myopathies; these myopathies are known to encompass a clinical continuum with Ullrich congenital muscular dystrophy (UCMD) and Bethlem myopathy (BM) at each end of the spectrum, originally described separately.[6–8] Intermediate phenotypes, named as mild UCMD or severe BM, have been known but less well defined as there is currently no clear-cut boundary between two major phenotypes. In addition, it should be noted that genotype–phenotype correlation is very difficult to establish; for example, both extremes of the clinical spectrum are seen in patients with p.Gly284Arg mutation in the *COL6A1* gene, the most commonly observed mutation, while half had an intermediate phenotype.[9] In this context, several researchers have proposed the clinical stratification of patients with collagen VI-related myopathies (figure 1A).[10–12]

The hallmarks of UCMD include marked distal joint hyperlaxity associated with proximal joint contractures, a rigid spine and normal intelligence. Furthermore, children presenting UCMD phenotype have been referred to as having 'early severe' or 'moderately progressive' course of early-onset collagen VI-related myopathies according to maximal motor ability and disease progression.[10–12] Thus, UCMD is relatively well defined as compared with BM or intermediate phenotypes.

CLINICAL PICTURE

In 1930, Otto Ullrich described two boys with an unusual congenital myopathy characterised by muscle weakness and wasting, marked distal joint looseness and contracture of the proximal joints since birth or early infancy and termed this new condition "Kongenitale, atonisch-skelerotische Muskeldystrophie, ein weiterer Typus der heredodegenerativen Erkrankungen des neuromuskulären Systems".[6 7] Subsequent publications confirmed a likely autosomal-recessive inheritance and a recognisable pattern of disease.[13] The diagnostic clinical and molecular criteria for UCMD have been proposed by the European Neuromuscular Centre.[14]

Epidemiology

UCMD is the second most common congenital muscular dystrophy (CMD) after CMD with laminin α2 deficiency (also known merosin-deficient CMD; or MDC1A) in Europe,[15] after Fukuyama CMD in Japan[16] and after α-dystroglycanopathies in Australia.[17] The prevalence of UCMD is reported to be 1.3 per million in Northern England.[18]

Perinatal features and development

Prenatal movements might be reduced in fetuses with UCMD.[19] Some patients have congenital hip dislocation, torticollis and transient kyphotic deformity.[19 20] Multiple joint contractures may be evident at birth, affecting the elbows, knees, spine

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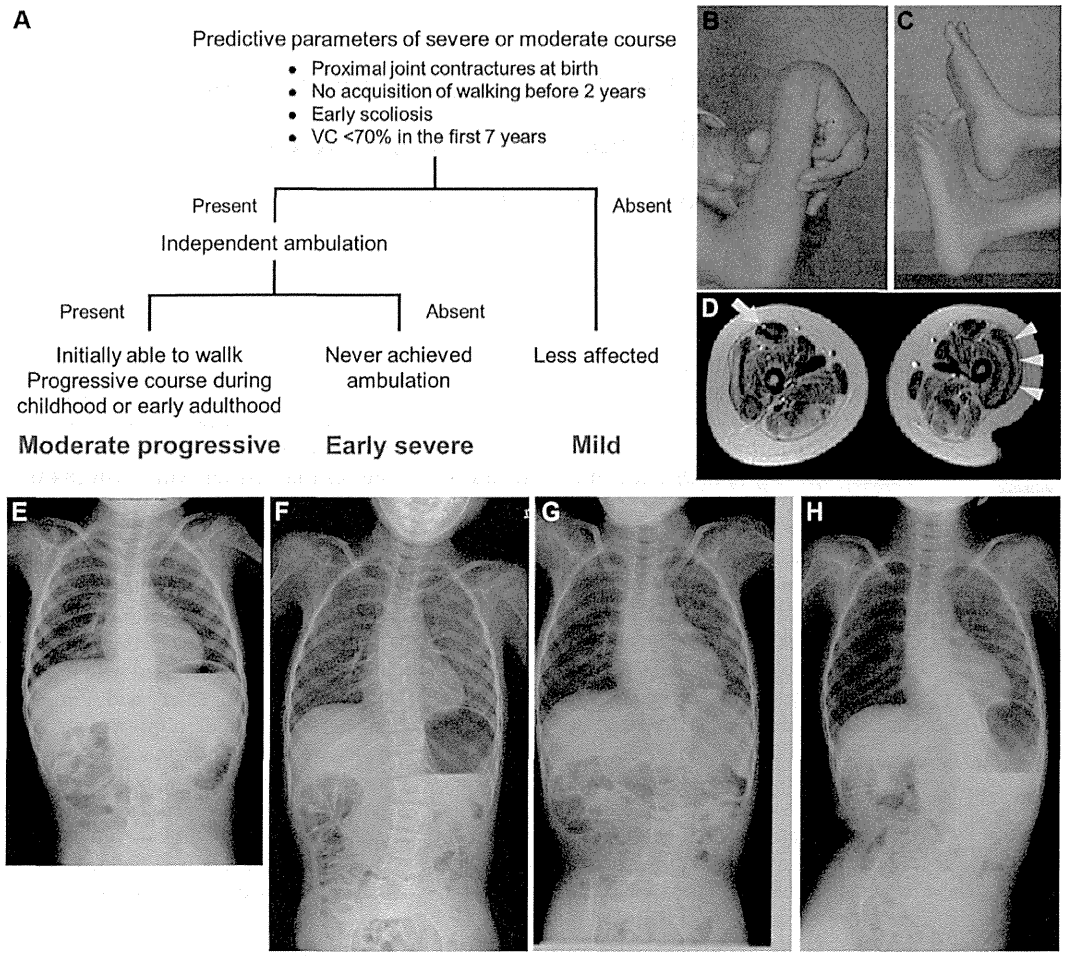

Figure 1 Phenotypic stratification of early-onset collagen VI-related myopathies; modification from Quijano-Roy *et al*[10] and Briñas *et al*[11] (A). Typical distal hyperlaxity and protruding calcanei in a patient with Ullrich congenital muscular dystrophy (UCMD) (B,C). Diagnostic muscle MRI findings of the thigh (D): fatty regeneration along the fascia in the centre of the rectus femoris (arrow) and in the periphery of the vastus lateralis with relative sparing of its central part (arrowheads). Early-onset and rapidly progressive scoliosis in UCMD (E–H). E, 3.7; F, 6.1; G, 6.9; H, 7.9 years of age at assessment. (Images courtesy of Drs Ikuya Nonaka and Akihiko Ishiyama).

(kyphoscoliosis) and ankles. Arthrogryposis multiplex are seen in 20.0% of patients.[19] Some transient feeding difficulties or poor sucking might occur in the neonatal period.[19 20]

The most common presentations are delayed motor milestone and proximal muscle weakness. Patients with UCMD usually become able to sit independently with or without delay.[19] The majority of patients with typical UCMD achieve the ability to walk by 2 years of age. The age at independent ambulation is reported to be 1.7±0.8 and 1.7±0.5 years in English and Japanese natural history studies, respectively.[19 20] This group is referred to as having 'moderate progressive' disease in the phenotypic stratification of early-onset collagen VI-related myopathy (figure 1A).[10 11] In the remaining patients, ambulation is never achieved. Those with the most severe presentation, accounting for 19.4–25.7% of UCMD patients,[11 19] are referred to as having 'early-severe' disease (figure 1A). However, even these severely affected children can usually learn to roll, crawl and maintain a sitting position. Patients who never walk due to severe contractures that prevent an upright posture may walk on their knees for a certain period of time. Achievement of speaking phrases is not delayed, ranging from 12 to 25 months of age.[19]

Clinical manifestations

Patients show generalised muscle weakness predominantly in trunk and proximal limbs. Neck flexors are weak. Facial weakness is reported respectively in 24.1% and 30.8% of patients in two different studies.[19 20] The most striking feature is hyperlaxity of the distal joints (figure 1B), although it can be absent in severely affected individuals. Spinal rigidity, scoliosis and various proximal joint contractures develop with progression of the disease. Typically, initially flexible distal joints, such as fingers, wrists and ankles, eventually become contractured with time. Interestingly, calcanei are often protruded posteriorly (figure 1C). Distal joint hyperlaxity, proximal joint contractures, scoliosis and protruding calcaneous are observed in >50% patients in a Japanese cohort.[19] Many patients have a characteristic facial appearance with round-shaped face with slight drooping of the lower lid and prominent ears.[15] Intelligence is normal. Other features include follicular hyperkeratosis over the extensor surfaces of upper and lower limbs, a softer consistency of the skin in the palms and soles and the tendency to keloid formation.[15] Respiratory insufficiency is not common at birth, although it becomes a critical complication of the disease as the

condition progresses. Cardiac involvement is not documented to date.[21 22]

Serum creatine kinase (CK) activity in patients with UCMD is usually within normal range or only mildly elevated,[19–21] which is unusual in other (congenital) muscular dystrophies. Electromyography shows action potentials of low amplitude and short duration.[13] Muscle MRI shows a characteristic pattern on transverse T1-weighted images—diffuse involvement of the thigh muscles with relative sparing of the medial muscles (sartorius, gracilis and adductor longus).[23] Rectus femoris is variably involved with a typical central area of high signal, called 'central shadow'.[23] In vastus lateralis, the peripheral part is mainly involved and signal intensity is markedly increased while the central part is relatively spared (figure 1D).[23]

Muscle pathology and collagen VI immunohistochemistry

Variable degrees of histological changes can be observed in muscle biopsies from patients with UCMD. The spectrum includes fibre size variation affecting both fast and slow fibres, type 1 fibre predominance, increased endomysial connective tissue or adipose tissue, increased number of internal nuclei and mild necrotic and regenerating process along with indirect evidence of regenerating fibres such as the presence of fibres containing fetal myosin.[21 24] One report described that, early in the disease, UCMD presents as a non-dystrophic myopathy with predominant fibre atrophy, showing a bimodal size distribution of type 1 fibres or a diagnostic pattern of congenital fibre-type disproportion.[25] Unlike other muscular dystrophies, interstitial fibrosis seems disproportionately prominent considering the relative paucity of necrotic and regenerating fibres in UCMD.

Collagen VI is widely distributed throughout ECMs in various tissues. In skeletal muscle, collagen VI is found in the epimysial, perimysial and endomysial interstitium, but it is concentrated in particular in and adjacent to basement membranes of myofibers, blood vessels and intramuscular nerves. Muscle biopsies from UCMD patients can show anything from mild reduction of endomysial or basal lamina collagen VI staining to complete deficiency (CD) of collagen VI in the ECM. We previously showed that, in the majority of patients with UCMD, collagen VI is present in the interstitium but is absent from the sarcolemma by using double immunostaining for collagen IV and VI (figure 2), and named it 'sarcolemma specific collagen VI deficiency (SSCD)'.[26] Electron microscopic findings support a lack of connection between collagen VI microfibrils in the interstitium and the basal lamina, leading to SSCD.[26] Space is observed between muscle fibres and connective tissue that are normally closely attached. Basal lamina appear intact even in degenerating muscle fibres with disorganised myofibrils. These findings suggest a loose connection between the basal lamina and other ECM collagens in UCMD.[27] Collagen VI is deficient also in capillaries in muscle.[13] The absence or alteration of collagen VI can be demonstrated by immunocytochemistry of cultured skin fibroblasts, although this analysis is available only in limited laboratories. In skin, collagen VI expression is decreased in the papillary dermis and skin hair follicles, but not in vessels, peripheral nerves, smooth muscle and sweat glands.[13] One report described that collagen VI levels were greatly decreased in peripheral blood macrophages from three patients with UCMD.[28]

Natural history of disease

Muscle weakness is slowly progressive. Most affected children become able to walk independently but eventually lose ambulation often by early teenage years. Loss of ambulation is reported to occur at 10.7±4.8, 10.1±4.4 and 8.8±2.9 years of age in English, French and Japanese group of patients with UCMD, respectively.[11 19 20] However, this can be widely variable: some patients never walk while others can still walk even beyond late teens. After loss of ambulation, progression of muscle weakness becomes less prominent. In contrast, the contractures can still be progressive, particularly in the ankles, knees, hips and elbows, aggravating physical disability. These clinical features may well be in line with strikingly progressive interstitial fibrosis on muscle pathology.

Respiratory insufficiency usually occurs after the loss of ambulation. It is noteworthy, however, some patients have impending respiratory dysfunction while they are still ambulant. Progressive decline in the predicted forced vital capacity (FVC) or vital capacity (VC) is observed from the preschool age to the early teens.[19 20] Of note, restrictive respiratory dysfunction develops rapidly in the first decade of life; indeed, %predicted FVC declines by 6.6±1.9%/year from 6 to 10 years of age compared to by 0.4±3.0%/year from 11 to 15 years of age.[20] Similarly, VC declines exponentially with a sharp decrease by 10 years of age.[19] This may well be associated with proximal joint and vertebral contractures together with weakness of the diaphragm. The introduction of non-invasive ventilation (NIV) is usually sufficient to treat this situation effectively for many years. The percentage of patients with NIV increases with age; half require NIV by age 11–12 years.[12 19] Natural history study from UK reported that age at initiation of NIV was 14.3 ±4.7 years, with a mean FVC of 20%.[20] The other two studies have recently reported similar findings: an estimated predicted VC of 36% at the time of initiation of NIV at 11.2±3.6 years in a Japanese cohort[19] and an average FVC of 34% just before NIV initiation at 11.3±4.0 years in a large international cohort,[12] demonstrating a remarkable consistency of the pulmonary function declining in patients with UCMD among different cohorts, regardless of different approaches to data acquisition.

Scoliosis, which may require surgical correction, is a common complication.[15 20] Substantial scoliosis appears as early as preschool years and its onset precedes loss of ambulation.[19 20] Development of scoliosis in Duchenne muscular dystrophy (DMD) is strongly related to the loss of walking ability—scoliosis is not typically evident in ambulatory patients and develops after they become wheelchair dependent. In contrast, in UCMD, scoliosis develops even when patients are still ambulant and is progressive from early stage. In our cohort, a maximum progression rate of Cobb angle was 16.2±10.0°/year.[19] Importantly, scoliosis progresses rapidly within years, once it starts (figure 1E–H). The early-onset and rapidly progressive scoliosis in UCMD may well accelerate physical disability, such as difficulty sitting, standing and walking, and cause pain. More importantly, scoliosis can aggravate respiratory function by reducing the rib cage compliance in combination with other proximal joint contractures.

Differential diagnosis

Differential diagnosis includes wide range of neuromuscular disorders in infants and children, such as other forms of muscular dystrophy, congenital myopathies, spinal muscular atrophy (SMA), especially type 2 and 3, and other diseases of connective tissue such as Ehlers–Danlos syndrome (EDS). However, combination of normal or minimally elevated CK levels, lack of cardiac manifestation and specific pattern of thigh muscle involvement is often suggestive of UCMD. Brain MRI is usually normal unlike other CMD forms such as MDC1A, Walker–

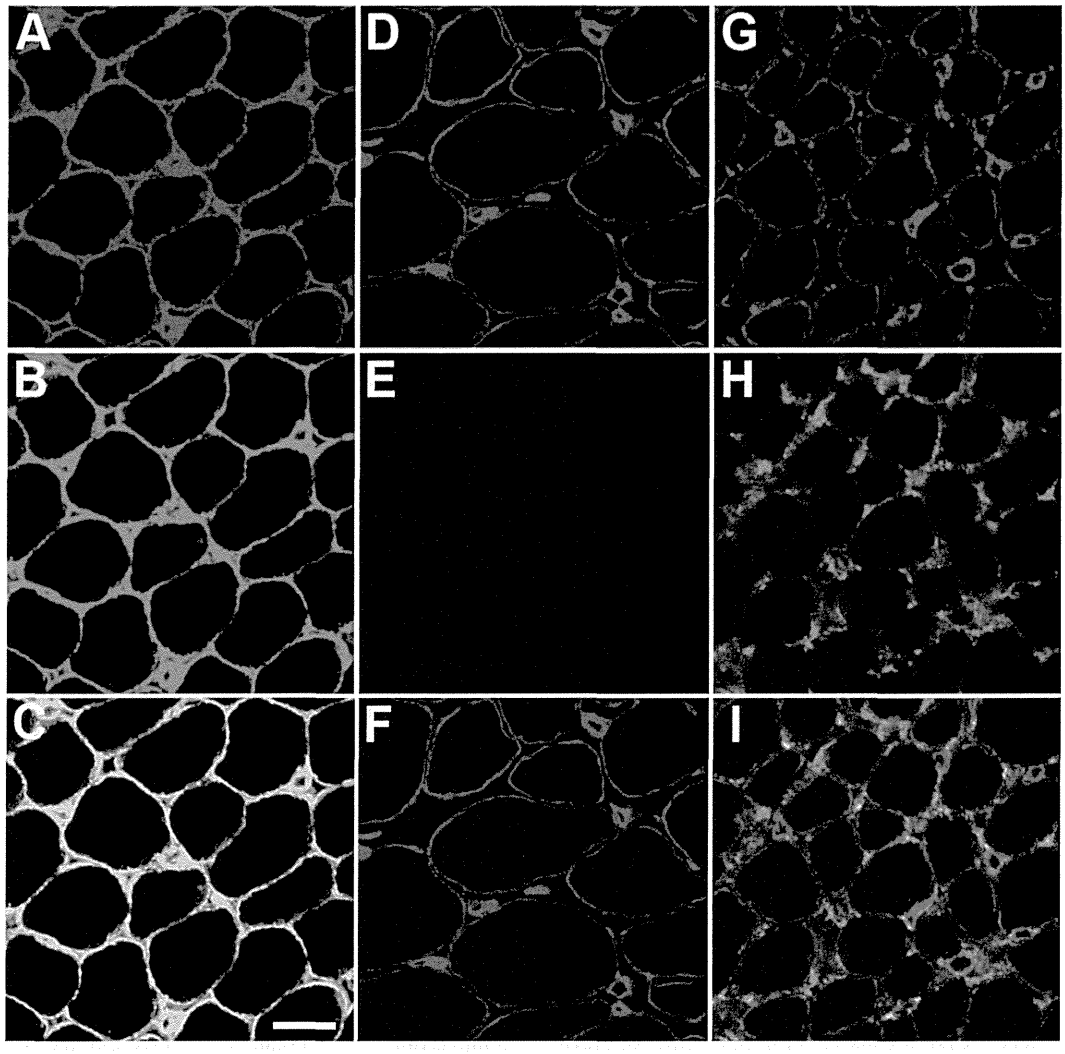

Figure 2 Double immunostaining for collagen IV and VI. (A–C) Patient with non-diagnostic muscle pathology; (D–F) UCMD patient with CD; (G–I) UCMD patient with SSCD. (A,D,G) Immunostaining for collagen IV. Collagen IV is present in the sarcolemma. (B,E,H) Immunostaining for collagen VI. Collagen VI is absent in a patient with CD, while it is present in the interstitium but markedly reduced in a patient with SSCD. (C,F,I) Merged images. Both collagen IV and VI are present in the sarcolemma in a patient with non-diagnostic pathology, as indicated by yellow; in contrast, collagen VI is absent in the sarcolemma in a patient with SSCD, as indicated by only red. UCMD, Ullrich congenital muscular dystrophy; CD, complete collagen VI deficiency; SSCD, sarcolemma-specific collagen VI deficiency, bar 20 μm.

Warburg syndrome, muscle-eye-brain disease and Fukuyama CMD, which may show structural abnormalities or white matter changes. *RYR1*-related core myopathy, including central core disease (CCD) and multiminicore disease, may also show similar clinical features as they can multiple joint contractures and spinal deformity. Progressive respiratory muscle involvement, often disproportionate to the skeletal muscle weakness, is also seen in multiminicore disease. However, significant respiratory insufficiency is unusual in CCD. Interestingly, a recent report demonstrated that muscle pathology in a patient with a heterozygous *COL6A3* gene mutation included cores, rods and lobulated fibres.[29] SMA is characterised by fasciculation and diagnosed by identifying mutations in the *SMN* gene. EDS may mimic UCMD in terms of joint laxity. Furthermore, various types of EDS are commonly associated with mild to moderate muscle weakness. Muscle pathology can demonstrate mild myopathic features but necrotic fibres and fibrosis are absent and collagen VI staining is normal.[30]

Of particular importance is rigid spine with muscular dystrophy type 1 (RSMD1) in forms of CMD, which results from mutations in the *SEPN1* gene, because it can show a significant clinical overlap in the late stage of the disease. RSMD1 patients typically show combination of mild or moderate proximal muscle weakness, Achilles tendon tightness, spinal rigidity and scoliosis, and require ventilator assistance in the first decade of life,[15] similarly to UCMD.

Management

Treatment for patients with UCMD is supportive for symptoms such as respiratory insufficiency and scoliosis, and is dependent on age of the individual and severity of symptoms. Respiratory failure is a common complication of UCMD and careful follow-up with regular assessments of respiratory function, including spirometry and nocturnal oximetry studies, is important to detect asymptomatic decline in patients. In a recent review based on expert consensus on the standard care for CMD, cough assistance using

mechanical insufflation-exsufflation is generally accepted as the method to improve cough efficiency.[22] Other methods such as breath stacking with Ambu bag maintain thoracic compliance and reduce the risk of chronic atelectasis.[22] Respiratory support with nocturnal NIV usually becomes necessary in the first or second decade of life for patients[12 19] and might be effective in reducing symptoms and promoting quality of life. There are times when chronic ventilation can require an invasive application via tracheostomy.

For scoliosis, conservative management including standing frame, positioning and bracing is widely used, although controversial whether those approaches are preventive.[22] However, scoliosis may require active management including spinal surgery to prevent progression, although there have been no formal studies on the efficacy of scoliosis surgery. The choice of the instrumentation such as growing rods depends on the age of the individual, his/her ability to grow and the severity of scoliosis.[22] Suggested contraindications to spinal surgery includes family decision, very poor or deteriorating cardiac status and/or respiratory status, very young age, potential loss of function after spinal fixation and severe scoliosis.[22] Severe respiratory insufficiency may not be a contraindication in certain high specialised centres. In fact, one study on surgical correction of spinal deformity from Japan reported that scoliosis surgery was successfully performed in three patients with UCMD at 11, 13 and 17 years of age, respectively[31]; spinal surgery, however, did not prevent deterioration of respiratory function in these patients, suggesting that at such older ages pulmonary and chest wall compliance might be too severely compromised for patients to benefit from scoliosis surgery, and earlier surgical intervention may be more beneficial. Indeed, a single case report described that slower decline of predicted VC in a patient after scoliosis surgery performed at 5 years of age compared with another patient who had undergone surgical correction of scoliosis at 9 years of age.[19] Further studies are necessary to conclude the efficacy of early scoliosis surgery.

Equipment recommended for assistance in standing, ambulation and/or other forms includes walking frames, standing frames, swivel walker, knee-ankle-foot orthoses, ankle-foot orthoses, scooters and wheelchairs.[22] The joint contractures of patients with UCMD in particular seem to be progressive and regular stretching is recommended to maintain a certain level of mobility of the joints. In addition, feeding and swallowing difficulties can be encountered in UCMD.[20] Issues of feeding and nutrition are multifactorial and closely related to other areas of care; for example, nocturnal hypoventilation can affect appetite and growth and respiratory insufficiency can result in easy fatigue and difficulty in swallowing.[22] Consultation with a nutrition specialist is often helpful to boost energy intake. Some children may need a temporary or permanent gastric feeding tube support to maintain an adequate nutritional and fluid intake.[20 21] Survival has not been fully documented under the current standards of medical care, but failure to introduce adequate respiratory support might lead to the death of teenagers with UCMD.[11 21] With the availability of effective respiratory interventions, patients commonly survive into adulthood to date, and other potential aspects of the disease could surface.

MOLECULAR DIAGNOSIS, PATHOGENESIS AND THERAPEUTIC AVENUES

Collagen VI is a ubiquitously expressed ECM protein composed of three α-chains, α1, α2 and α3. The three chains are encoded by the genes *COL6A1* and *COL6A2* on chromosome 21q22.3 and *COL6A3* on chromosome 2q37.[13] The basic monomer, made up of two globular domains connected by a triple helical structure, is composed of one of each of the these α-chain subunits (1:1:1 ratio). Prior to secretion into the extracellular space, the two basic monomers assemble into dimers (two antiparallel, overlapping monomers) and such dimers associate in a staggered parallel orientation to form tetramers (four monomers) in the cytoplasm.[13 32] Outside the cell, these tetramers, the secreted form of collagen VI, associate in an end-to-end fashion to form collagen VI microfibrillar structures, which interacts with collagen IV and other ECM components including proteoglycans decorin and biglycan, collagen I, hyaluronan, heparin and integrin.[13 33]

Collagen VI gene mutations in UCMD

UCMD used to be regarded as an autosomal-recessive disorder. However, soon after the initial discovery of recessive mutations in the *COL6A2* gene,[34 35] a total of four patients with sporadic UCMD were found to carry *de novo* autosomal-dominant mutations in either *COL6A1*, *COL6A2* or *COL6A3*.[36 37] UCMD is now known to be caused by either recessive or dominant genetic mechanism, the latter most typically occur as *de novo* mutations.[32] This is most likely because these dominant mutations are associated with too severe phenotype to allow patients to produce their offspring. In contrast, in BM, the phenotype is typically mild enough for patients to produce children, resulting in autosomal-dominant inheritance condition.[8 13]

The most common types of mutations are point mutations, exon skipping and mutations leading to premature termination codons (PTCs).[32] Among point mutations, missense changes affecting glycine residues in the Gly-Xaa-Yaa motifs of the N-terminal triple helical domains are the most common and are often dominant *de novo*.[32 33] Splice mutations resulting in in-frame exon skipping are generally dominant *de novo* mutations.[32 33] These dominant mutations can result in secretion of some mutant-containing tetramers into the extracellular space; in the multistep collagen VI intracellular assembly process, only 1/16 of the tetramers produced by patients with dominant mutations could be composed entirely of normal α-chains, thus exerting a dominant negative effect.[37] This leads to loss of normal localisation of collagen VI in the basement membrane and eventually results in a severe phenotype. Nonsense mutations and small deletions or insertions inducing PTCs with consequent nonsense-mediated mRNA decay (NMD), an mRNA quality control mechanism that degrades aberrant mRNAs containing PTCs, and loss of the mutated chain are mostly inherited as recessive mutations.[32 33] Patients with these mutations are unable to assemble or secrete functional collagen VI protein, as all three α-chains are required to form a collagen VI monomer. Thus, such functional null alleles, which underlie typical UCMD, mostly lead to CD mode of collagen VI in skeletal muscles.[16 26 33 34] On the other hand, complete deletions of one copy of these genes also act in a recessive fashion. In support of this notion, carriers of the deletion are in fact clinically asymptomatic, indicating that complete haploinsufficiency of any of the three collagen VI genes does not cause the disease. Interestingly, two reports demonstrated that autosomal-recessive inheritance can also underlie BM, in which patients carried a truncating or null *COL6A2* mutation associated with missense changes in the partnering allele lying within the C2 domain of the α2 chain.[38 39] Furthermore, myosclerosis syndrome was reported to be responsible for a homozygous nonsense *COL6A2* mutation.[40] Unlike nonsense mutations associated with UCMD, the mutated mRNA escaped NMD and was translated into a truncated α2 chain, but secreted collagen VI was reduced and

structurally abnormal and thus did not correctly localise in the basement membrane of myofibers. These facts suggest that the severity of collagen VI gene mutations and the resulting functional abnormality of collagen VI in the ECM dictate a phenotypic spectrum of collagen VI-related myopathies, meaning that a fundamentally different genetic and biochemical mechanism among these myopathies can no longer be assumed.

We previously showed that there are two modes of collagen VI deficiency, CD and SSCD,[26] which respectively result from recessive and *de novo* dominant mutations in the collagen VI genes.[16] There is no straightforward correlation between protein levels and phenotypes; CD, however, is most likely to be associated with the more severe phenotype than SSCD.[11 19] Unlike patients with CD, a great heterogeneity in the maximal motor capacity was observed in patients with SSCD, ranging from no acquisition of walking ability to retaining ambulation throughout childhood.

Properties of collagen VI and its pathological roles

Collagen VI is widely distributed throughout ECMs in various tissues, including muscle, skin, tendon, cartilage, intervertebral discs, lung, blood vessels and adipose tissue.[4] Given the clinical features seen in patients with collagen VI-related myopathies, the tissues in which collagen VI has the most important roles include muscle and tendon. In muscle, the cell source producing collagen VI is the interstitial mesenchymal cell.[41 42] In tendons, abundant collagen VI is present in immediate pericellular ECM of the resident tendon fibroblasts.[43]

Collagen VI contributes to the properties of the local ECM microenvironment by forming a discrete network of beaded microfilaments, which interact with a large number of matrix molecules and cell surface receptors. One possible molecule mediating its interaction would be collagen type IV, the most important collagenous component of basement membranes.[13] On the other hand, collagen VI might be indirectly linked to muscle cell surface receptors via biglycan and the dystrophin-associated protein complex, as collagen VI binds to biglycan,[13] which interacts with the sarcoglycan and dystroglycan complex.[33] The functions of collagen VI pertaining to various cell types also include the promotion of adhesion,[1 2] proliferation,[3] migration[4] and survival.[5 44 45] Attachment of cells to the ECM is important for preventing apoptosis,[46] which could be particularly relevant for muscle disorders that directly involve interactions between matrix and muscle, as is the case for high early implanted cell death, partially due to 'anoikis', in cell transplantation treatment of DMD.[47] Studies with cultured fibroblasts from patients with UCMD have shown that mutant cells or mutated collagen VI exhibit decreased adherence to their surroundings, emphasising that loss of cell ECM interactions is the key mechanism of collagen VI-related myopathies.[2 48] Collagen VI also has crucial roles in the regulation and differentiation of adipocytes.[49]

Studies on muscle fibres from *Col6a1*$^{-/-}$ mice, engineered by genetic ablation of the *Col6a1* gene,[50] and human myoblast cultures has suggested that collagen VI may be involved in preventing myofiber apoptosis, which seems to be mediated by regulating the mitochondrion-mediated cell death cascade[5 51]; a key event appears to be inappropriate opening of the mitochondrial permeability transition pore. These findings therefore link a defect of the ECM to mitochondrial dysfunction followed by apoptosis that is preventable by inactivation of cyclophilin D by using cyclosporine A, its derivative Debio025 or genetic inactivation of cyclophilin D.[51–53] However, there are contradictory reports in which researchers did not find evidence of myofiber apoptosis in biopsied muscles from UCMD patients or *Col6a3* mutant mice muscles,[54 55] suggesting that muscle cell death by apoptosis is not a universal phenomenon in all patients and collagen VI-deficient mice.

In addition, a study of the autophagic process in muscles of *Col6a1*$^{-/-}$ mice revealed that autophagy was not induced efficiently, which determines the presence of dysfunctional organelles in muscle fibres.[56] A similar alteration of autophagy was also detected in muscle biopsies derived from nine patients with UCMD or BM.[56] This defective autophagy provides the link between the previously described mitochondrial dysfunction and myofiber degeneration. These data thus provide a basis for novel therapeutic targets to reactivate of the autophagic flux by either nutritional approaches[57] or by pharmacological and genetic tools in collagen VI deficient skeletal muscle.

Therapeutic advances

The events responsible for myofiber atrophy and loss might be different in UCMD than in other forms of muscular dystrophy with prominent membrane fragility such as DMD. To date, no single hypothesis can fully explain variation in fibre size, ongoing interstitial fibrogenesis and adipogenesis even in mild necrotic and regenerating process in UCMD or provide all targets for therapies, although important clues have been discovered.

Pathological hypotheses leading to myofiber degeneration in collagen VI-deficient skeletal muscle have been proposed and therapeutic targets have been suggested. There have been pilot studies on patients with UCMD based upon the theory of mitochondrial dysfunction or impaired autophagy.[51–53 57] A recent report has shown that collagen VI is a key component of satellite cell niche and lack of collagen VI causes impaired muscle regeneration and reduced satellite cell self-renewal capability after injury in *Col6a1*$^{-/-}$ mice.[58] Additionally, when normal collagen VI is supplied *in vivo* by grafting wild-type interstitial mesenchymal cells, the biochemical properties of collagen VI-deficient muscles are ameliorated and satellite cell defects also rescued.[58] These results can open new venues for a better understanding of the pathomechanism underlying collagen VI-related myopathies. Furthermore, multipotent mesenchymal stem cell (MSC) is the most common type of adult stem cells and is isolated from several sources such as bone marrow and adipose tissue. Another report has recently shown that transplanted human adipose-derived stem cells, with phenotypic and functional features of mesenchymal progenitors, secrete collagen VI protein in *Col6a1*$^{-/-}$ mice.[59] Thus, MSC-based therapy can be an attractive option as transplanted cells are able to self-renew and to differentiate into collagen VI-producing cells in skeletal muscle.[41 42]

Advances in molecular genetics provide gene-based therapies; that is, antisense oligonucleotide or small interfering RNA (siRNA) inhibition of mutant transcripts exerting dominant negative effects[60–62] and upregulation of mutant transcripts by specific inhibition of NMD.[63] As 60–80% of UCMD cases are attributed to dominant negative mutations,[11 16] the allele-specific antisense approach can be applied to the majority of patients. Recent reports have shown that siRNA-mediated knockdown of SMG-1 and Upf1, essential components for NMD, or SMG-8, a subunit of SMG-1 kinase, gives rise to the upregulation of mutant triple-helical collagen VI, thus ameliorating mutant phenotypes from UCMD fibroblasts with a homozygous frameshift mutation causing a PTC in the *COL6A2* gene.[64 65]

CONCLUSION

UCMD is caused by mutations in either *COL6A1*, *COL6A2* or *COL6A3* gene, thereby leading to collagen VI deficiency in the ECM. We here presented the clinicopathological features, robust natural history and the current supportive care for symptoms. Of special interest is progressive interstitial fibrosis even in very mild necrotic and regenerating process in muscle. Patients with UCMD have unique manifestations attributable to both muscle and connective tissue disorders. Collagen VI contributes to the properties of the local ECM microenvironment by forming a discrete network of beaded microfilaments, which interact with a large number of matrix molecules and cell surface receptors. Advanced researches have provide important clues to explain how collagen VI deficiency in the ECM can cause the development of muscle weakness in *Col6a1*$^{-/-}$ mice or patients with UCMD, although the link between the ECM defect and phenotype remains to be fully elucidated. Further studies are necessary to elucidate exactly how collagen VI deficiency in the ECM makes muscle cells vulnerable to apoptosis or interstitial fibrogenesis and adipogenesis strikingly progressive.

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Ullrich congenital muscular dystrophy: clinicopathological features, natural history and pathomechanism(s)

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