Therapy of Collagen VI-Related Myopathies (Bethlem and Ullrich)

Luciano Merlini*† and Paolo Bernardi‡

**Laboratory of Myology, Department of Medical Genetics, University of Ferrara, Ferrara, Italy 44100; †Laboratory of Cell Biology, Istituto Ortopedico Rizzoli, Bologna, Italy 40136; and ‡Department of Biomedical Sciences, University of Padova, Padova, Italy 35121*

Summary: The collagen VI-related myopathies comprise two major forms, Bethlem myopathy (BM) and Ullrich congenital muscular dystrophy (UCMD), which show a variable combination of muscle wasting and weakness, joint contractures, distal laxity, and respiratory compromise. Specific diagnosis requires molecular genetic testing showing mutation in one of the three genes involved. This review summarizes current treatments, in particular indication for physiotherapy, orthopedic treatment for correction of foot deformity, scoliosis, and flexion contractures of elbows, and treatment of respiratory failure. The turning point in basic research on collagen VI myopathies was the discovery of an unexpected mitochondrial dysfunction as a pathogenetic mechanism underlying the myopathic syndrome seen in *Col6a1* null mice. Treatment of *Col6a1*$^{-/-}$ mice with cyclosporin A (CsA) rescued the mitochondrial dysfunction and decreased apoptosis. Similar mitochondrial defects were revealed in cultures of UCMD patients. The results of an open pilot trial with CsA in five patients with collagen VI-related myopathies are summarized and discussed. With the availability of new potential effective treatments, several challenges must be addressed in conducting trials in orphan diseases and in neuromuscular disorders in particular. Outcome measures are discussed in the context of the expected effect of the cure. Randomized clinical trials often are not feasible for rare diseases, and sometimes would be ethically inappropriate. The need to develop alternative outcome measures or biomarkers using platforms such as genomics and proteomics is stressed in this context. **Key Words:** Bethlem myopathy, Ullrich congenital muscular dystrophy, collagen VI, outcome measures, therapy.

INTRODUCTION

Mutations in the genes encoding collagen VI (*COL6A1*, *COL6A2*, and *COL6A3*) cause the collagen VI-related myopathies, which comprise two major clinical forms, Bethlem myopathy (BM [MIM 158810])[1] and Ullrich congenital muscular dystrophy (UCMD [MIM 254090]),[2] but also the limb girdle[3] and the myosclerosis variants.[4] Bethlem myopathy is an autosomal dominant disorder characterized by slowly progressive axial and proximal muscle weakness with finger flexion contractures.[5] Bethlem myopathy shows interfamilial variability and different clinical onset, from prenatal to mid-adulthood. Prenatal onset is characterized by decreased fetal movements; neonatal onset with hypotonia or torticollis; early-childhood onset with delayed motor milestones and muscle weakness and contractures; and adult onset with proximal weakness, and Achilles tendon and finger contractures. Bethlem myopathy is usually mild,[5] sometimes slowly progressive, with some affected individuals over 50 years of age needing aids for mobility outdoors.[6] There is no evidence of cardiac involvement in BM.[7] Respiratory failure is part of the clinical spectrum and can occur in ambulatory patients.[7]

Ullrich congenital muscular dystrophy is an autosomal recessive severe disorder characterized by congenital muscle weakness with axial and proximal joint contractures and coexisting distal joint hypermobility.[8] The presentation is usually at birth, with hypotonia, congenital hip dislocation, prominent calcanei, and a transient kyphotic deformity. Motor milestones are delayed, and most of the children never acquire the ability to walk independently. Follicular hyperkeratosis over the extensor surfaces of upper and lower limbs and keloid and cigarette paper scar formation are common. Axial muscle involvement is severe, resulting in progressive scoliosis with spine rigidity. Early and severe respiratory involvement may require artificial ventilatory support in the first

Address correspondence and reprint requests to: Luciano Merlini, M.D., Laboratory of Myology, Department of Medical Genetics, S. Anna Hospital, University of Ferrara, Via Fossato di Mortara 74, 44100 Ferrara, Italy. E-mail: luciano.merlini@unife.it.

or second decade of life. Patients with UCMD have a normal intelligence, and magnetic resonance imaging shows normal brain development. Patients with *de novo* heterozygous mutations may have a severe phenotype,[9,10] whereas patients with recessive mutations may present a milder Bethlem-type disease.[11] In addition, in some patients with the UCMD phenotype, the role of collagen VI molecules has been excluded, suggesting genetic heterogeneity even for this condition.[9–13] Prevalence has been estimated as 0.5:100,000 for BM and 0.1:100,000 for UCMD,[14] but these disorders are probably underdiagnosed. No muscular dystrophy has yet been connected to mutations in the newly identified collagen VI chains α4, α5, and α6.[15]

DIAGNOSIS

These disorders are rare, but also are frequently misdiagnosed, or remain undiagnosed. They have no, or few, specific clinical and laboratory findings; and the correct diagnosis requires molecular genetic testing, which is currently available only at a few medical centers. Diagnosis depends on typical clinical features, with the serum creatine kinase concentration usually being normal or only mildly elevated and muscle biopsy showing myopathic or dystrophic changes.[8] In BM, collagen VI immunolabeling of muscle is usually normal or shows only subtle alterations. In older individuals, a secondary reduction of laminin β1 labeling may be observed.[16] In UCMD, collagen VI immunolabeling is absent or markedly reduced from the endomysium and basal lamina, but may be normal around capillaries.[17] If muscle is not available for collagen immunolabeling, loss of collagen VI in skin fibroblast cultures may be a useful adjunct to the diagnosis.[18–20]

CURRENT TREATMENT

Physiotherapy follows the standard used for slowly progressive neuromuscular diseases and consists mainly of advice regarding stretching, splinting, and mobility aids. Orthopedic treatment is considered for correction of foot deformity, scoliosis, and flexion contractures of elbows. In pediatric patients with Achilles tendon contractures interfering with stable walking, surgery may be considered. It is recommended that the patient receive intensive physical therapy after foot surgery.

Patients with UCMD have an axial weakness and are prone to develop scoliosis, but because of the rarity of this condition the experience in treatment of UCMD-related scoliosis is limited. Our suggestion, therefore, is to follow the rules that apply to a condition with a similar range of clinical severity, namely, the intermediate–mild form of spinal muscular atrophy (SMA2-3).[21] Because the scoliosis develops from early infancy, when the chest wall is normally very compliant, in UCMD and SMA2 the distortion of the thorax tends to be very severe and causes severe impairment of lung growth. Bracing is not a definitive method of preventing progression in SMA, but it may be used for improving trunk support or seating comfort.[21,22] Braces may also cause further pulmonary restriction, however, and may interfere with feeding, especially gastrostomy tube feeding. In early childhood, satisfactory results were not observed after telescope rod implantation.[22] For SMA2 patients younger than 10 years with progressive scoliosis, the current therapeutic recommendation is a corset until the age of 10 to 12 years, followed by definitive surgical correction using multisegmental instrumentation.[22] Nonetheless, decision making for surgery is challenging in the presence of pulmonary dysfunction, poor nutritional status, and feeding disorders. "Who should be treated?" and "when?" are the two questions best answered on a patient-by-patient basis. As in other comparable neuromuscular disorders, the patients in the mild spectrum of the disease are the ones who benefit more from spine surgery, with less risk of surgical and respiratory-associated life-threatening complications. In general, patients with neuromuscular scoliosis are likely to experience a deterioration of their pulmonary function despite scoliosis surgery.[21]

Comparable results have been reported with both arthroscopic and open methods for correction of post-traumatic diminished elbow extension.[23,24] However, there is no specific experience in surgical correction of elbow flexion contractures secondary to muscular dystrophy. At the Rizzoli Orthopedic Institute in Bologna, Italy, a 14-year-old boy affected with BM received an open release of the right elbow. The boy had a mild muscle weakness, but severe contractures. Elbows were flexed at 110° and moved in further flexion for only 10°. Right anterior release with capsulectomy yielded a full elbow extension with passive flexion of 65° and active flexion of 50°. In dystrophic elbow contractures, however, it should be kept in mind that after surgery active flexion will still be limited by the weakness of the flexor muscles of the elbow.

TREATMENT OF RESPIRATORY FAILURE

Assessment and treatment of respiratory impairment in various childhood neuromuscular disorders, including UCMD, was the focus of the 117th ENMC Workshop.[25] General rules to follow are respiratory surveillance for possible nocturnal hypoventilation, prophylaxis of chest infections with vaccination and physiotherapy, and aggressive treatment of pulmonary infections. In UCMD, respiratory failure typically occurs during childhood, when most of the severely affected patients are wheelchair-bound. However, a minority of these patients are at specific risk of respiratory failure even while ambulant

and should be monitored for diaphragmatic impairment.[2] Assisted cough, noninvasive ventilation, and tracheostomy ventilation are treatments successfully applied in specific situations. Respiratory impairment in UCMD patients may also be complicated by nutritional problems. Assessment of these children should therefore take this into account, with swallowing and dietetic assessment and timely use of gastrostomy being vital to overall management.

THE COLLAGEN VI–MITOCHONDRIA CONNECTION

Mitochondria play a critical role in initiating both apoptotic and necrotic cell death.[26] A major player in this process is the mitochondrial permeability transition pore (PTP), a selective channel that opens in the inner mitochondrial membrane under conditions of elevated matrix concentration of Ca^{2+}, especially when this is accompanied by oxidative stress and depletion of adenine nucleotides.[27,28] Opening of the PTP causes massive swelling of mitochondria, rupture of the outer membrane, and release of intermembrane components that induce apoptosis. In addition, after PTP opening, the inner membrane becomes permeable to protons, resulting in the uncoupling of oxidative phosphorylation, which in turn causes the reverse mode activation of ATPase, leading to ATP hydrolysis rather than ATP synthesis. Pore opening is inhibited by cyclosporin A (CsA), and CsA analogs devoid of immunosuppressive activity, with the same affinity, inhibit the peptidyl-prolyl cis-trans isomerase activity of mitochondrial cyclophilin (CyP-D).[29]

Irwin et al.[27] have revealed an unexpected mitochondrial dysfunction as a pathogenetic mechanism underlying the myopathic syndrome seen in *Col6a1* null mice.[26,27] Mice with targeted disruption of the *Col6a1* gene display a muscle phenotype that strongly resembles Bethlem myopathy.[5,30] *Col6a1*$^{-/-}$ muscles have a loss of contractile strength associated with ultrastructural alterations, consisting of marked dilations of the sarcoplasmic reticulum (SR), and of mitochondrial alterations that ranged from tubular cristae, to electron-dense inclusions, to overt swelling.[27] Remarkably, myofibers with mitochondrial-SR alterations also displayed nuclear features of apoptosis, suggesting a link between organellar changes and increased incidence of cell death. A latent mitochondrial dysfunction was demonstrated in myofibers of *Col6a1*$^{-/-}$ mice on incubation with the selective F1F(O)-ATPase inhibitor oligomycin, which caused mitochondrial depolarization, Ca^{2+} deregulation, and increased apoptosis. These defects were reversible, and could be normalized by plating *Col6a1*$^{-/-}$ myofibers on collagen VI or by addition of CsA. Treatment of *Col6a1*$^{-/-}$ mice with CsA rescued the ultrastructural defects in muscle and markedly decreased the number of apoptotic nuclei *in vivo*.

These exciting studies have now been taken one important step further towards the clinical goal of developing a therapy for collagen VI-based myopathies in humans.[26] To establish whether mitochondria are also involved in the pathogenesis of UCMD and BM, the same group studied muscle biopsies and myoblasts from patients.[31] UCMD patients displayed an increased rate of apoptosis in skeletal muscle *in vivo* and in primary myoblast cultures.[31] The latter also displayed a measurable fraction of altered mitochondria (with morphological alterations ranging from shape changes to overt swelling) and the presence of a latent mitochondrial dysfunction that could be revealed by the addition of oligomycin, which caused depolarization only in mitochondria from patients.[31] The mitochondrial defect could be revealed in cultures from UCMD patients irrespective of whether the primary genetic defect was in the *COL6A1* or the *COL6A3* gene, and in both homozygous and heterozygous mutations.[31] These findings suggest that PTP opening plays a key role in all collagen VI myopathies, which opens new perspectives for the pharmacological treatment of patients.[16,31,32]

PILOT CLINICAL TRIAL WITH CsA

In an open trial with CsA, five patients representing the clinical and molecular variety of collagen VI myopathies were enrolled.[33] Four were affected by UCMD and one by BM; three were of pediatric age and two were adults. Their collagen VI ranged from almost normal to marked depletion, and the pathogenic mutation involved each of the three *COL6* genes. All patients were treated for 1 month with CsA. Muscle biopsy was done before the onset of treatment, and mitochondria within the cells isolated from patients depolarized after the addition of oligomycin, confirming *in vivo* the presence of the latent dysfunction previously identified in muscle fibers from *Col6a1*$^{-/-}$ mice and in cultures from UCMD patients.[31] On the other hand, the vast majority of mitochondria in cells prepared from muscle biopsies of two healthy individuals did not depolarize upon the addition of oligomycin. One month after oral administration of CsA at a dose of 5 mg/kg per day in two divided doses, a new biopsy was taken at the contralateral site, and the experiment was repeated.

Strikingly, the mitochondrial membrane potential response to oligomycin was largely normalized in the muscle cells from all patients, indicating that at the dose used the CsA reached pharmacologically active concentrations in muscle.[33] In addition, unlike samples from normal donors, in which apoptosis was undetectable, all samples from patients had a sizeable number of apoptotic nuclei in the first biopsy, and treatment with CsA considerably decreased the occurrence of apoptosis in all patients, indicating a cause-and-effect relationship be-

tween normalization of mitochondrial function and decreased cell death. After treatment with CsA, an increased number of regenerating myofibers were detected, particularly prominent in the three younger patients included in the study.

It is quite encouraging that CsA decreased apoptosis in all treated patients within 1 month,[33] because this finding suggests that assessing the effects of CsA on the clinical course of the disease may be feasible in future clinical trials. Some reason for hope also comes from the observation that treatment with CsA increased muscle regeneration. An appealing explanation is that in collagen VI diseases differentiating muscle cells are undergoing early apoptosis together with mature fibers, a situation that would be similar to the ineffective erythropoiesis seen in many hemoglobinopathies. By decreasing ineffective myogenesis, CsA could increase the overall efficiency of muscle regeneration. This is another promising indication that long-term PTP desensitization may do more than just stop the progression of the disease. Additional studies and a larger sample size will be required before firm conclusions can be made about this issue and about the correlation between regeneration and patient age observed here.

This study[33] shows that treatment with moderate doses of CsA favorably affects mitochondrial function in collagen VI myopathies *in vivo,* and dramatically decreases the frequency of muscle cell death in the patients. Whether this will eventually stop progression of the disease or translate into a better muscle performance depends on multiple factors, such as muscle loss and extent of fibrosis at the time of treatment, and potential for muscle regeneration in individual patients. Furthermore, the potential benefit must be carefully weighed against increased occurrence of infections that may follow immunosuppression during treatment with CsA. We note, however, that other cyclosporins (e.g., MeAla^{3}EtVal^{4}–cyclosporin; Debio-025, DebioPharm, Lausanne, Switzerland) may represent an alternative to CsA.[31] It has been shown that Debio-025, a nonimmunosuppressive cyclosporin that retains the PTP-desensitizing properties of CsA, is as effective as CsA at protecting mitochondrial function and preventing apoptosis in muscle cultures from UCMD patients—a finding that holds great promise for the treatment of collagen VI disorders.[31] The result of this trial represent an important proof of principle, that hereditary muscle diseases can be cured with proper drugs downstream of the genetic lesion if the pathogenetic mechanisms are understood.

OTHER IN VITRO TREATMENT APPROACHES

Another possibility for pathogenetic mechanism and therapeutic intervention involves nonsense-mediated decay and its specific inhibition. Nonsense-mediated decay may be responsible for some of the collagen mutations in patients with UCMD. Usuki et al.[34] have shown that siRNA-mediated knockdown of SMG-1 or Upf1, both essential proteins of nonsense-mediated decay, led to upregulation of the mutant triple-helical collagen VI in the fibroblasts from a patient with homozygous frameshift mutation with a premature translation termination codon in the *COL6A2* gene, resulting in the formation of partially functional extracellular matrix.

OUTCOME MEASURES

Up until now, hereditary neuromuscular diseases had no cure and clinical trials were tailored to track down the correction of a decline in function, as in steroids in Duchenne muscular dystrophy,[35] or to better delineate the natural history of a disease like most spinal muscular atrophy trials.[36] With the availability of enzyme replacement therapy for Pompe disease,[37] and the indication that antisense-mediated exon skipping may be a potential approach to restoring dystrophin synthesis in the muscles of patients with Duchenne muscular dystrophy,[38] the scenario has rapidly changed. It has been realized that new cures are coming, and that they will have something in common: lifelong duration, frequent administration, side effects, and high cost for the health care system. Because no cure has the same effect in all affected populations, it is imperative to be able to document benefits at the individual level, to justify demanding, risky, and expensive treatments. This requirement should be reflected in proper trial design.

A crucial point to consider is, "what kind of effect are we anticipating with the cure or treatment?" Is it dramatic (penicillin-like)? or moderate to mild improvement? or stabilization? It seems as if, in an ideal scenario (i.e., a cure which completely restores the missing protein), patients with the same disease may have a different outcome. A dramatic effect could be seen only in less affected or presymptomatic patients, cases in which the loss of muscle fiber and the connective substitution are minimal and the regeneration capacity is maximal. In contrast, in a situation in which there is diffuse connective substitution with little or no functional muscle tissue left, and regeneration has been exhausted, only minimal improvement or stabilization can be reasonably expected.

Most myopathies, including collagen VI-related myopathies, are slowly progressive disorders characterized by muscle wasting and weakness that compromise motor and respiratory function. If a cure is available, it should have a positive effect on muscle strength, recovering muscle and respiratory function. In addition, these myopathies are clinically heterogeneous, with patients in whom motor function severity ranges from children who have never been able to walk to still ambulant adults.

Survival rates are likewise highly variable. In summary, even in an ideal scenario in which a cure is available, it is not easy to anticipate at the individual level what kind of improvement will be achievable and how long it would take to get there.

How can we make sure, then, that a cure works? The randomized, controlled trial (RCT) is the gold standard for establishing the effect of an intervention for common diseases.[39,40] This approach, however, is often not feasible for rare diseases such as congenital muscular dystrophies.[40] The U.S. Congress has defined a rare disease as a disease affecting fewer than 200,000 individuals in the United States. Currently, the global number of genetically defined UCMD patients is below 100, which is just half the minimal number of patients usually required to conduct a sufficiently large RCT. In addition, trials are considered ethical when prior belief is in equipoise; that is, randomization is sound when utility is expected to be the same with each treatment. Once it has been shown that a pathological biomarker is positively affected by the treatment (restoration of the missing protein, correction of a downstream alteration), the equipoise belief is disproved, and an RCT is ethically questionable. Furthermore, if there is no alternative treatment, an RCT is unnecessary.[40]

In brief, what kind of a clinical trial is feasible once the cure is found in a very rare disease with variable prognosis and markedly different outcomes expected? Is there a way out of the conundrum?

Due to the nature of rare disease, clinicians have decided that open protocol, open-label, historical control, cross-over trials, withdrawal design trials, or surrogate endpoints are the most appropriate to their studies. In the European Medicines Agency (EMEA) guidelines on clinical trials in small populations, it is stated that in very rare diseases, the combined evaluation of single case studies may be the only way to provide evidence.[41] In such situations, treatment conditions and data collection should be standardized, studies should be prospectively planned, and every patient should contribute as much information as possible. The usual approach of prespecifying the primary endpoint may be too conservative, and more knowledge may be gained from collecting all sensible or possible endpoints and then presenting all the data in the final study report.

Several challenges must be addressed in conducting trials in orphan diseases and in neuromuscular disorders in particular. There is a clear need for newer, more sensitive outcome measures to detect small changes in disease progression in addition to muscle strength evaluation for impairment, and forced vital capacity, timed tests, and functional scales for disability. A key goal is to develop alternative outcome measures or biomarkers that can identify whether a treatment has an effect over much shorter time periods—a few months, instead of a few years. Molecular biomarkers may be discovered using platforms such as genomics and proteomics. There is the hope that genomic profiling can aid in identifying patients who are likely to benefit from a given therapeutic, because the disease that the compound aims to treat is heterogeneous in nature and a single therapy for such disease may be effective in only a small subset of cases. From a clinical perspective, proteomics may be most useful when protein expression profile of samples from two or more different biological states are compared (disease *versus* normal; drug-treated *versus* placebo) to identify proteins that are differentially expressed in one population over the other.[42]

With the availability of new potentially effective treatments, the clinicians who care for patients with neuromuscular orphan diseases should take charge in the conduct of clinical trials and devise proper new ways to show if these treatments work.

Acknowledgments: L.M. was supported by a grant of the Italian Health Ministry (RF 2005).

REFERENCES

1. Bethlem J, Wijngaarden GK. Benign myopathy, with autosomal dominant inheritance: a report on three pedigrees. Brain 1976;99: 91–100.
2. Camacho Vanegas O, Bertini E, Zhang RZ, et al. Ullrich scleroatonic muscular dystrophy is caused by recessive mutations in collagen type VI. Proc Natl Acad Sci U S A 2001;98:7516–7521.
3. Scacheri PC, Gillanders EM, Subramony SH, et al. Novel mutations in collagen VI genes: expansion of the Bethlem myopathy phenotype. Neurology 2002;58:593–602.
4. Merlini L, Martoni E, Grumati BS, et al. Autosomal recessive myosclerosis myopathy is a collagen VI disorder. Neurology 2008 (in press).
5. Merlini L, Morandi L, Granata C, Ballestrazzi A. Bethlem myopathy: early-onset benign autosomal dominant myopathy with contractures: description of two new families. Neuromuscul Disord 1994;4:503–511.
6. Pepe G, Giusti B, Bertini E, et al. A heterozygous splice site mutation in *COL6A1* leading to an in-frame deletion of the α1(VI) collagen chain in an Italian family affected by Bethlem myopathy. Biochem Biophys Res Commun 1999;258:802–807.
7. van der Kooi AJ, de Voogt WG, Bertini E, et al. Cardiac and pulmonary investigations in Bethlem myopathy. Arch Neurol 2006;63:1617–1621.
8. Pepe G, Bertini E, Bonaldo P, et al. Bethlem myopathy (BETHLEM) and Ullrich scleroatonic muscular dystrophy: 100th ENMC international workshop, 23–24 November 2001, Naarden, The Netherlands. Neuromuscul Disord 2002;12:984–993.
9. Pan TC, Zhang RZ, Sudano DG, Marie SK, Bonnemann CG, Chu ML. New molecular mechanism for Ullrich congenital muscular dystrophy: a heterozygous in-frame deletion in the *COL6A1* gene causes a severe phenotype. Am J Hum Genet 2003;73:355–369.
10. Baker NL, Morgelin M, Peat R, et al. Dominant collagen VI mutations are a common cause of Ullrich congenital muscular dystrophy. Hum Mol Genet 2005;14:279–293.
11. Demir E, Sabatelli P, Allamand V, et al. Mutations in *COL6A3* cause severe and mild phenotypes of Ullrich congenital muscular dystrophy. Am J Hum Genet 2002;70:1446–1458.
12. Lampe AK, Dunn DM, von Niederhausern AC, et al. Automated genomic sequence analysis of the three collagen VI genes: applications to Ullrich congenital muscular dystrophy and Bethlem myopathy. J Med Genet 2005;42:108–120.

13. Lucioli S, Giusti B, Mercuri E, et al. Detection of common and private mutations in the *COL6A1* gene of patients with Bethlem myopathy. Neurology 2005;64:1931–1937.
14. Lampe AK, Bushby KM. Collagen VI related muscle disorders. J Med Genet 2005;42:673–685.
15. Gara SK, Grumati P, Urciuolo A, et al. Three novel collagen VI chains with high homology to the α3 chain. J Biol Chem 2008; 283:10658–10670.
16. Merlini L, Villanova M, Sabatelli P, Malandrini A, Maraldi NM. Decreased expression of laminin β1 in chromosome 21-linked Bethlem myopathy. Neuromuscul Disord 1999;9:326–329.
17. Higuchi I, Horikiri T, Niiyama T, et al. Pathological characteristics of skeletal muscle in Ullrich's disease with collagen VI deficiency. Neuromuscul Disord 2003;13:310–316.
18. Jimenez-Mallebrera C, Maioli MA, Kim J, et al. A comparative analysis of collagen VI production in muscle, skin and fibroblasts from 14 Ullrich congenital muscular dystrophy patients with dominant and recessive *COL6A* mutations. Neuromuscul Disord 2006; 16:571–582.
19. Zou Y, Zhang RZ, Sabatelli P, Chu ML, Bonnemann CG. Muscle interstitial fibroblasts are the main source of collagen VI synthesis in skeletal muscle: implications for congenital muscular dystrophy types Ullrich and Bethlem. J Neuropathol Exp Neurol 2008.
20. Hicks D, Lampe AK, Barresi R, et al. A refined diagnostic algorithm for Bethlem myopathy. Neurology 2008;70:1192–1199.
21. Merlini L, Granata C, Bonfiglioli S, Marini ML, Cervellati S, Savini R. Scoliosis in spinal muscular atrophy: natural history and management. Dev Med Child Neurol 1989;31:501–508.
22. Fujak A, Ingenhorst A, Heuser K, Forst R, Forst J. Treatment of scoliosis in intermediate spinal muscular atrophy (SMA type II) in childhood. Ortop Traumatol Rehabil 2005;7:175–179.
23. Aldridge JM 3rd, Atkins TA, Gunneson EE, Urbaniak JR. Anterior release of the elbow for extension loss. J Bone Joint Surg Am 2004;86A:1955–1960.
24. Nguyen D, Proper SI, MacDermid JC, King GJ, Faber KJ. Functional outcomes of arthroscopic capsular release of the elbow. Arthroscopy 2006;22:842–849.
25. Wallgren-Pettersson C, Bushby K, Mellies U, Simonds A; ENMC. 117th ENMC workshop: ventilatory support in congenital neuromuscular disorders: congenital myopathies, congenital muscular dystrophies, congenital myotonic dystrophy and SMA (II) 4–6 April 2003, Naarden, The Netherlands. Neuromuscul Disord 2004; 14:56–69.
26. Hohenester BR. A therapy for myopathy caused by collagen VI mutations? Matrix Biol 2007;26:145.
27. Irwin WA, Bergamin N, Sabatelli P, et al. Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency. Nat Genet 2003;35:367–371.
28. Bernardi P, Petronilli V, Di Lisa F, Forte M. A mitochondrial perspective on cell death. Trends Biochem Sci 2001;26:112–117.
29. Halestrap A. Biochemistry: a pore way to die. Nature 2005;434: 578–579.
30. Bonaldo P, Braghetta P, Zanetti M, Piccolo S, Volpin D, Bressan GM. Collagen VI deficiency induces early onset myopathy in the mouse: an animal model for Bethlem myopathy. Hum Mol Genet 1998;7:2135–2140.
31. Angelin A, Tiepolo T, Sabatelli P, et al. Mitochondrial dysfunction in the pathogenesis of Ullrich congenital muscular dystrophy and prospective therapy with cyclosporins. Proc Natl Acad Sci U S A 2007;104:991–996.
32. Rizzuto R. The collagen–mitochondria connection. Nat Genet 2003;35:300–301.
33. Merlini L, Angelin A, Tiepolo T, et al. Cyclosporin A corrects mitochondrial dysfunction and muscle apoptosis in patients with collagen VI myopathies. Proc Natl Acad Sci U S A 2008;105: 5225–5229.
34. Usuki F, Yamashita A, Kashima I, Higuchi I, Osame M, Ohno S. Specific inhibition of nonsense-mediated mRNA decay components, SMG-1 or Upf1, rescues the phenotype of Ullrich disease fibroblasts. Mol Ther 2006;14:351–360.
35. Moxley RT 3rd, Ashwal S, Pandya S, et al. Practice parameter: corticosteroid treatment of Duchenne dystrophy: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Neurology 2005;64:13–20.
36. Merlini L, Estournet-Mathiaud B, Iannaccone S, et al.; ENMC. 90th ENMC international workshop: European Spinal Muscular Atrophy Randomised Trial (EuroSMART) 9–10 February 2001, Naarden, The Netherlands. Neuromuscul Disord 2002;12:201–210.
37. van der Beek NA, Hagemans ML, van der Ploeg AT, Reuser AJ, van Doorn PA. Pompe disease (glycogen storage disease type II): clinical features and enzyme replacement therapy. Acta Neurol Belg 2006;106:82–86.
38. van Deutekom JC, Janson AA, Ginjaar IB, et al. Local dystrophin restoration with antisense oligonucleotide PRO051. N Engl J Med 2007;357:2677–2686.
39. Behera M, Kumar A, Soares HP, Sokol L, Djulbegovic B. Evidence-based medicine for rare diseases: implications for data interpretation and clinical trial design. Cancer Control 2007;14:160–166.
40. Lilford RJ, Thornton JG, Braunholtz D. Clinical trials and rare diseases: a way out of a conundrum. BMJ 1995;311:1621–1625.
41. Committee for Medicinal Products for Human Use, European Medicines Agency (CHMP–EMEA). Guideline on clinical trials in small populations. Adopted 27 July 2006. Available at: http://www.emea.europa.eu/pdfs/human/ewp/8356105en.pdf.
42. De Palma S, Morandi L, Mariani E, et al. Proteomic investigation of the molecular pathophysiology of dysferlinopathy. Proteomics 2006;6:379–385.