

ABSTRACT: Hereditary inclusion-body myopathy (h-IBM), or distal myopathy with rimmed vacuoles (DMRV), is an autosomal recessive disorder with onset in early adult life and a progressive course leading to severe disability. h-IBM/DMRV is due to mutations of a gene (*GNE*) that codes for a rate-limiting enzyme in the sialic acid biosynthetic pathway. Despite the identification of the causative gene defect, it has not been unambiguously clarified how *GNE* gene mutations impair muscle metabolism. Although numerous studies have indicated a key role of hyposialylation of glycoproteins in h-IBM/DMRV pathogenesis, others have demonstrated new and unpredicted functions of the *GNE* gene, outside the sialic acid biosynthetic pathway, that may also be relevant. This review illustrates the clinical and pathologic characteristics of h-IBM/DMRV and the main clues available to date concerning the possible pathogenic mechanisms and therapeutic perspectives of this disorder.

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HEREDITARY INCLUSION-BODY MYOPATHY: CLUES ON PATHOGENESIS AND POSSIBLE THERAPY

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The term “hereditary inclusion-body myopathy” refers to several syndromes with autosomal recessive or dominant inheritance. Although the clinical presentation may vary among different forms, they all have a progressive course leading to severe disability and share common pathologic findings on muscle biopsy. The most common form of hereditary inclusion-body myopathy (h-IBM, MIM# 600737) was originally described in Persian-Jewish families. It is characterized by onset in the second–third decade of life with weakness and atrophy of distal lower limb muscles followed by

proximal progression with relative sparing of the quadriceps.^{1,2}

h-IBM is associated with mutations in the UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (*GNE*) gene on chromosome 9.³ A homozygous T to C substitution at nucleotide position 2186 in the *GNE* gene, converting methionine to threonine at codon 712 (p.M712T), has been found in all Middle Eastern families of both Jewish and non-Jewish descent, whereas affected individuals of other ethnicities are usually compound heterozygous or homozygous for different mutations.³ Because all Middle Eastern patients share a common recombinant haplotype, a genetic common founder hypothesis has been proposed. Later, *GNE* mutations were also found in patients with distal myopathy with rimmed vacuoles (DMRV; OMIM# 605820), also known as Nonaka myopathy. This is an autosomal recessive disorder that was previously suspected to be allelic to h-IBM based on the common disease locus on chromosome 9 and similar clinical involvement and muscle pathology.⁴ Similar to h-IBM Middle Eastern patients, strong linkage disequilibrium has also been demonstrated in Japanese patients with DMRV who carry the homozygous missense mutation p.V572L.⁵ To date, more

Abbreviations: α -DG, α -dystroglycan; A β , Amyloid β ; A β PP, Amyloid β precursor protein; CK, creatine kinase; DMRV, distal myopathy with rimmed vacuoles; ER, endoplasmic reticulum; *GNE*, UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase gene; *GNE*/MNK, UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase enzyme; h-IBM, hereditary inclusion-body myopathy; IVIG, intravenous immune globulin G; ManNAc, N-acetyl-D-mannosamine; NCAM, neural cell adhesion molecule; NEP, neprilysin; NeuAc, N-Acetylneuraminic acid; NMJ, neuromuscular junction; PSA-NCAM, polysialylated-neural cell adhesion molecule; s-IBM, sporadic inclusion-body myositis

Key words: hereditary inclusion-body myopathy; distal myopathy with rimmed vacuoles; h-IBM; DMRV; *GNE*

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than 50 different mutations (including point mutations, deletions, and small insertions) of the *GNE* gene have been identified in h-IBM/DMRV patients worldwide.^{6–17}

CLINICAL AND PATHOLOGIC FEATURES OF h-IBM/DMRV

As outlined above, the typical phenotypic features of h-IBM/DMRV include onset in late teenage or early adulthood years with initial distal muscle weakness and subsequent distal-proximal progression. Distinctively in this disorder, the quadriceps muscles retain normal or nearly normal strength despite the diffuse and pronounced involvement of other hip muscles. Upper limb muscle involvement is usually observed in the more advanced stages of the disease.^{1,18} Electromyographic examination usually reveals mixed features compatible with myopathy and neurogenic disease. The serum creatine kinase (CK) level is usually normal or slightly elevated. However, the identification of the causative gene defect has allowed recognition of phenotypic variants of this disorder. These include a minority of patients who lack distal weakness or have distinctive quadriceps involvement, as well as patients with unusual facial weakness.¹⁹ On the contrary, it has been shown that sparing of the quadriceps is not a unique clinical feature of h-IBM/DMRV, as it has also been described in patients who have a myopathy but do not have mutations of the *GNE* gene.²⁰ Furthermore, the age at onset of symptoms is sometimes delayed even to late adulthood. Isolated patients who carry either the p.M712T or the p.A578T mutation and remain asymptomatic in their 6th–7th decade of life have also been described.^{6,19} This evidence clearly suggests that in h-IBM/DMRV the age at onset, degree of progression of symptoms, and distribution of muscle weakness may be variable, but it is not known whether this is because different *GNE* mutations are not functionally equivalent or other factors modulate the clinical phenotype. Moreover, selective involvement of skeletal muscle is particularly puzzling considering the fact that this tissue expresses relatively low levels of the enzyme in comparison to other tissues like liver, lung, and kidney,²¹ which remain unaffected. This suggests the existence of putative susceptibility factors of skeletal muscle to a generalized metabolic impairment.

Morphologic abnormalities of h-IBM/DMRV muscle biopsy include (1) increased scatter of muscle fiber diameter and centralization of myonuclei;

(2) muscle fibers with rimmed vacuoles; (3) intracytoplasmic and intranuclear filamentous inclusions by electron microscopy; and (4) variable amounts of angulated atrophic fibers^{1,18} (Fig. 1A,B). The molecular phenotype of h-IBM/DMRV muscle remarkably resembles that of sporadic inclusion-body myositis (s-IBM), the most frequent myopathy in elderly patients. In fact, besides the presence of muscle fibers bearing cytoplasmic rimmed vacuoles, in both disorders there is abnormal accumulation of an array of proteins commonly associated with Alzheimer's disease brain pathology including amyloid β ($A\beta$)^{22,23} and paired helical filaments immunoreactive with the SMI-31 antibody that recognizes hyperphosphorylated tau^{24,25} (Fig. 1C). Although in isolated h-IBM/DMRV cases collections of muscle perimysial or endomysial inflammatory cells have also been reported,^{12,19,26} the key pathologic features that differentiate h-IBM/DMRV muscle from that of s-IBM include the lack of inflammation and congophilic inclusions within the muscle fibers.^{22,25,27} The similarities between h-IBM/DMRV and s-IBM muscles suggest that, despite different etiologies, both disorders may share some common downstream pathogenic mechanisms that contribute to progressive muscle fiber degeneration.

CONSIDERATIONS ON PATHOGENESIS

Despite identification of the causative gene defect, the pathogenic mechanisms that are activated by mutations of the *GNE* gene and lead to muscle fiber vacuolization and degeneration are still elusive.

GNE codes for a bifunctional enzyme, the UDP-N-acetylglucosamine (UDP-GlcNAc) 2-epimerase/N-acetylmannosamine (ManNAc) kinase (GNE/MNK), with both epimerase and kinase activities, which is expressed in different tissues and has a critical role in the biosynthesis of sialic acid. GNE/MNK catalyzes the conversion of UDP-GlcNAc to ManNAc and consecutive phosphorylation to form ManNAc 6-phosphate (Fig. 2). The two domains of the enzyme are functionally independent. In fact, it has been shown by site-directed mutagenesis experiments that selective targeting of the epimerase domain of the enzyme does not affect the kinase active site and vice versa.²⁸ *GNE* has been also previously associated with sialuria (MIM# 269921), an inborn error of metabolism characterized by slight to moderate developmental delay, hepatosplenomegaly, coarse facial features, and increased production and urinary secretion of

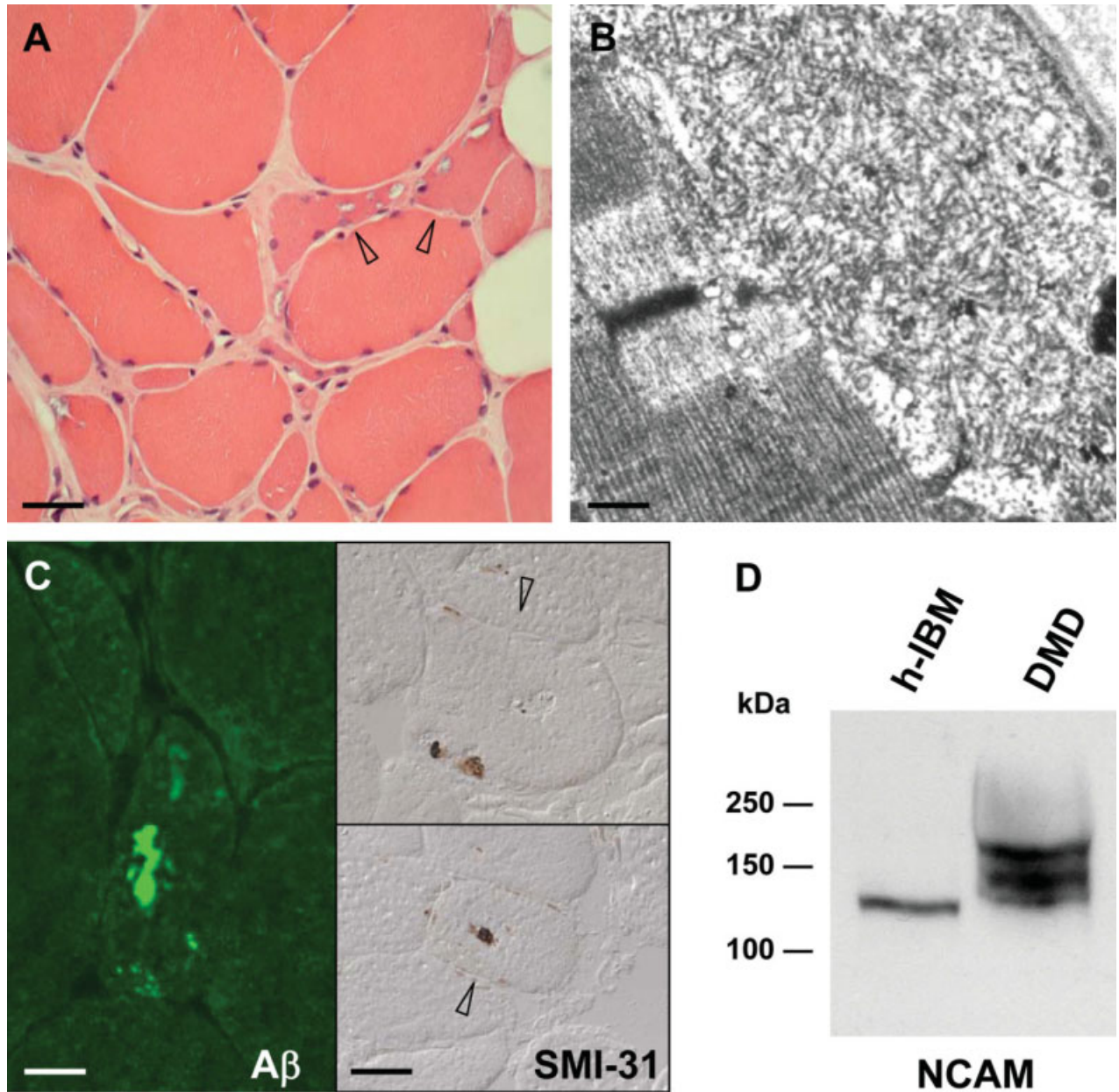


FIGURE 1. Hematoxylin and eosin stain of a representative h-IBM/DMRV muscle biopsy. There is increased variation in fiber size, angulated atrophic fibers, and fibers bearing cytoplasmic vacuoles (arrowheads). Scale bar = 20 μm . **(B)** Electron microscopy micrograph showing a subsarcolemmal collection of typical 15–21 nm filaments. Scale bar = 300 nm. **(C)** Left, confocal microscope image showing an h-IBM/DMRV abnormal muscle fiber with a cytoplasmic inclusion immunoreactive with anti-amyloid β ($A\beta$) antibody. Scale bar = 10 μm . Right, Two abnormal muscle fibers (arrowhead) with cytoplasmic inclusions immunopositive with the SMI-31 antibody recognizing hyperphosphorylated tau protein. Scale bar = 20 μm . **(D)** By Western blot analysis, in h-IBM/DMRV muscle, NCAM migrates as a discrete band of ≈ 130 kDa, whereas in control myopathies (Duchenne muscular dystrophy in this case) NCAM migrates as a broad band of ≈ 150 –200 kDa. This evidence suggests abnormal sialylation of NCAM in h-IBM/DMRV muscle.

sialic acid.²⁹ In patients with sialuria, three distinct *GNE* missense mutations that differ from those associated with h-IBM/DMRV have been identified in the allosteric site of the enzyme. These mutations lead to a loss of feedback control by CMP-N-acetylneuraminic acid (CMP-NeuAc) on enzyme

activity and consequent increased cytosolic and glycan-bound sialic acid.³⁰

Sialic acid is normally present on the distal ends of N- and O-glycans and is involved in many biological functions including cellular adhesion, formation or masking of recognition determinants,

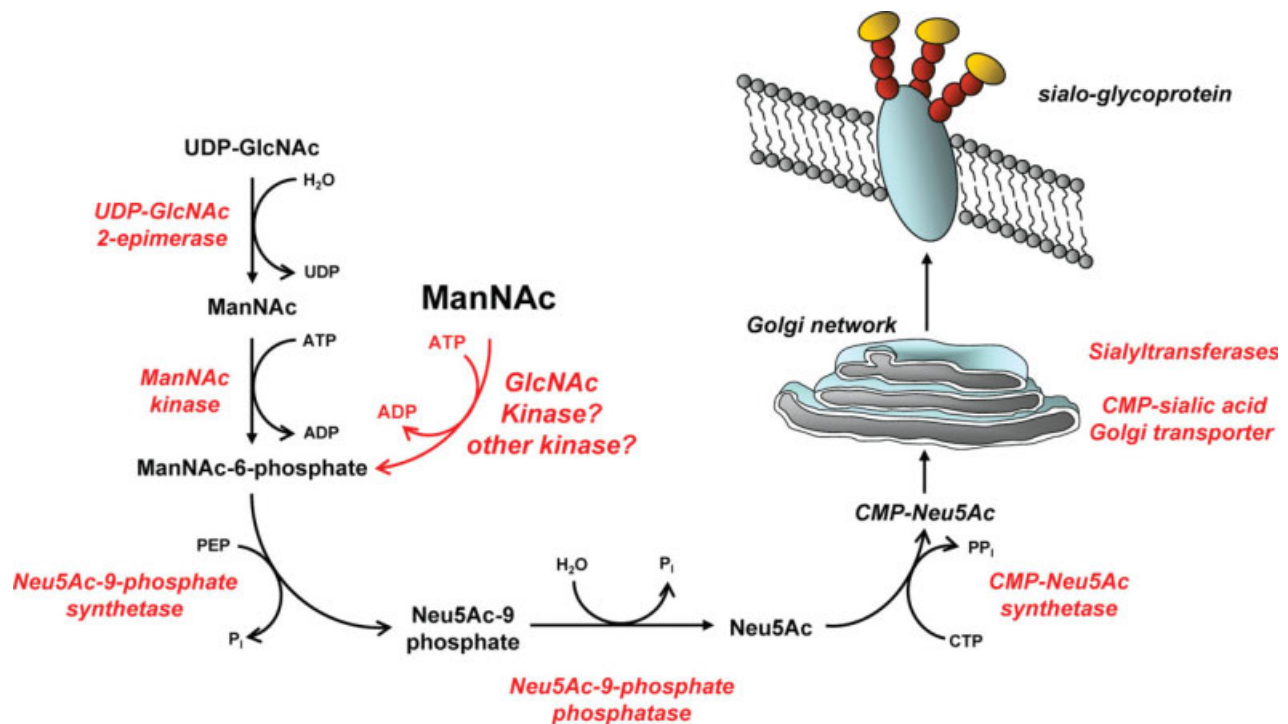


FIGURE 2. Schematic representation of sialic acid metabolism and possible rescue of the metabolic impairment determined in h-IBM/DMRV by N-acetyl-D-mannosamine (ManNAc) supplementation. UDP-N-acetylglucosamine (UDP-GlcNAc) 2-epimerase/N-acetylmannosamine kinase catalyzes the first two consecutive and rate-limiting steps of the sialic acid biosynthetic pathway. ManNAc can enter the sialic acid pathway immediately downstream from the metabolic block consequent to mutations of the *GNE* gene after phosphorylation by a different enzyme with ManNAc kinase activity (most likely N-acetylglucosamine kinase). ManNAc-6-phosphate is then metabolized further to sialic acid.

stabilization of glycoproteins structure, and signal transduction.^{31,28} Whether mutations of the *GNE* gene lead to hyposialylation of muscle glycoproteins and perform a pivotal role in the molecular pathogenesis of h-IBM/DMRV is still a matter of debate. Although numerous lines of evidence suggest hyposialylation of muscle glycoproteins in h-IBM/DMRV muscle, other investigators believe that this represents only a minor byproduct of a metabolic impairment that may instead crucially affect other subcellular compartments and may not be exclusively associated with the synthesis of sialic acid.

Below we illustrate the main findings in favor of each hypothesis.

Does Abnormal Sialylation of Muscle Glycoproteins Play a Role in the Pathogenic Cascade Unfolding in h-IBM/DMRV Muscle? By *in vitro* experiments, it has been shown that two independent lines of Lec3 Chinese hamster ovary cell glycosylation mutants that carry mutations in the *GNE* gene lack UDP-GlcNAc 2-epimerase activity and have extremely

low levels of polysialylated-neural cell adhesion molecule (PSA-NCAM) on the cell surface.³² In h-IBM/DMRV muscle biopsies and cultured muscle cells, *GNE*/MNK protein level and its subcellular distribution appear to be normal, thus suggesting that impaired function of the enzyme, rather than a lack of expression, may underlie the pathogenesis of this disorder.³³ This is further confirmed by demonstration of a reduction of the epimerase activity in lymphocytes from h-IBM/DMRV patients.⁶ Substantial evidence suggests that indeed the muscle of h-IBM/DMRV patients is characterized by reduced sialylation of muscle glycoproteins.^{13,20,34-37} Very recently it has been shown that a transgenic mouse model of the disease that expresses the human *GNE* gene with the p.D176V mutation on a *Gne* knockout background (*Gne*^{-/-}hGNED176V-Tg) is characterized by a reduced level of sialic acid in serum and other tissues and develops a myopathy with a molecular phenotype very similar to that observed in h-IBM/DMRV.³⁸ Indeed, these transgenic mice appear normal at birth but by 30 weeks of age they start showing progressively reduced muscle power and increased serum CK

compared to their normal littermates. They weigh less than normal and by gross examination some muscles, especially the gastrocnemius, appear atrophic. The histological analysis of affected muscles reveals increased scatter of fiber size variability and the presence of rimmed vacuoles and cytoplasmic inclusions within the muscle fibers, similar to what is observed in h-IBM/DMRV. Interestingly, abnormal muscle fibers of *Gne*^(-/-) hGNED176V-Tg mice also accumulate A β precursor protein (A β PP) and its toxic fragment A β (1-42), mainly in the form of cytoplasmic deposits, and this abnormality seems to precede the appearance of other pathologic changes including rimmed vacuoles. In this animal model the measurement of total sialic content in different tissues shows a more pronounced reduction in liver, spleen, and kidney compared to that of skeletal muscle. Nonetheless, skeletal muscle appears to be the only tissue that becomes affected following this metabolic perturbation, as a gross pathologic evaluation of other organs is unremarkable in this animal model.³⁸ This evidence provides further strength to the hypothesis that in h-IBM/DMRV, skeletal muscle may be more sensitive to perturbations in the sialic acid pathway, as suggested earlier in this review. However, no data are available to date regarding the existence of muscle-specific metabolic pathways that render this tissue more susceptible to a reduction of sialic acid content.

Despite the evidence of a possible link between a widespread reduction of sialic acid and the onset of a myopathy, to date few clues are available regarding specific proteins or cellular processes that become deranged due to this metabolic impairment and contribute to progressive muscle fiber degeneration.

Muscle glycoproteins presumed to have a role in h-IBM/DMRV pathogenesis are discussed below.

α -Dystroglycan. In the attempt to identify possible target glycoproteins whose function may become impaired secondary to abnormal sialylation, initial attention has been given to α -dystroglycan (α -DG), a structural protein that provides a tight connection between the extracellular matrix and the cellular cytoskeleton.³⁹ α -DG undergoes posttranslational N-linked and O-linked glycosylation. In particular, sialylated O-linked glycans appear to play a critical role in binding ligands such as laminin, agrin, neurexin, and perlecan. Abnormal glycosylation of α -DG, as seen in some congenital muscular dystrophies, results in impairment of its laminin-binding activity and possibly promotes muscle fiber degeneration.³⁹ A similar

defect was hypothesized initially to underlie the pathogenic mechanism of h-IBM/DMRV. However, despite an early report that showed abnormal glycosylation of α -DG,⁴⁰ in h-IBM/DMRV muscle α -DG appears to be only and inconstantly hyposialylated, and its laminin binding capacity is never impaired.³⁵ In our opinion this latter evidence rules out any major implication of α -DG in the pathogenesis of h-IBM/DMRV.

Neural Cell Adhesion Molecule (NCAM). NCAM is a member of the immunoglobulin superfamily of adhesion molecules that binds long linear homopolymers of 2,8 linked sialic acid residues, thus forming PSA-NCAM. In skeletal muscle PSA-NCAM plays a role during muscle fiber development and regeneration and in the organization and function of the neuromuscular junction (NMJ).^{41,42} Independent investigators have found that in h-IBM/DMRV muscle NCAM is consistently hyposialylated,^{20,43} as suggested by its increased electrophoretic mobility by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis (Fig. 1D). Whether this defect is pathogenically relevant in h-IBM/DMRV remains to be determined. PSA-NCAM has been shown to have a role in NMJ physiology, as mice lacking NCAM show structural and functional abnormalities of this cellular site.⁴² Interestingly, in vitro it has been shown that cultured h-IBM/DMRV myotubes cannot be properly innervated by neurites of rat spinal cord explants, and a mechanism of "myogenous dysreception to innervation" has been proposed.⁴⁴ Moreover, h-IBM/DMRV muscle biopsies are often characterized by a variable amount of angulated atrophic fibers that suggest, along with the presence of spontaneous activity and fibrillation potentials on electromyographic examination, an ongoing process of denervation.^{1,18} Therefore, we may speculate that the underlying metabolic defect in mature muscle results in impairment of NMJ stabilization, as fibers are initially well innervated but later probably lose their contact with the nerve terminal. However, further studies are necessary to verify whether such a mechanism plays a role in the pathogenesis of h-IBM/DMRV.

Nephrilysin. One of the most peculiar aspects of the pathology of h-IBM/DMRV is accumulation of A β PP and its toxic fragment A β within muscle fibers.^{22,23,37,45} It is conceivable that this abnormality may contribute to the pathologic cascade that leads to muscle degeneration as has been previously demonstrated for s-IBM.⁴⁵⁻⁴⁸ Very recently we have shown that neprilysin (NEP), an endopeptidase capable of cleaving A β at multiple

sites,^{49,50} is hyposialylated in h-IBM/DMRV muscle. NEP is characterized by the presence of several N-glycosylation sites and contains large amounts of sialic acid. Previous studies have shown that changes in the sugar moieties of the protein affect its stability and enzymatic activity.⁵¹ Indeed, it appears that, in h-IBM/DMRV muscle, hyposialylation of NEP results in a significant reduction of its expression and enzymatic activity. In vitro, the experimental removal of sialic acid from glycoproteins of cultured human normal myotubes results in reduced NEP stability and enzymatic activity. The reduced expression of NEP in such an experimental setting is mainly due to reduced stability of the protein rather than to reduced transcription of the gene. Moreover, provided that the experimental de-sialylation of muscle glycoproteins in vitro results in reduced expression and enzymatic activity of NEP, we have also found that this is associated with an increased expression of A β -positive cytoplasmic foci within cultured myotubes.³⁷ We do not know whether the functional defect of NEP, as found in h-IBM/DMRV muscle, is per se sufficient to trigger A β accumulation. In fact, h-IBM muscle is also characterized by increased expression of A β PP mRNA and protein,^{22,23,45} promoted by abnormal cellular mechanisms possibly connected with mutations in the *GNE* gene. However, in the complex and not yet completely uncovered molecular pathogenic scenario of h-IBM/DMRV muscle, it is possible that hyposialylated and dysfunctional NEP has a role in hampering the cellular A β clearing system, thus contributing to its abnormal accumulation within vulnerable fibers.

How abnormal sialylation of NEP affects its stability is not known, although one could imagine that it may interfere with the proper processing of the protein in the endoplasmic reticulum (ER), thus favoring more rapid degradation. In general the possibility exists that, in h-IBM/DMRV muscle, hyposialylation of glycoproteins may perturb their proper folding and trafficking through the ER and Golgi network and the translocation to the plasma membrane. This would possibly activate a mechanism of ER stress that is intended to cope with accumulation of abnormal proteins.^{52,53} Once an ER stress condition is established, the misfolded and unfolded proteins trapped in the ER are retrotranslocated to the cytoplasm and degraded by either the ubiquitin-proteasome system or the autophagic process.⁵² It has been already established that ubiquitinated proteins accumulate within h-IBM/DMRV muscle fibers,²² thus strengthening the hypothesis that abnormal pro-

tein processing does indeed play a role in this disorder. Moreover, Malicdan et al.^{38,54} have shown activation of the autophagic process in the muscle of *Gne*^(-/-)hGNED176V-Tg mice and suggested that this may be a downstream event to hyposialylation of glycoproteins and A β deposition. Therefore, it is possible that the persistent activation of these cellular degradation systems plays a key role in progressive muscle fiber degeneration. Although the evidence accumulated so far argues in favor of this hypothesis, further studies are necessary to clarify this issue.

Are There Other Roles of GNE/MNK Outside the Sialic Acid Biosynthetic Pathway Possibly Significant for h-IBM/DMRV Pathogenesis? An alternative hypothesis pursued so far is that mutations of the *GNE* gene also result in impairment of other cellular processes that are not necessarily associated with synthesis of sialic acid. In this respect, in h-IBM/DMRV muscle hyposialylation of glycoproteins would represent only a minor side effect of a metabolic defect that may instead crucially affect other subcellular compartments.

It has been shown that GNE/MNK is able to interact with other factors such as the collapsin response mediator protein 1 and the promyelocytic leukemia zinc finger protein, but none of these molecular partners has been proven to have a role in the pathogenesis of h-IBM/DMRV.⁵⁵ More recently, GNE/MNK has been shown to physically interact also with α -actinin 1, and these two proteins partially colocalize in the sarcomere of mature muscle fibers. However, in vitro no gross difference has been observed between the interaction of α -actinin 1 with wildtype GNE/MNK and with GNE/MNK carrying the p.M712T mutation, respectively.⁵⁶ Although this line of evidence provides novel interesting clues on additional roles of GNE/MNK outside the sialic acid biosynthetic pathway, it remains to be determined how even a minor impairment of the interaction between α -actinin 1 and GNE/MNK, as hypothesized in h-IBM/DMRV, may impact the viability of muscle fibers.

In h-IBM/DMRV, necrosis of muscle fibers has hardly ever been shown by routine histological examination except in anecdotal descriptions.⁵⁷ Thus, the terminating cellular process of fibers, which determines the progressive reduction of muscle bulk, has not been fully clarified to date. In an isolated report, features associated with the apoptotic process have been shown in a small

subpopulation of abnormal muscle fibers. These included the presence of fragmented nuclear DNA in 1% of total myonuclei and increased expression of factors involved in the programmed cell death cascade (i.e., apoptotic-specific protein, c-Jun, and Fas).⁵⁸ In this respect, it has been recently shown that cultured primary muscle cells obtained from h-IBM/DMRV patients show increased expression of the active forms of caspase-3 and caspase-9 and diminished phosphorylated Akt following exposure to staurosporine.⁵⁶ These data suggest impairment of the apoptotic cascade and possibly of the insulin-like growth factor-I/Akt signaling pathway in cultured h-IBM/DMRV muscle cells. Although no major mitochondrial abnormalities have ever been described in h-IBM/DMRV muscle, a recent study that used a gene expression profiling technique has shown dysregulation of genes involved in various mitochondrial processes, including modulation of apoptosis. In addition, primary h-IBM/DMRV myoblasts display slightly abnormal mitochondria that appear significantly more branched than in control cells.⁵⁹ These abnormalities are clearly different from the mitochondrial changes of s-IBM muscle, where cytochrome c oxidase-deficient muscle fibers and large-scale mitochondrial DNA deletions are encountered.^{60,61} In s-IBM the possibility exists that mitochondrial abnormalities are a consequence of A β overexpression and increased oxidative stress in the cellular milieu of aged muscle.^{47,62} In h-IBM/DMRV muscle it has not been demonstrated that mitochondrial abnormalities and dysregulation of the apoptotic cascade are directly activated by mutated GNE/MNK. However, a report by Wang et al.⁶³ has convincingly demonstrated that GNE/MNK is able to modulate the expression of the ST3Gal5 and ST8Sial sialyltransferase and thus to influence the cellular levels of the GM3 and GD3 gangliosides, respectively, and this mechanism is independent from the amount of sialic acid within the cell. Interestingly, GM3 and GD3 gangliosides regulate the mRNA level of BiP, a master regulator protein involved in ER stress, and they appear to have a role in diverse cellular processes such as proliferation, senescence, and apoptosis.⁶⁴⁻⁶⁶ In more detail, it has been demonstrated that GD3 is able to elicit a burst of reactive oxygen species production from complex III of the mitochondrial electron transport chain, which triggers the opening of the mitochondrial permeability transition pore, leading to cytochrome c-dependent caspase 3 activation.⁶⁷ Nevertheless, to date the molecular mechanisms through which GNE/MNK influences the level of expres-

sion of GM3 and GD3 gangliosides are not understood and, more importantly, no studies have been conducted on how this functional relationship becomes modified by mutations of the *GNE* gene in the h-IBM/DMRV cellular environment.

If future studies prove that GNE/MNK has a role in cellular pathways other than that of sialic acid and possibly more relevant for maintaining skeletal muscle homeostasis, then this will also provide valuable clues to understand the specific susceptibility of muscle to a generalized metabolic impairment that is peculiar to h-IBM/DMRV.

THERAPEUTIC PERSPECTIVES

The identification of the key pathogenic mechanism(s) triggered by mutations of the *GNE* gene and responsible for the h-IBM/DMRV phenotype is a fundamental prerequisite for the design of any attempt at treatment of this muscle disorder.

If indeed hyposialylation of glycoproteins plays a pivotal role in the relentless muscle degeneration observed in h-IBM/DMRV, then it is plausible that the restoration of normal sialylation status within the cellular environment may be instrumental in recovering homeostasis of muscle fibers.

To date only one short-term pilot study of administration of sialic acid to h-IBM/DMRV patients has been performed.⁴³ Four patients were enrolled in an open-label trial and received sialic acid via intravenous immune globulin G (IVIG), a glycoprotein that contains 8 μ mol of this sugar per gram, for 1 month. Although no evidence of increased sialylation of specific glycoproteins such as NCAM could be detected in the muscle of the four patients at the end of the treatment, the systemic supplementation of sialic acid via IVIG was associated with a temporary improvement of objective and subjective measures of muscle strength and function. With all the limitations of a small and short-term study, the authors reasoned that the provision of sialic acid may hold therapeutic promise and suggested that the use of sialic acid precursors such as N-acetyl-D-mannosamine (ManNAc) could be a suitable alternative source of sialic acid as therapy for h-IBM/DMRV.⁴³

Previous reports have shown that the exogenous supplementation of ManNAc can increase the sialylation level of a subclone of B lymphoma cell line lacking GNE/MNK activity in a dose-dependent manner. This is because ManNAc enters the sialic acid biosynthetic pathway immediately downstream from the metabolic block determined by mutations of the *GNE* gene, and it is

readily phosphorylated by a different enzyme with ManNAc kinase activity (most likely N-acetylglucosamine kinase).⁶⁸ Then ManNAc-6-phosphate can be further metabolized to sialic acid (Fig. 2). Basic evidence that such a strategy may be effective for h-IBM/DMRV muscle has been provided in vitro by Noguchi et al.³⁴ Indeed, by feeding h-IBM/DMRV cultured myotubes and fibroblasts with ManNAc as well as with NeuAc, sialic acid concentrations in the cells increased to normal levels. This has been shown by direct measurement of total sialic acid content and increased binding of wheat germ agglutinin, a lectin that specifically recognizes a cluster structure of sialic acids, to the plasma membrane of cultured h-IBM/DMRV myotubes up to a level comparable to normal controls. In this respect, further information is provided by a gene-targeted knock-in mouse homozygous for the p.M712T *GNE* mutation. Interestingly, this mouse model shows early postnatal lethality due to severe renal abnormalities but no muscle symptoms. The reason why these mice fail to develop a myopathy is not known, but the fact that their average survival time is very short may be a possible explanation. Nonetheless, supplementation with ManNAc resulted in extended survival and ameliorated renal pathology. This line of evidence further strengthens the possibility of using sialic acid precursors such as ManNAc to overcome the metabolic impairment caused by mutations of the *GNE* gene.⁶⁹ Whether the supplementation of sialic acid precursors can also ameliorate muscle symptoms and pathology in the h-IBM/DMRV mouse model carrying the human p.D176V mutation remains to be determined. We believe that these data may be fundamental in designing a future feasible therapeutic strategy for this crippling disorder.

NOTE ADDED IN PROOF

While this review was in production, Malicdan et al. have demonstrated convincingly that the oral prophylactic supplementation of ManAC in *Gne*^(-/-)hGNED176V-Tg mice results in an increase of sialic acid in muscle to a nearly normal level and prevents development of the muscle phenotype. In particular, ManAC-treated mice have increased strength, muscle mass, body weight and overall survival compared to untreated control litter mates. This is associated with an increase of the mean muscle fiber cross sectional area and a reduction of the amount of atrophic fibers and of

fibers with cytoplasmic rimmed vacuoles and amyloid-beta inclusions.⁷⁰ This line of evidence provides additional strength to the hypothesis that reduced sialylation of muscle glycoproteins plays a pivotal role in the h-IBM/DMRV muscle phenotype. Although direct transfer of these findings to the human disorder requires further safety studies, we believe these data may be important for attempting treatment of this muscle disorder.

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