

Recent advances in distal myopathy with rimmed vacuoles (DMRV) or hIBM: treatment perspectives

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Current Opinion in Neurology 2008, 21:596–600

Purpose of review

Distal myopathy with rimmed vacuoles or hereditary inclusion body myopathy is an adult-onset autosomal recessive, slowly progressive and debilitating myopathy due to mutations in the gene that regulates the synthesis of sialic acid. This review aims to update our knowledge of this myopathy and to review studies about pathomechanism and therapeutic strategies.

Recent findings

Owing to the mutated gene, it was expected that the pathomechanism of this myopathy would be based on hyposialylation, a highly controversial phenomenon. This concept has been supported by findings in two recently generated animal models. In addition, the intracellular amyloid- β accumulation in a distal myopathy with rimmed vacuole mouse model is relevant to similar findings in patients.

Summary

Clarifying the role of hyposialylation in distal myopathy with rimmed vacuole/hereditary inclusion body myopathy could potentially lead to a therapeutic strategy for this progressive myopathy. In addition, strategies aimed at preventing amyloid- β deposition or enhancing its clearance could also be beneficial, as this epiphenomenon is now known to occur early in the course of the disease.

Keywords

amyloid, muscle atrophy, sialic acid

Curr Opin Neurol 21:596–600
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1350-7540

Introduction

Distal myopathy with rimmed vacuoles (DMRV), otherwise known as Nonaka myopathy or hereditary inclusion body myopathy (hIBM) is an early adult-onset, slowly progressive myopathy. In general, patients become non-ambulatory 12 years after the onset [1]. As the name implies, the disease is characterized clinically by the early involvement of distal muscles and pathologically by the so-called rimmed vacuoles, which are immunoreactive to various proteins, by intracellular protein accumulation, scattered angular fibers, and by tubulo-filamentous inclusions both in the nucleus and in the cytoplasm.

DMRV/hIBM is due to mutations in the uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc) 2-epimerase/*N*-acetylmannosamine (ManNAc) kinase (*GNE*) gene [2–4], which encodes a bifunctional enzyme that catalyzes the two exclusive rate-limiting reactions of sialic acid synthesis in the cytosol [5]. Sialic acids are the most abundant terminal monosaccharides of glycoconjugates in eukaryotic cell surfaces, and – besides conferring stability to glycoproteins – they are involved in a variety of cellular functions [6,7]. Mutations in the gene respon-

sible for DMRV/hIBM can affect the kinase or epimerase domain, occur in homozygous or compound heterozygous mode, and are not correlated to disease phenotype or severity. Mutations decrease the enzymatic activity by 70–90% [8]. The identification of the genetic cause has not explained why mutations in a gene involved in sialic acid synthesis cause a myopathy. Various theories have been proposed, and in recent years there has been increased research to verify them. We will review these theories and highlight recent attempts to clarify the pathophysiology of DMRV/hIBM.

Amyloid deposition . . . another Alzheimer's disease phenotype in muscle?

Intracellular amyloid- β deposition in myofibers has been documented only in few muscle diseases, namely sporadic inclusion body myositis (sIBM) and DMRV/hIBM [9]. Although the causative gene for DMRV/hIBM has been identified, the gene or specific factor responsible for IBM remains elusive. Pathologically, the features of DMRV/hIBM are very similar to those of sIBM, except for the presence of inflammation in sIBM. Both diseases are vacuolar myopathies, and, more strikingly, both have

similar intracellular deposits that are congophilic and immunoreactive to amyloid- β precursor protein (A β PP), amyloid- β , phosphorylated tau, presenilin-1, α -synuclein, proteins related to oxidative stress, and others. Therefore, it has been proposed that, in spite of different causes, both diseases may have similar pathogenic mechanisms, at least as far as muscle is concerned.

Several studies have attempted to clarify the mechanism of amyloid deposition in IBM, comparing it with the pathomechanistic theories proposed in Alzheimer's disease, including increased mRNA expression of A β PP, mitochondrial abnormalities, and neuromuscular junction anomaly [10,11]. The role of amyloid deposition in the pathogenesis of muscle diseases has been highlighted in a sIBM mouse model, in which a correlation of intracellular amyloid levels and motor weakness was seen [11]. Increased intracellular amyloid- β can cause abnormal signal transduction, modulation of other genes, induction of mitochondrial dysfunction and oxidative stress, proteosomal activation, alteration of calcium homeostasis, and hyperphosphorylation of tau. The intracellular accumulation of amyloid- β has been difficult to conceptualize and has been controversial even in Alzheimer's disease, though recent studies have made this concept more acceptable in the field [12,13]. As a prerequisite to discuss this problem, it is important to know where amyloid- β is produced from A β PP, its precursor protein. A β PP is localized at the plasma membrane, but recently it has been shown to localize also at the trans-Golgi network, endolysosomal, lysosomal, and mitochondrial membranes, and endoplasmic reticulum. Thus, the liberation, and consequent deposition, of amyloid- β can occur wherever A β PP and the β - and γ -secretases involved in its processing are located [14,15–18,19]. However, the mechanism by which amyloid- β remains intracellular is still controversial. It has been proposed that newly generated amyloid- β is not secreted and remains intracellular [20,21], or that secreted amyloid- β is taken up again by the cell, as amyloid- β can bind to various biomolecules and be transported back into the intracellular pool [22–26].

As of now, however, little is known about the role of this amyloid epiphenomenon in DMRV/hIBM. It has been originally proposed that the genetic defect could somehow predispose the muscle to enter a premature state of 'ageing milieu' [9], but the mechanism by which *GNE* mutations could lead to such state is far from understood. Despite the lack of evidence connecting directly amyloidogenesis and DMRV/hIBM, the fact that amyloid deposition is an early pathological finding makes it an attractive therapeutic target.

One of the endopeptidases implicated in A β PP processing is neprilysin. This type II membrane metalloendopeptidase has an active domain containing a zinc binding motif,

which is capable of degrading the monomeric and oligomeric forms of amyloid- β peptide [27]. An inverse relationship between the levels of neprilysin and amyloid- β peptide has been observed, and neprilysin has been shown to catabolize amyloid- β peptides and reduce toxicity in the brain [28]. As in muscle monomeric, oligomeric, and fibrillar forms of amyloid- β have been detected [29], neprilysin has been considered a candidate molecule that could contribute to our understanding of amyloidogenesis in DMRV/hIBM. In hereditary myopathies, Broccolini *et al.* [30] demonstrated that neprilysin expression was directly associated with the degree of muscle fiber regeneration and that in IBM neprilysin colocalized with intracellular amyloid- β deposits. Furthermore, they showed that neprilysin participates in muscle cell differentiation and regeneration, and its expression is regulated at the posttranscriptional level, showing a rapid increase in the early stage of myoblast differentiation followed by a gradual reduction thereafter. Inhibition of neprilysin activity resulted in impaired muscle differentiation that was mainly associated with an abnormal regulation of Akt activation. They suggested that neprilysin is capable of cleaving the insulin growth factor binding protein 5, thus modulating the activation of insulin growth factor-I (IGF-I)/Akt pathways within muscle fibers. These data suggest that enhancing neprilysin activity in muscle may reduce the steady-state level of amyloid- β in vulnerable fibers and promote the regeneration capacity of myofibers through the modulation of IGF-I dependent pathways.

To date, many strategies have been aimed at clearing amyloid deposition, but a discussion of these therapeutic attempts is beyond the scope of this review.

Hyposialylation: a central role in the pathomechanism of distal myopathy with rimmed vacuole/hereditary inclusion body myopathy?

As *GNE* is involved in sialic acid biosynthesis, it follows that decreased sialic acid, or hyposialylation, should be considered to play a role in the symptomatology or disease progression in DMRV/hIBM. *GNE* mutations reduce the UDP-GlcNAc 2-epimerase and ManNAc kinase activities, and the extent of the reduction is mutation dependent [8,31,32]. Presumably, these reduced enzymatic activities would reduce sialic acid concentration because the two enzymes are rate limiting in sialic acid biosynthesis.

Lessons from in-vitro studies

Studies analyzing the sialylation of glycoproteins have had varied results. α -Dystroglycan, a functional protein of the sarcolemma, is highly sialylated but a defect in its sialylation, albeit controversial [8,33–35], does not seem to contribute to the pathomechanism of DMRV/hIBM. This is

understandable, because a defect in the sugar chains of α -dystroglycan would result in muscle necrosis, a situation that has not been seen in DMRV/hIBM patients, except for anecdotal reports [36]. Another glycoprotein that was implicated in DMRV/hIBM is the neural cell adhesion molecule (NCAM), the most abundant polysialylated protein in mammalian cells. The presence of polysialic acid (PSA) on NCAM has been shown to decrease cell adhesion and is critical for a variety of processes, including brain development, synaptic plasticity, axon guidance and pathfinding, neurite outgrowth, and general cell migration. The expression of NCAM and PSA-NCAM in muscle is a good index of muscle regeneration. In DMRV/hIBM, NCAM has been shown to be hyposialylated [37] and its protein expression is enhanced in regenerating fibers. The upregulation of NCAM in DMRV/hIBM could be a secondary response to the presence of degenerating fibers, though it may not be effective in promoting muscle regeneration because of hyposialylation.

Neprilysin, which was mentioned earlier in this review, also showed altered sialylation. Broccolini *et al.* [38**] showed that in various inflammatory and hereditary myopathies, including hIBM, the immunoreactivity to neprilysin was increased in rare vacuolated fibers and colocalized with the amyloid- β (A β 140) signals, besides being upregulated in regenerating fibers. Intriguingly, the amount of neprilysin is lower in hIBM than in control muscles, in contrast to the increased protein expression in inflammatory myopathies (polymyositis and dermatomyositis) and in muscular dystrophies (both Duchenne and Becker). These findings were corroborated by enzymatic analysis of neprilysin. As neprilysin is characterized by the presence of several *N*-glycosylation sites and contains large amounts of sialic acid, changes in these sugar moieties affect its stability and enzymatic activity. Interestingly, in glycoprotein-enriched hIBM muscles, the affinity of neprilysin to *Macckia amurensis* lectin (MAL), which binds to glycoproteins through specific sialylated structures, is remarkably reduced, a sign of hyposialylation. This finding was supported by further in-vitro studies, whereby differentiated myotubes from hIBM patients and normal controls treated with neuraminidase (an enzyme that cleaves sialic acid from cell surface) showed reduced neprilysin enzymatic activity and reduced binding to MAL. In addition, myotubes experimentally de-sialylated with neuraminidase showed deposition of amyloid- β , which colocalized with neprilysin immunosignal. This work has clearly shown that neprilysin is indeed hyposialylated, and this is accompanied by reduction of enzymatic activity. It is, however, not clear why this phenomenon (at least in experimental de-sialylation of cell membrane in myotubes) should lead to increased intracellular amyloid- β accumulation, because, though neprilysin can cleave A β PP, its physiological location in the plasma membrane does not explain

the intracellular cleavage of A β PP. This raises the possibility that experimental de-sialylation might have triggered other mechanisms capable of inducing intracellular accumulation of amyloid- β .

Clues from animal models

The importance of the *GNE* gene in growth and development was highlighted by the discovery that inactivation of the *Gne* gene is embryonically lethal in mice [39]. Although various strategies had been used to generate an animal model for this disease, to date only few animal models have been published. In the knock-in mice carrying M712T, the most common *GNE* mutation among Iranian Jews, homozygous pups (harboring the mutation in both alleles) did not survive beyond the third postnatal day [40**]. Despite the reduced epimerase enzymatic activity, no obvious myopathic phenotype was noted, at least by morphological analysis of muscle. Instead, and rather surprisingly, these mice developed a severe renal phenotype characterized by proteinuria, severe glomerular disease, and podocytopathy that led to their early demise. Interestingly, the mice also exhibited reduced sialylation of podocalyxin, a major podocyte sialoprotein. Administration of the sialic acid precursor ManNAc to pregnant mice increased the survival rate of pups beyond P3 and corrected the hyposialylation of podocalyxin. The role of sialic acid in glomerular disease has been attributed to the reduction of sialic acid and not the loss of sialoglycoproteins [41]; however, no kidney abnormality has ever been reported in DMRV/hIBM patients. Other factors may contribute to the M712T phenotype, as *GNE* also enhances the activity of the sialyltransferases, GM3 synthase, and GD3 synthase, thereby increasing the synthesis of gangliosides GM3 and GD3 [42], which are also expressed in podocytes and contribute to the charge characteristics of the filtration barrier. Thus, knock-in mice carrying the M712T mutation showed the importance of sialic acid in maintaining the glomerular filtration barrier. It would be interesting to know if organs other than the kidneys were involved in these mice and if such involvement could have contributed to their early demise.

Yet another mouse model showed how hyposialylation may play an important role in the pathogenesis of DMRV/hIBM. This mouse harbored the *GNE* D176V mutation on a *Gne* knockout background (*GNE* D176V transgenic in *Gne*^{-/-} mouse; DMRV/hIBM mouse) and developed myopathic features similar to those of the DMRV/hIBM phenotype in humans [43**], with hyposialylation of serum and other organs, lower body mass than control littermates, and clinical weakness. At a younger age, these mice had unremarkable findings in muscle pathology, but with time they developed the pathological hallmarks of DMRV/hIBM, including intracellular deposition of amyloid- β and formation of rimmed vacuoles.

Similar to the situation in humans, muscle bulk is reduced even before pathological changes become manifest; thus it would be informative to document if these animals indeed have muscle atrophy.

In contrast to the M712T mice, the DMRV/hIBM mice do not show gross kidney abnormalities, at least at the light microscopy level. Another peculiar finding in these DMRV/hIBM mice is that intracellular amyloid- β deposition seemingly predates rimmed vacuole formation and myofibrillar degeneration [43**]. These findings are in agreement with the observation cited above that in cultured hIBM myocytes, A β PP overexpression precedes IBM-like abnormalities [44].

These DMRV mice also raise several questions. First, some animals had reduced survival rate, suggesting that we should pay closer attention at other organs. More importantly, correcting the hyposialylation in these mice could be a relevant therapeutic strategy to be ultimately used in patients.

The very different phenotypes expressed by the two mouse models need to be explained. In humans, the phenotype associated with the D176V mutations is similar to that associated with the M712T mutation. It is likely that mutation of the endogenous *GNE* gene in the M712T knock-in mouse is more severe, whereas in the DMRV/hIBM mouse, higher expression of the transgenic mutant *GNE* rescues the severe phenotype and allows the mice to develop milder progression and a myopathic phenotype. This notion is bolstered by the observation that mouse tissues may require a higher level of sialic acid than human tissues, as the normal serum level of sialic acids in mouse is 1.5 times higher than in humans. This concept clearly needs further investigation.

A step closer to developing therapy for distal myopathy with rimmed vacuole/hereditary inclusion body myopathy

Although the concept of hyposialylation in DMRV is still controversial, the data from animal models and various *in vitro* analyses cannot be disregarded and have led to the development of various attempts to treat this crippling disorder. Noguchi *et al.* [8] showed that exposing DMRV cells to sialic acid and its precursor or both restored the sialylation status of the cells to a remarkable extent. Similarly, administration of sialic acids to DMRV/hIBM patients could help normalize the sialylation status of muscle glycoproteins and provide clinical benefit.

A pilot study employing one method to deliver sialic acid to the cells was carried out in four patients with DMRV/hIBM [45*], who were given intravenous (*i.v.*) immunoglobulin G (IVIG), a glycoprotein that contains 8 μ moles

of sialic acid per gram. The authors claimed that the administration of a single loading dose (1 g/kg) of IVIG by continuous *i.v.* infusion for 2 days led to some improvement in quantitative muscle testing, qualitative improvements in activities of daily living, improved muscle strength and endurance in all four patients. However, this so-called improvement was not correlated to the degree of sialylation of the glycoproteins NCAM, transferrin, and α -dystroglycan, raising the possibilities that short-term therapy may not change the sialylation of glycoproteins. Alternatively, glycoproteins other than those included in their study need to be analyzed. Nevertheless, the possibility that the subjective improvement after therapy could be attributed to other effects of IVIG should still be considered. After this initial pilot study, no other DMRV/hIBM patients were treated with IVIG, thus the results in this study remain to be verified.

In the M712T mice, though it is remarkable that administration of ManNAc improved survival and rescued the severe renal phenotype, it is not conceivable to use this as a basis of therapy for DMRV/hIBM patients, because the efficacy of such an agent has never been demonstrated *in vivo* due to the lack of a disease phenotype in the M712T mice. Administration of ManNAc or sialic acid itself to DMRV/hIBM mice is expected to clarify issues surrounding the therapy in this disabling disease.

Conclusion

From these recent studies, it is clear that hyposialylation has a central role in the pathogenesis of this myopathy, though specific details are lacking as to how this phenomenon causes disease. Of greater practical importance, this phenomenon should not be disregarded because it opens an avenue to therapy, and trials addressing this step in the DMRV/hIBM mouse are much anticipated. Another issue that may be worth exploring is the early epiphenomenon of amyloidogenesis, which can be targeted for therapy.

Acknowledgements

The study is supported partly by the 'Research on Psychiatric and Neurological Diseases and Mental Health' from the Japanese Health Sciences Foundation, the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO), the 'Research Grant (17A-10, 19A-7) for Nervous and Mental Disorders' from the Ministry of Health Labour and Welfare, and the Neuromuscular Disease Foundation.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 621–622).

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