The hereditary inclusion-body myopathies encompass several syndromes with autosomal recessive or dominant inheritance. Despite a different clinical presentation they all have a progressive course leading to severe disability and share similar pathologic findings at the muscle biopsy. Quadriceps-sparing autosomal recessive hereditary inclusion-body myopathy (h-IBM) is the commonest form and is tied to mutations of the UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (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 clarified how mutations of the GNE gene impair muscle homeostasis. Although several lines of evidence argue in favor of an abnormal sialylation of muscle glycoproteins playing a key role in h-IBM pathogenesis, others studies have demonstrated new 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 and the main clues available to date concerning the possible pathogenic mechanisms of this disorder. Understanding the molecular mechanism underlying h-IBM pathology is a fundamental requisite to plan a future attempt to therapy.

Key words: h-IBM, quadriceps sparing myopathy, GNE mutations

The hereditary inclusion-body myopathies encompass several syndromes with autosomal recessive or dominant inheritance. Despite a different clinical presentation they all have a progressive course leading to severe disability and share similar pathologic findings at the muscle biopsy. Quadriceps-sparing autosomal recessive hereditary inclusion-body myopathy (h-IBM, IBM2, OMIM# 600737) is the commonest form and was originally described in patients of Persian Jewish (PJ) heritage (1, 2). Typically, h-IBM is characterized by onset in the second-third decade of life with weakness and atrophy of distal lower limb muscles that eventually spreads proximally with a relative and selective sparing of the quadriceps. Involvement of upper limb muscles is observed in the more advanced stages of the disease (1). h-IBM is tied to mutations of the UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE) gene that codes for a bifunctional and rate-limiting enzyme (the UDP-GlcNAc epimerase/ManNAc kinase, GNE/MNK) in the sialic acid biosynthetic cascade (3, 4). h-IBM patients of PJ descent share a common founder mutation consisting in a T to C shift at nucleotide position c.2135 of the GNE gene that results in a methionine to threonine change at codon 712 (p.M712T) (3), whereas affected individuals of different ethnicities are usually compound heterozygous or homozygous for different mutations (5-10). Shortly after the identification of the h-IBM causative gene defect, mutations of the GNE gene have been demonstrated to be responsible also for distal myopathy with rimmed vacuoles or Nonaka myopathy (DMRV, OMIM# 605820) (11, 12), a disorder described in Japanese patients and characterized by clinical and pathologic features remarkably similar to h-IBM, thus proving that h-IBM and DMRV are indeed the same entity as previously suggested by Askanas and Engel (13). To date more than 50 mutations distributed throughout the GNE gene and associated to h-IBM have been described worldwide.

As previously sketched out, the h-IBM canonical phenotype is characterized by onset in late teenage or early adulthood years with initial distal muscle weakness and subsequent disto-proximal progression. Distinctively in this disorder, the quadriceps muscles retain a normal or close-to-normal strength even when other muscle compartments become severely affected. Nonetheless, the identification of the causative gene defect has allowed
the recognition of phenotypic variants, such as patients lacking distal weakness or with unusual quadriceps involvement (2, 14). On the contrary, it has been shown that sparing of the quadriceps is not unique to h-IBM as it has also been reported in patients affected by a non-GNE-related myopathy (15). Furthermore, the age at onset of symptoms is sometimes postponed even to late adulthood as isolated patients, carrying either the p.M712T or the p.A578T mutations and still asymptomatic in their 6th-7th decade of life, have also been described (2, 11). This collection of evidence leads to diverse considerations. First, the prevalence of this disorder in patients of non-PJ inheritance is probably higher than what previously assumed, as numerous cases with atypical presentation may remain undiagnosed. Moreover, a wider range of clinical phenotypes associated with GNE mutations would suggest that different mutations are not functionally equivalent, id est different mutations may differently impact cell metabolism and viability. Contrariwise, the fact that a different clinical course can be observed in patients harboring the same mutations argue in favor of epigenetic factors conditioning the phenotypic outcome. However, no conclusive genotype-phenotype studies are available to date. Finally, the selective involvement of skeletal muscle is particularly puzzling in consideration of the fact that this tissue expresses relatively low levels of the enzyme in comparison to other tissues, like liver, lung and kidney, that remain unaffected (16). This suggests the existence of putative susceptibility factors of skeletal muscle to a generalized metabolic impairment.

Morphologic abnormalities of h-IBM muscle biopsy include i) increased scatter of muscle fiber diameter and centralization of myonuclei, ii) muscle fibers with rimmed vacuoles, iii) intracytoplasmic and intranuclear filamentous inclusions by electron microscopy, and iv) variable amount of angulated atrophic fibers. The molecular phenotype of h-IBM muscle remarkably resembles that of sporadic inclusion-body myositis (s-IBM), the most frequent myopathy occurring in elderly patients. In fact, besides the presence of muscle fibers bearing cytoplasmic “rimmed vacuoles”, in both disorders there is the abnormal accumulation of an array of proteins commonly associated with Alzheimer’s disease brain pathology including Amyloid β (Aβ) and paired helical filaments containing hyperphosphorylated tau (13). Although in isolated h-IBM cases the presence of muscle perimysial or endomysial collection of inflammatory cells has also been reported (2, 17, 18), the key pathologic features differentiating h-IBM muscle from that of s-IBM include the lack of inflammation and congophilic inclusions within the muscle fibers (19, 20). The similarities between the molecular features of h-IBM and s-IBM suggest that, despite different etiologies, possibly both disorders share some common downstream pathogenic mechanisms leading to progressive muscle fiber degeneration (21).

To date the cellular pathogenic mechanism activated by mutations of the GNE gene is far to be elucidated. Sialic acid is a monosaccharide decorating the terminal ends of the glycan chains of glycoproteins. Differential sialylation of cell surface molecules is crucial for their functions in physiological as well as pathological processes (4). An object of controversy is whether GNE mutations lead to reduced sialylation of muscle glycoproteins and this has a pivotal role in h-IBM pathogenesis. The GNE/MNK has two functionally independent domains and it has been shown that the selective targeting of the epimerase domain of the enzyme does not affect the kinase active site and vice versa (22, 23). A reduction of the epimerase activity has been demonstrated in lymphocytes from h-IBM patients, thus suggesting a partial loss of function of the enzyme (11). In addition, it has been shown by in-vitro experiments that two independent lines of Lec3 Chinese hamster ovary cell glycosylation mutants, carrying either a nonsense p.E45stop or a p.G135E missense mutations in the GNE gene, lack UDP-GlCNα epimerase activity and have extremely low levels of polysialylated-neural cell adhesion molecule (PSA-NCAM) on the cell surface (24). In keeping with this line of evidence is the fact that a transgenic mouse model expressing the human GNE gene with the p.D176V mutation on a GNE knockout background is characterized by a reduced level of sialic acid in serum and other tissues and develops a myopathy that resembles h-IBM (25). Moreover, in this animal model the prophylactic supplementation of sialic acid metabolites prevents the development of the myopathic phenotype, thus strengthening the hypothesis that a reduced amount of cellular sialic acid underlies disease pathogenesis (26). However, to date few clues are available regarding specific proteins or cellular processes whose function becomes impaired following this metabolic defect. We have previously shown an abnormal, although inconsistent among different h-IBM patients, sialylation of α-dystroglycan (α-DG), a structural protein that provides a connection between proteins of the cellular cytoskeleton and the extracellular matrix such as laminin (27). Provided that such abnormality does not impact the capacity of α-DG to bind laminin, we believe that the abnormal sialylation of α-DG does not have a relevant role in the pathogenic cascade activated in h-IBM muscle.

We have also shown that in h-IBM muscle NCAM is consistently hyposialylated and this results in increased electrophoretic mobility of the protein by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Indeed, by western blot analysis in h-IBM muscle NCAM appears as a sharp band of approximately 130 kDa rather than a broader band with a molecular weight ranging between
150 and 200 kDa as observed in all other myopathies (15).

The possible pathogenic relevance of hyposialylated NCAM in h-IBM is not known. PSA-NCAM has a role in NMI physiology (28) and this is potentially interesting in view of the fact that in vitro cultured primary h-IBM myotubes cannot be innervated by neuritis emerging from rat spinal cord fragments, thus suggesting a mechanism of “myogenous dysreception to innervation” (29). Whether hyposialylated NCAM plays some role in this abnormality remains to be elucidated. Nonetheless, despite a possible role in h-IBM pathogenesis, in our experience hyposialylated NCAM can be used as a pre-genetic cellular marker to identify patients with a GNE-related myopathy in the routine diagnostic workup of muscle biopsy in the laboratory. This can be helpful to differentiate h-IBM patients from others affected by a phenotypically similar myopathy not due to mutations of the GNE gene or to identify h-IBM patients with uncommon clinical or pathologic features (14).

Furthermore, we have demonstrated that the expression and enzymatic activity of nephrilysin (NEP), a sialoglycoprotein capable of cleaving Aβ at multiple sites, are reduced in h-IBM muscle, possibly in connection with its abnormal sialylation. In fact, we have found that in vitro the enzymatic removal of sialic acid from muscle glycoproteins results in reduced expression and enzymatic activity of NEP along with the intracytoplasmic accumulation of Aβ (30). We do not know whether this functional defect of NEP is per se sufficient to trigger Aβ accumulation. In fact, h-IBM muscle is also characterized by increased expression of the Aβ precursor protein possibly due to the abnormal cellular mechanisms connected with mutations of the GNE gene (19, 31, 32). However, in the complex and still undisclosed scenario of h-IBM muscle, it is possible that hyposialylated and dysfunctional NEP has a role in hampering the cellular Aβ clearing system, thus contributing to its accumulation within vulnerable fibers. How hyposialylation of NEP affects its stability is not known, although interference with the correct processing of the protein in the endoplasmic reticulum (ER) leading to a more rapid degradation can be hypothesized. In normal conditions nascent proteins destined for the secretory pathway are translocated from the cytosol into the ER and then engaged in the folding machinery, which aids in achievement of the native conformation, posttranslational modifications and multimeric protein assembly (33). Such a complex protein processing is subjected to a stringent quality control system so that defective and misfolded proteins remain in the ER and then degraded through the ER-associated degradation process (34). Changes in cellular homeostasis, involving for example the redox status or glycosylation mechanisms, can result in accumulation of unfolded or misfolded proteins within the ER, thus leading to a condition of ER stress (34, 35). Once ER stress condition is established, the misfolded and unfolded proteins trapped in the ER are retrotranslocated to the cytoplasm and degraded by the ubiquitin-proteasome system (36). It has been already established that ubiquitinated proteins accumulate within h-IBM muscle fibers, thus strengthening the hypothesis that an abnormal protein processing does indeed play a role also in this disorder (21, 37). In general terms, the possibility exists that, in h-IBM, 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 activate a mechanism of ER stress that is intended to manage the accumulation of abnormal proteins. Once a 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 (38). Nevertheless, further studies are necessary to verify this hypothesis.

Another line of reasoning pursued so far is that hyposialylation of muscle glycoproteins represents only a minor byproduct of a metabolic impairment that may instead crucially affect other subcellular compartments. In vitro studies have shown that GNE/MNK is able to interact with factors such as the collapsin response mediator protein 1 and the promyelocytic leukemia zinc finger protein (39), but none of these proteins has been proven so far to be involved in the pathogenic cascade of h-IBM muscle. More recently, GNE/MNK has also been demonstrated to partially co-localize with α-actinin 1 in the sarcomere of mature muscle fibers. However, such evidence does not appear relevant to h-IBM pathophysiology as, in vitro, no gross difference has been observed between the interaction of α-actinin 1 with wild type GNE/MNK and mutated GNE/MNK, respectively (40).

In h-IBM the terminating cellular process, either necrotic or apoptotic, that is primarily responsible for the progressive reduction of muscle bulk, has not been unequivocally elucidated. Indeed, h-IBM muscle fiber necrosis is infrequently found and features of apoptosis have so far only an anecdotic relevance (41, 42). Nonetheless, a possible role of mutated GNE/MNK in the activation of the apoptotic cascade has recently arisen by showing that in vitro cultured primary h-IBM muscle cells are more prone to staurosporine-induced apoptosis possibly in connection with an intrinsic impairment of the insulin-like growth factor-I/Akt pathway (43). Nevertheless, an unambiguous demonstration that apoptotic mechanisms are activated in h-IBM muscle biopsies is still missing.

A recent work has shown that GNE/MNK regulates the expression of the ST3GaI5 and ST8Sia1 sialyltransferase that control the cellular levels of the GM3 and
GD3 gangliosides, respectively (44). Interestingly, GM3 and GD3 gangliosides regulate the mRNA level of BiP, a master regulator protein involved in ER stress as well as in other cellular processes such as proliferation, senescence and apoptosis (45-47). More in detail, GD3 elicits production of reactive oxygen species from complex III of the mitochondrial electron transport chain that leads to the opening of the mitochondrial permeability transition pore and the activation of cytochrome c-dependent caspase 3 (48). Nevertheless, the molecular mechanisms through which GNE/MNK influences the level of expression 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.

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 of h-IBM.

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