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Hereditary Inclusion Body Myopathy: A Decade of Progress

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Abstract

Hereditary Inclusion Body Myopathy (HIBM) is an autosomal recessive, quadriceps sparing type commonly referred to as HIBM but also termed h-IBM or Inclusion Body Myopathy 2 (IBM2). The clinical manifestations begin with muscle weakness progressing over the next 10–20 years uniquely sparing the quadriceps until the most advanced stage of the disease. Histopathology of an HIBM muscle biopsy shows rimmed vacuoles on Gomori's trichrome stain, small fibers in groups and tubulofilaments without evidence of inflammation. In affected individuals distinct mutations have been identified in the *GNE* gene, which encodes the bifunctional enzyme uridine diphospho-N-acetylglucosamine (UDP-GlcNAc) 2-epimerase/N-acetyl-mannosamine (ManNAc) kinase (GNE/MNK). GNE/MNK catalyzes the first two committed steps in the biosynthesis of acetylneuraminic acid (Neu5Ac), an abundant and functionally important sugar. The generation of HIBM animal models has led to novel insights into both the disease and the role of GNE/MNK in pathophysiology. Recent advances in therapeutic approaches for HIBM, including administration of N-acetyl mannosamine (ManNAc), a precursor of Neu5Ac will be discussed.

Keywords

muscular dystrophy; sialic acid synthesis; UDP-GlcNAc 2-epimerase/ManNAc kinase; hyposialylation; HIBM mouse model; ManNAc therapy; *GNE* mutations

1. Introduction

Inclusion Body Myositis (IBM) was described by Yunis and Samaha on the basis of distinctive inclusions containing tubulofilaments in a subset of patients with polymyositis [1]. IBM, defined by the pathologic presence of rimmed vacuoles and tubulofilaments on muscle histology is further classified into "sporadic inclusion body myositis" (s-IBM; OMIM#137421), which invariably has inflammation, and "hereditary inclusion body myopathy" which shows familial inheritance and no inflammation [2,3]. This review will focus on the molecular basis, pathophysiology and clinical features of a specific type of Hereditary Inclusion Body Myopathy, the autosomal recessive, quadriceps sparing type commonly referred to as HIBM but also termed h-IBM, or Inclusion Body Myopathy 2 (IBM2) (OMIM#600737), which is allelic to the Japanese disorder Distal Myopathy with Rimmed Vacuoles (DMRV) or Nonaka Myopathy (OMIM#605820) [4,5]. We henceforth refer to this disorder as HIBM.

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2. Clinical features and pathology

2.1. Clinical features

Argov and Yarom [6] first described the disorder HIBM in Jews of Persian descent characterized clinically by progressive proximal and distal muscle weakness and wasting of the upper and lower limbs usually beginning after age 20. Apart from the Persian-Jewish population, affected individuals have now been described worldwide, including patients of Caucasian, Indian, Thai, Japanese and African descent [5,7–10].

The clinical course of HIBM is relentless. Progression of muscle weakness after onset continues over the next 10 to 20 years. Typically, however, there is sparing of the quadriceps muscles, partially or completely, even in the advanced stages of the disease, a unique feature of this disorder [6]. Weakness and atrophy of the foot extensors manifests as impaired foot dorsiflexion at an early stage of the disease presenting as gait difficulties. Subsequently, forearm flexors, girdle and axial muscles become more involved. The progressive course is gradual without involvement of the ocular, pharyngeal, and respiratory muscles. Cognition, cranial nerves, sensation and coordination remain normal. In more advanced stages of this disorder the muscles of the shoulder girdle are severely affected, with relative sparing of the deltoid, biceps, and triceps. As lower extremity weakness becomes widespread the most characteristic clinical finding, sparing of the quadriceps, becomes obvious. Even as muscle weakness progresses in other groups, the quadriceps remains strong so that affected individuals are able to stand and walk until the clinical pathology is quite advanced [5,8]. By two to three decades after diagnosis affected individuals require a wheelchair for mobility. HIBM has also been associated with cardiac involvement in a small number of affected patients with severe muscle disease.

Creatine kinase levels are normal or only mildly elevated and nerve conduction velocity is typically normal. MRI T1 weighted images of the thighs showed fatty or fibrous replacement of the hamstring muscles with sparing of the quadriceps (Fig. 1). The diagnosis of HIBM is based on both clinical symptoms as well as the histopathology of a muscle biopsy.

2.2. Histopathology

Histopathology of a muscle biopsy from an HIBM affected individual typically demonstrates red rimmed vacuoles with Gomori’s trichrome stain, small fibers in groups, occasionally amyloid deposit, seen with Congo-red staining visualized with rhodamine filters, and 15 to18nm tubulofilaments [2,6,11]. These “non-storage vacuoles” have granular staining, basophilic on H&E and red on Gomori trichrome stains. It was suggested that these vacuoles are autophagic [12]. Presumptive evidence of an autophagocytic process in the rimmed vacuole areas is supported by high acid phosphatase activity, reactivity with lysosomal markers, and the presence of multilammelar bodies on electron microscopy [13].

HIBM muscle immunohistochemistry shows normal cytoskeletal and membrane protein staining patterns. Many degenerating, vacuolated muscle cells show immunoreactivity to neural cell adhesion molecule, NCAM1, which is a fetal muscle antigen. NCAM1 is almost undetectable in normal control muscles, however, it is detectable in regenerating fibers [14]. There is no apparent autoimmune basis for the myopathy as only macrophages around necrotic fibers were noted without the presence of lymphocytes [4]. Electron microscopy on muscle biopsy reveals cytoplasmic and nuclear inclusion bodies containing membrane degradation products with some proliferation of mitochondria with irregular size and shape as well as cytoplasmic tubulofilaments.

3. Molecular genetics

3.1. GNE gene identification

Initial genome-wide linkage analyses in nine Persian Jewish families with HIBM revealed evidence for both autosomal recessive inheritance as well as linkage to 9p1-q1 [15]. Linkage results to the same region in Japanese families with autosomal recessive distal myopathy suggested that this disorder, Nonaka myopathy or DMRV, and HIBM were allelic [16]. The gene was further localized to a 700kb region within 9p13-p12 in Middle Eastern Jews and haplotype analysis of the chromosomal region in 104 affected people from 47 Middle Eastern families revealed one unique ancestral founder chromosome [17,18]. Single non-Jewish families from India, U.S., and the Bahamas with linkage to the same region, had three distinct haplotypes. Using a candidate gene approach in Middle Eastern patients, Eisenberg et al. identified a shared single homozygous missense mutations in the *GNE* gene, p.M712T; while affected individuals of other ethnic origins were compound heterozygotes for other distinct mutations [19].

The *GNE* gene (GenBank NM_005476) spans ~ 44kb of genomic DNA and its major transcript consists of 13 exons, exons 1 and 13 are non-coding. *GNE* is ubiquitously expressed, with the highest levels in liver.

3.1. GNE mutation analysis

Over the last 8 years, the genetic heterogeneity of HIBM has expanded. There are now over 60 *GNE* mutations described worldwide associated with HIBM/DMRV in patients of different ethnic backgrounds (listed in Table 1). These mutations are predominantly missense (82%) and scattered throughout the *GNE* gene.

Of all 62 reported *GNE* mutations associated with HIBM so far, only 11 (18%) are ‘null’ mutations, nonsense or frame shift mutations, highlighted in gray in Table 1, which likely result in nonsense mediated RNA decay and limited or no protein expression. *GNE* null mutations have never been identified on both alleles in a patient; this would most likely be lethal, also suggested by a *Gne* knock-out mouse model, which did not survive past the embryonic stage [20].

Among the *GNE* missense mutations there appear to exist at least 3 founder mutations. p.M712T is the most predominant, identified in patients of Persian-Jewish decent [19,21,22], however, surprisingly this mutation has also been described in an Italian patient [23], a Japanese patient [24], and two unrelated Middle Eastern Moslem families [21]. The second most common *GNE* mutation is p.V572L, predominantly identified in patients of Japanese descent, but also found in some other Asian patients [25–27]. A third *GNE* founder mutation is p.D176V, occurring in the Japanese population [5,25].

Other interesting findings among *GNE* mutations suggest a presence of genetic “hotspots” for mutation. Several distinct amino acids are mutated in different ways: p.G206S or p.G206fsX4 [28], p.R246W or p.R246Q [19,22,28–30], p.303V or p.303X [19,25], p.P511H or p.P511L [10,31], and p.A631T and p.A631V [5,19,21,25,32]. Also some specific *GNE* mutations arose presumably independently in multiple ethnicities: p.R246Q in Italy, Bahamas and Taiwan [19,28,29], p.D378Y in Japan and Ireland [5,21], p.A524V in Thailand, Mexico and France [10,22,32], p.I557T in Italy and Japan [21,24], p.A631V in Germany, Ireland and Japan [5, 21,25], and p.V696M in Thailand, India and Algeria [10,19,32,33].

4. Protein function and biochemistry

GNE mRNA is translated into the 722 amino acid bifunctional enzyme uridine diphospho-N-acetylglucosamine (UDP-GlcNAc) 2-epimerase/N-acetyl-mannosamine (ManNAc) kinase (GNE/MNK) [34–36]. GNE/MNK is ubiquitously expressed and catalyzes the first 2 committed, rate-limiting steps in the biosynthesis of 5-N-acetylneuraminic acid (Neu5Ac), also known as sialic acid. Neu5Ac, further referred to as 'sialic acid', is the most abundant mammalian sialic acid and is typically found as the terminal sugar on glycoconjugates, where it plays a role in a variety of cellular signaling functions [37].

The N-terminal portion of GNE/MNK (amino acids 1–378) has UDP-GlcNAc 2-epimerase catalytic activity (EC 5.1.3.14) [38], which catalyzes the epimerization of UDP-GlcNAc to ManNAc with release of UDP. The C-terminal portion (amino acids 410–722) has ManNAc kinase catalytic activity (EC 2.7.1.60), which phosphorylates ManNAc to ManNAc-6-P and phosphoenolpyruvate. ManNAc-6-P is then further condensed to sialic acid (Fig. 2). The exact locations of the active sites within these domains remain to be determined. The activated form of sialic acid, cytidine monophosphate (CMP)-sialic acid, is utilized as substrate for sialyltransferases by the Golgi-complex in the sialylation of glycoconjugates [39]. Cytosolic CMP-sialic acid regulates GNE/MNK epimerase catalytic activity through a negative feedback mechanism at its allosteric site (amino acids 263–266) [40–42]. Interestingly, heterozygous missense mutations within the GNE/MNK allosteric site lead to another human disorder, sialuria (OMIM#269921). Sialuria cells have lost GNE/MNK feedback inhibition by CMP-sialic acid, resulting in cytoplasmic accumulation and urinary excretion of large quantities of free sialic acid [40–42].

GNE/MNK is a soluble protein, localizing to the cytoplasm, the Golgi-region and the cell nucleus [43]. The role of GNE/MNK in the nucleus remains elusive. GNE/MNK is not predicted to undergo glycosylation, but it has several potential phosphorylation sites [44]. In addition, the GNE/MNK enzyme forms a homohexamer by oligomerization. As a monomer, GNE/MNK has no enzymatic activity; its dimer exhibits only MNK activity, and the hexameric state displays both GNE and MNK activities [34,38,45].

5. HIBM Pathology

Since mutations in the *GNE* gene are associated with HIBM, the presumed mechanism of pathophysiology would be: *GNE* mutations (mostly missense, Table 1) lead to decreased GNE/MNK enzymatic activities resulting in decreased production of sialic acid. The decrease in intracellular sialic acid content would then lead to the muscle degeneration in HIBM. Although excellent experimental work has been done in pursuit of supportive evidence of this hypothesis, the exact cellular mechanisms behind the development of the myopathy in HIBM has remained elusive.

The effects of *GNE* mutations on the enzymatic properties of GNE/MNK were assessed by assays of both GNE-epimerase and MNK-kinase activities, which were reduced, but not absent, in HIBM muscle biopsies, as well as in cultured HIBM fibroblasts, lymphoblasts, and myoblasts [5,30,45,46]. *In vitro* studies, in which specific human *GNE* mutations were expressed in Sf6 insect cells [38,47], in COS-7 cells [45], or in a cell-free *in vitro* transcription-translation system [30], revealed that the reduction in GNE and MNK enzymatic activity is mutation-dependent. Moreover, mutations in one enzymatic domain affect not only that domain's enzyme activity but also the activity of the other domain. Compared with enzyme activities in a cell-free system, fibroblasts exhibited higher residual activities of both GNE and MNK, suggesting the presence in fibroblasts of additional sugar epimerases and kinases with overlapping substrate specificity [30,48].

These experiments revealed that the mechanism of pathology is not that of a typical autosomal recessive disorder with low enzyme activity in the gene product. Rather the total functional activity of GNE/MNK activity in a cell may be dependent both on the location or domain of the mutation in the GNE/MNK protein as well as the activity of other enzymes in metabolically interconnected pathways.

Equally enigmatic are the results of several investigators analyzing sialic acid levels in tissue from individuals affected with HIBM. Hinderlich et al. demonstrated normal membrane bound sialic acid levels in lymphoblastoid lines with the p.M712T mutation [49]. Yet, cultured muscle cells from patients with a variation of *GNE* mutations, showed variable sialylation, ranging from the normal range to significantly decreased [45,46,50]. These studies suggested that muscle cells with a strong reduction in epimerase activity, below 35% of normal, resulting from at least one *GNE* mutation in the epimerase domain, consistently showed measurable decreased sialyation [45,46,50]. However, isoelectric focusing studies of serum transferrin, which contains only N-GlcNAc linked glycans, and serum apolipoprotein CIII, which contains only O-GalNAc linked glycans, appeared normal in all HIBM patients tested so far. This suggests that unaffected serum N-GlcNAc linked and O-GalNAc linked glycosylation in hepatically derived serum glycoproteins in individuals with HIBM [51,52]. Hyposialylation of specific glycosylated proteins in HIBM muscle was reported for PSA, polysialic acid, on NCAM [14] and for α-dystroglycan [33,53], but was reported to be unaffected in other studies [46,54]. Hyposialylation of O-linked glycans in HIBM muscle cells was also demonstrated by use of specific lectins [45,53]. Importantly, Noguchi et al. showed that the hyposialylation of these cells can be reversed by the addition of ManNAc, a substrate in sialic acid synthesis, or sialic acid itself to the media of the cells [45].

Since HIBM is an adult onset disease, and patients have residual GNE/MNK enzymatic activity, the effects of sialic acid deficiency may appear gradually. Some glycoconjugates, for example N-linked, might be more readily sialylated than others, for example O-linked or PSA linked. Thus, when a shortage of sialic acid occurs, specific proteins may be inadequately glycosylated, such as PSA-NCAM or alpha-dystroglycan, contributing to the pathology of HIBM.

Apart from hyposialylation, other hypotheses have arisen for the role of mutated GNE/MNK in the pathology of HIBM. These include the unusual compartmentalization of GNE/MNK in cells [43], leading to speculation of additional GNE/MNK enzymatic activities in cells. Exploration of this phenomenon showed no difference in the compartmentalization of GNE/MNK in either skeletal muscle or primary myoblasts from individuals affected with HIBM [55]. In addition, two novel isoforms of GNE/MNK (GNE1) were identified, which have extended (GNE2) or partially deleted N-termini (GNE3), and display tissue-specific expression [56], which may contribute to the pathology of HIBM. Furthermore, impaired apoptotic signaling in HIBM cells was reported, implicating involvement of apoptotic pathways in HIBM pathophysiology [57]. Another intriguing finding is that GNE/MNK may control sialyltransferase expression, ganglioside production and modulation of proliferation and apoptosis, independent of sialic acid production [58]. In another study, microarray RNA expression and muscle morphology analysis indicated that mitochondrial processes may be affected in HIBM muscle [59]. And recently, co-immunoprecipitation assays identified alpha-actinin 1, an actin binding and crosslinking protein, as a ligand of GNE/MNK [60]. The relevance of α-actinin 1 function in skeletal muscle and its role in HIBM pathophysiology remains elusive.

The above findings underscore that there is more to be learned about the cellularsite of pathology and the mechanism of muscle cell degeneration in HIBM. To further analyze these

pathways on a whole animal, as well as explore potential treatment methods, efforts to study HIBM mouse models are ongoing.

6. HIBM mouse models

Generating a mouse model for HIBM has been complicated because initial trials showed that a complete knock-out of the *Gne* gene led to embryonic lethality [20]. Despite this hurdle, two models were generated by different groups.

Malicdan et al. developed an animal model for HIBM showing the pathologic muscle phenotype over time. They generated a transgenic mouse which expressed the human *GNE* cDNA with the p.D176V epimerase domain mutation, common among Japanese patients, on a mouse background with a disrupted mouse *Gne* gene; $Gne^{(-/-)}$h*GNE*D176V-Tg [61,62]. The mutant offspring of this cross appeared normal at birth but had decreased levels of sialic acid in serum and different organs. These mice developed poor motor performance and increasing serum creatine kinase levels mimicking some aspects of the muscle phenotype seen in the human disease. By 40 weeks of age the mice showed significant changes in muscle pathology with intracytoplasmic rimmed vacuoles which were immunoreactive to lysosomal markers, amyloid and phosphorylated tau and neurofilaments. Ultrastructural and immunohistochemical studies confirmed the presence of autophagosomes in affected mouse muscle. Of interest, the cardiac muscle of these mice also showed rimmed vacuoles suggesting the possible involvement of other muscles types besides skeletal muscle. The phenotype of this p.D176V transgenic mouse model appeared reminiscent of the clinical, pathologic and biochemical features of HIBM/DMRV in humans [61,62].

A second HIBM mouse model created by our group produced an intriguing, unexpected outcome. This *Gne* knock-in mouse was created by homologous recombination which introduced the p.M712T kinase domain mutation, common among Persian-Jews, into the endogenous mouse *Gne* gene; $Gne^{M712T/M712T}$ [63]. Surprisingly, mutant mice died within 72 hours of birth with severe glomerular disease including proteinuria, podocytopathy, segmental splitting of the glomerular basement membrane and effacement of the podocyte foot processes. Biochemical analysis of the mutant mice kidneys revealed decreased Gne/Mnk expression and activity and deficient sialylation of the major podocyte sialoprotein, podocalyxin, suggesting that decreased production of sialic acid may lead to lethality in these mice.

We then proceeded with oral administration of the sialic acid precursor N-acetylmannosamine (ManNAc) to the pregnant and nursing mothers, which resulted in survival of 43% of the mutant $Gne^{M712T/M712T}$ pups beyond 72 hours. Mutant survivors exhibited improved renal histology, increased sialylation of podocalyxin, and increased Gne/Mnk protein expression and Gne-epimerase activities. These findings established this $Gne^{M712T/M712T}$ knock-in mouse as the first genetic model of podocyte injury due to hyposialylation [63,64].

In HIBM patients, no indications of renal abnormalities have been reported. Humans and mice may differ in the relative importance of sialic acid to the kidney, and protein glycosylation patterns also vary; it is known that podoxalyxin differs among species in the contingent of O- and N-linked glycosylation sites [65]. The type of sialic acid present also differs, most mammalian species utilize the sialic acid N-glycolylneuraminic acid (Neu5Gc), but humans have lost the ability to synthesize Neu5Gc [66], and mainly utilize N-acetyl neuraminic acid (Neu5Ac).

Our recent findings of abnormal histology in muscle tissue starting at 6 months of age in surviving mutant $Gne^{M712T/M712T}$ mice (not receiving ManNAc) are encouraging in that this may mimic the human disorder (authors unpublished data). Further research is required to

elucidate phenotypic differences between the transgenic *Gne*$^{(-/-)}$h*GNE*D176V-Tg model and the knock-in *Gne*$^{M712T/M712T}$ model.

In spite of these still obscure phenotypic differences between HIBM mouse models, the encouraging results of the ManNAc supplementation of the murine knock-in model [63] support evaluation of ManNAc, a well-tolerated intervention, not only as a treatment for HIBM, but also as a treatment for renal disorders involving proteinuria and hematuria due to podocytopathy and/or segmental splitting of the glomerular basement membrane.

7. Treatment

No therapies are currently available for HIBM. Dietary modifications were proposed, including avoidance of excess selenium, copper and zinc (inhibitors of GNE/MNK activity), reduced consumption of ethanol, ethanol promotes hydrolysis of sialoconjugates, and dietary promotion of magnesium, an essential co-factor of GNE/MNK [67].

Other suggested strategies of therapy for HIBM invoke the concept that hyposialylation is the basis of the pathophysiology in affected individuals. The hypothesis that increasing total body sialic acid through exogenous means will lead to clinical benefit, was recently tested in our center through a pilot study on four affected patients (http://clinicaltrials.gov: Identifier NCT00195637) [68]. The HIBM patients were loaded with 1g/kg intravenous immunoglobulin G (IVIG) on two consecutive days followed by 3 doses of 400mg/kd at weekly intervals. It was hypothesized that the large sialic acid content on IgG (~ 8 μmol of sialic acid/g) could be utilized to sialylate other glycoproteins. This study showed improvement in mean quadriceps strength both after loading (+22%) and at the end of the study (+35%). Mean shoulder strength showed similar findings at (+44%) and (+46%) respectively. A composite of 8 other muscle groups showed improvements as well after loading (+8%) and (+19) at the end of the study [68].

Although immunohistochemical staining and immunoblotting of muscle biopsies for alpha-dystroglycan and NCAM did not show indisputable evidence of increased sialylation after IVIG treatment, patients did report subjective improvement in their ability to perform routine daily activities. Though follow-up studies are needed, this protocol reveals issues surrounding therapeutic strategies for HIBM and opens the door to other possible therapeutic options. These include the administration of other forms of sialic acid, in particular the sialic acid precursor ManNAc.

ManNAc is an uncharged, natural compound and feeds into the sialic acid biosynthesis pathway distal to the rate-limiting GNE-epimerase step (Fig. 2). Residual MNK activity in HIBM patients, or ancillary kinases (e.g., GlcNAc kinase) [48], might convert ManNAc into ManNAc-6P and aid synthesis of free sialic acid. Hyposialylated, *Gne*-deficient mouse embryonic stem cells [20] and human HIBM/DMRV cultured myotubes [45] became resialylated after the growth medium was supplemented with ManNAc. Furthermore, incubation of cultured cells with ‘unnatural’ ManNAc derivatives (ManLev, N-levulinoylmannosamine or ManNAz, N-azidoacetylmannosamine) resulted in incorporation of the downstream sialic acid analogs, SiaLev (N-levulinoyl sialic acid) or SiaNAz (N-azidoacetyl sialic acid), into cell surface glycoconjugates [69,70]. Of greatest significance, the salutary effect of oral ManNAc supplementation on survival and sialylation status of our HIBM knock-in mouse model [63] holds promise for potential benefit in a future human clinical treatment protocol. Our center is pursuing the design of a formal trial of ManNAc in humans, which has yet to be approved by regulatory authorities.

Apart from manipulating products and/or substrates in the GNE/MNK pathway, another future treatment option could be the delivery of a healthy *GNE* gene, gene therapy, or a healthy GNE/

MNK enzyme via stem cells, to patients' cells and tissues, in particular to the muscle. Continued work in this field will elucidate insights both into the pathophysiology of this devastating disorder as well as other human diseases caused by the perturbation of glycobiologic pathways.

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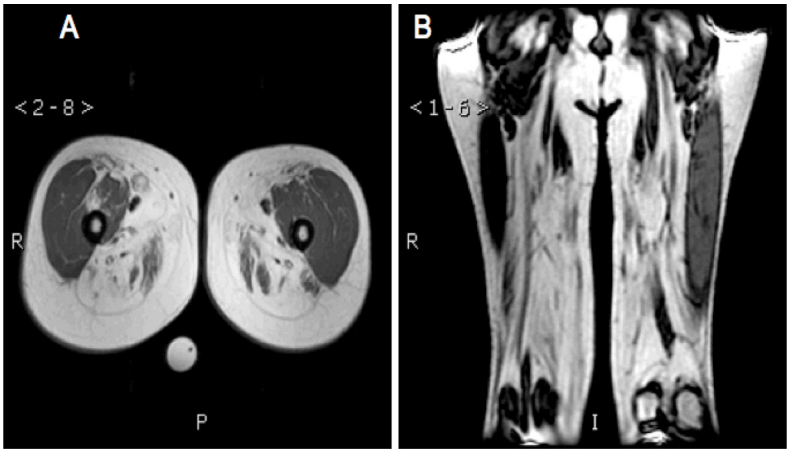

Fig. 1.

T1 weighted magnetic resonance images of the thigh of an individual affected with HIBM. (A) Axial image showing fibrotic muscles of the posterior compartment or “hamstring” muscles (H) with comparatively less involvement of the quadriceps femoris (Q). (B) Coronal image showing similar findings.

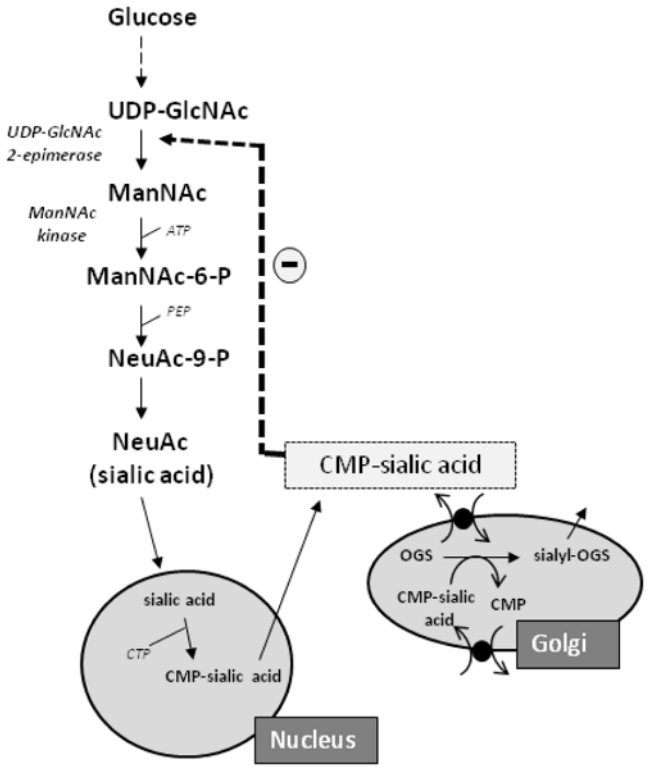

Fig. 2.

Sialic acid synthesis pathway. The biosynthesis of sialic acid (Neu5Ac) occurs in the cytosol, where glucose undergoes several modifications to become UDP-GlcNAc. The UDP-GlcNAC 2-epimerase activity of GNE/MNK then epimerises UDP-GlcNAc into ManNAc, after which its ManNAc kinase activity further converts this to ManNAc-6-P, which is then converted in several steps to the downstream product CMP-sialic acid. CMP-sialic acid is utilized by the Golgi complex to sialylate glycoconjugates. CMP-sialic acid can feedback-inhibit the UDP-GlcNAc 2-epimerase enzymatic activity in its allosteric site. For more details, see text.

Table 1

GNE mutations associated with HIBM

#	Nucleotide substitution	Amino acid substitution	*GNE* exon	Ethnicity	Protein domain	Refs
1	c.C31T	p.R11W	2	India	Epimerase	[33]
2	c.G38C	p.C13S	2	Japan, Korea	Epimerase	[24,27]
3	c.79C>T	p.P27S	2	Italy	Epimerase	[28]
4	c.86T>C	p.M29T	2	Korea	Epimerase	[27]
5	c.107C>T	p.P36L	2	Italy	Epimerase	[21]
6	ins10 bp	Frameshift *	2	Japan	Epimerase	[5]
7	c.265G>C	p.G89R	3	Thailand	Epimerase	[10]
8	c.386G>A	p.R129Q	3	Japan, Korea	Epimerase	[24,27]
9	c.396C>G	p.H132Q	3	Japan	Epimerase	[5]
10	c.404G>T	p.G135V	3	USA, Irish, English	Epimerase	[30]
11	c.484C>T	p.R162C	3	Italy	Epimerase	[71]
12	c.511A>G	p.M171V	3	Italy	Epimerase	[23]
13	c.578A>T	p.D176V	3	Japan	Epimerase	[5,24]
14	c.529C>T	p.R177C	3	Japan	Epimerase	[5]
15	c.598A>T	p.I200F	3	USA	Epimerase	[21]
16	c.605G>T	p.R202L	3	Greece	Epimerase	[33]
17	c.616G>A	p.G206S	3	Italy	Epimerase	[28]
18	c.617delG	p.G206fsX4	3	Italy	Epimerase	[28]
19	c.647T>C	p.V216A	4	USA	Epimerase	[33,72]
20	c.673G>A	p.D225N	4	Bahamas	Epimerase	[19]
21	c.722T>G	p.I241S	4	Taiwan	Epimerase	[29,73]
22	c.736C>T	p.R246W	4	USA	Epimerase	[22,30]
23	c.737G>A	p.R246Q	4	Bahamas, Italy, Taiwan	Epimerase	[19,28,29]
24	IVS4+4A>G	Exon 4 skipping	Intron 4	Japan	Epimerase	[5]
25	c.829C>T	p.R277C	5	France	Epimerase	[32]
26	c.847C>T	p.P283S	5	Japan	Epimerase	[24]
27	c.907–908TG>GT	p.C303V	5	Japan	Epimerase	[25]
28	c.909T>A	p.C303X	5	India	Epimerase	[19]
29	c.917G>A	p.R306Q	5	Japan	Epimerase	[5]

#	Nucleotide substitution	Amino acid substitution	*GNE* exon	Ethnicity	Protein domain	Refs
30	c.992T>C	p.V331A	6	Japan	Epimerase	[5]
31	c.1003C>T	p.R335W	6	USA	Epimerase	[74]
32	c.1039_1042 delCTGCinsA	p.L347del; H348N	6	USA	Epimerase	[74]
33	IVS6+2dupT (c.1070+2dupT)	p.352fsX15; p.Y355_C357del	6	Italian	Epimerase	[28]
34	c.1099G>A	p.V367I	7	Iran	Epimerase	[75]
35	c.1130delT	p.I377fsX16	7	Italian	Epimerase	[28]
36	c.1183G>T	p.D378Y	7	USA, Japan, Ireland	Epimerase**	[5,21]
37	c.1136T>A	p.L379H	7	Tunesia	Epimerase?**	[76]
38	c.1258C>T	p.R420X	7	Japan	Kinase	[24]
36	c.1262T>C	p.V421A	7	Japan	Kinase	[24]
37	c.1295delA	p.K432fsX17	8	India	Kinase	[77]
38	c.1379C>T	p.A460V	8	Japan	Kinase	[26]
39	c.1415T>C	p.I472T	9	Japan	Kinase	[5,78]
40	c.1532C>A	p.P511H	9	Japan	Kinase	[31]
41	c.1532C>T	p.P511L	9	Thailand	Kinase	[10]
42	c.1539G>A	p.W513X	9	Taiwan	Kinase	[73]
43	c.1556A>G	p.N519S	9	Italy	Kinase	[28]
44	c.1571C>T	p.A524V	9	Thailand, Mexico, South America, France	Kinase	[10,22,32]
45	c.1583T>G	p.F528C	9	Germany	Kinase	[21]
46	c.1670T>C	p.I557T	10	Italy, Japan	Kinase	[21,24]
47	c.1675G>C	p.G559R	10	Greece	Kinase	[33]
48	c.1714G>C	p.V572L	10	Japan, Korea, Asian	Kinase	[25–27]
49	c.1727G>A	p.G576E	10	USA	Kinase	[19]
50	c.1760T>C	p.I587T	10	USA, Italy, Algeria	Kinase	[21,32]
51	c.1771G>A	p.A591T	10	Korea	Kinase	[27]
52	c.1798G>A	p.A600T	10	Italy	Kinase	[28]
53	c.1888G>A	p.A630T	11	Japan	Kinase	[5]
54	c.1891G>A	p.A631T	11	USA, Senegal	Kinase	[19,32]
55	c.1892C>T	p.A631V	11	Germany, Ireland, Japan, USA	Kinase	[5,21,25,72]
56	c.1967T>A	p.I656N	11	Thailand	Kinase	[10]
57	c.2023T>C	p.Y675H	12	Mexico, South America	Kinase	[22]

#	Nucleotide substitution	Amino acid substitution	*GNE* exon	Ethnicity	Protein domain	Refs
58	c.2036T>G	p.V679G	12	France	Kinase	[32]
59	c.2086G>A	p.V696M	12	Thailand, India, Algeria	Kinase	[10,19,32,33]
60	c.2122G>A	p.G708S	12	Japan	Kinase	[24]
61	c.2135T>C	p.M712T	12	Persian Jewish	Kinase	[19,22–24,76]
62	del ex3-ex9 (>35.7kb)	Large deletion	3–9	Italy	Epimerase + Kinase	[71]

*Gray background; severe mutations, likely resulting in nonsense mediated RNA decay and limited GNE/MNK protein expression.

**GNE/MNK amino acid residues 1–378 are suggested to regulate epimerase enzymatic activity, and residues 410–722 regulate kinase enzymatic activity [38].