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216.1: GNE Myopathy

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Abstract

1. GNE myopathy is a rare muscle disease caused by mutations in *GNE*, the gene that encodes the rate-limiting enzyme of the biosynthetic pathway of sialic acid.
2. Uridine diphosphate (UDP)-N-acetylglucosamine (GlcNAc) 2-epimerase/N-acetylmannosamine (ManNAc) kinase (GNE) catalyzes the first 2 committed steps in sialic acid synthesis and is encoded by the *GNE* gene.
3. GNE myopathy patients have nonallosteric, biallelic, predominantly missense mutations in *GNE*. GNE myopathy has historically also been named hereditary inclusion body myopathy (HIBM), distal myopathy with rimmed vacuoles (DMRV), Nonaka myopathy, inclusion body myopathy type 2 (IBM2), and quadriceps-sparing myopathy (QSM).
4. GNE myopathy presents in early adulthood with lower extremity distal muscle weakness. The disease is characterized by a slow progression of muscle weakness and atrophy, from distal to proximal, initially in the lower extremities, with relative sparing of the quadriceps, and subsequently in the upper extremities. The disease leads to marked disability, wheelchair use, and dependent care.
5. The diagnosis of GNE myopathy is currently based upon clinical features, muscle pathology, and, ultimately, the presence of *GNE* gene mutations. Histopathology of muscle biopsies typically shows rimmed vacuoles and characteristic filamentous inclusions, but may be negative. Diagnosis is usually delayed or missed, likely because of the rare nature of the disease and the lack of inexpensive and noninvasive diagnostic tests.
6. Impaired sialylation of (muscle) glycans likely underlies the disease pathology. However, the exact pathophysiology of GNE myopathy remains unknown.
7. No approved therapies are currently available for GNE myopathy. Clinical trials are being conducted, including trials that increase sialic acid levels through exogenous means or through *GNE* gene therapy. Exogenous therapies include oral administration of the sialic acid precursor N-acetylmannosamine (ManNAc) or sialic acid (Neu5Ac) itself and intravenous administration of immunoglobulin (IVIG), a highly sialylated compound.

Background

Historical Aspects

Inclusion body myositis (IBM) is defined by the pathologic presence of rimmed vacuoles and tubulofilaments on muscle histology, and is further classified into sporadic inclusion body myositis (s-IBM; OMIM#147421), which invariably has inflammation, and hereditary inclusion body myopathies, which show familial inheritance and no inflammation.[1–3] This chapter describes the clinical features, molecular basis, and pathophysiology of a specific type of hereditary inclusion body myopathy, the autosomal recessive, quadriceps-sparing type, originally termed h-IBM, or inclusion body myopathy type 2 (IBM2; OMIM#600737).

The features of adult-onset myopathy with a predilection for distal muscles were originally described in 1981 in Japanese patients and termed Nonaka distal myopathy, now commonly referred to as distal myopathy with rimmed vacuoles (DMRV; OMIM#605820).[4] In 1984, a similar disorder in Iranian-Jewish patients was described as vacuolar myopathy sparing the quadriceps,[5] later commonly referred to as inclusion body myopathy 2 (IBM2) or hereditary inclusion body myopathy (HIBM).

Genome-wide linkage analyses in 1996 in 9 Persian Jewish families with HIBM revealed autosomal recessive inheritance and mapped the gene to 9p1–q1.[6] The next year, linkage to the same region in Japanese families with DMRV was described, suggesting that DMRV and HIBM were allelic.[7] Fine mapping using Middle Eastern Jewish HIBM families further narrowed the region to 9p13–p12, and haplotype analysis revealed one unique ancestral founder chromosome in Middle Eastern families.[8,9] In 2001, a candidate gene approach revealed a shared single homozygous missense mutation in the *GNE* gene in Middle Eastern patients, while affected individuals of other ethnic origins were compound heterozygotes for other distinct *GNE* mutations.[10] Once *GNE* was identified as the HIBM-causing gene, *GNE* mutations were also reported in DMRV patients.[11] Apart from the Persian Jewish and Japanese isolates, patients have now been described in a wide variety of ethnicities, including Caucasian, Indian, Thai, Mexican, and African (Table 258-1).

Table 258-1 *GNE* Mutations Associated with GNE Myopathy

Amino Acid Substitution[1]		**Nucleotide Substitution[2]**	***GNE* Exon[2]**	**GNE Protein Domain[3]**	**Ethnicity**	**Refs**
hGNE1	**hGNE2**	***mRNA variant 1***				
NP_005467	***NP_001121699***	***NM_001128227***				
p.E2G	p.E33G	c.98A>G	3	ep	*European*	[38]
p.R8X[4]	**p.R39X**	c.115C>T	3	ep	*Caucasian, Chinese, Japanese*	[38,40,97]
p.R11W	p.R42W	c.124C>T	3	ep	*Indian*	[76]

Amino Acid Substitution[1]		Nucleotide Substitution[2]	*GNE* Exon[2]	GNE Protein Domain[3]	Ethnicity	Refs
hGNE1 *NP_005467*	**hGNE2** *NP_001121699*	*mRNA variant 1* *NM_001128227*				
p.C13S	p.C44S	c.131G>C	3	ep	*Chinese, Japanese, Korean*	36,97–99
p.A26P	p.A57P	c.169G>C	3	ep	*Caucasian*	29
p.P27S	p.P58S	c.172C>T	3	ep	*Italian*	100
p.P27L	p.P58L	c.173C>T	3	ep	*Japanese*	40
p.M29T	p.M60T	c.179T>C	3	ep	*Korean*	99
p.E35K	p.E66K	c.196G>A	3	ep	*Chinese*	35,97
p.P36L	p.P67L	c.200C>T	3	ep	*Italian*	56
frameshift	**frameshift**	ins10 bp	3	ep	*Japanese*	11
p.I51M	p.I82M	c.246A>G	3	ep	*Chinese*	35,97
p.R71W	p.R102W	c.304A>T	4	ep	*Caucasian*	38
p.G89R	p.G120R	c.358G>C	4	ep	*Thai*	101
p.G89S	p.G120S	c.358G>A	4	ep	*Japanese*	40
p.R101C	p.R132C	c.394C>T	4	ep	*Korean*	36
p.I106T	p.I137T	c.410T>C	4	ep	*Chinese*	97
p.I128IfsX6	**p.I159IfsX6**	c.476insT	4	ep	*Japanese*	40
p.R129Q	p.R160Q	c.479G>A	4	ep-NES	*Japanese, Korean*	98,99
p.H132Q	p.H163Q	c.489C>G	4	ep-NES	*Japanese*	11
p.G135V	p.G166V	c.497G>T	4	ep-NES	*English, Irish, USA*	23
p.I142T	p.I173T	c.518T>C	4	ep	*Caucasian*	38
p.I150V	p.I181V	c.541A>G	4	ep	*European*	102
p.R162C	p.R193C	c.515C>T	4	ep	*Italian*	103
p.M171V	p.M202V	c.604A>G	4	ep	*Italian*	104
p.D176V	p.D207V	c.620A>T	4	ep	*Chinese, Japanese, Korean*	11,35,36,98,105
p.R177C	p.R208C	c.622C>T	4	ep	*Japanese*	11
p.L179F	p.L210F	c.628C>T	4	ep	*Italian*	41
p.Y186C	p.Y217C	c.650A>G	4	ep	*Pakistani*	102
p.D187G	p.D218G	c.653A>G	4	ep	*Japanese*	40
p.I200F	p.I231F	c.691A>T	4	ep	*USA*	56
p.R202L	p.R233L	c.698G>T	4	ep	*Greek*	76

Amino Acid Substitution[1]		Nucleotide Substitution[2]	*GNE* Exon[2]	GNE Protein Domain[3]	Ethnicity	Refs
hGNE1 *NP_005467*	**hGNE2** *NP_001121699*	*mRNA variant 1 NM_001128227*				
p.W204X	**p.W235X**	c.705G>A	4	ep	*Caucasian*	38
p.G206S	p.G237S	c.709G>A	4	ep	*Italian*	100
p.G206fsX4	**p.G237fsX4**	c.710delG	4	ep	*Italian*	100
p.V216A	p.V247A	c.740T>C	5	ep	*USA*	76,106
p.D225N	p.D256N	c.766G>A	5	ep	*Bahamian*	10
p.F233S	p.F264S	c.791T>C	5	ep	*Japanese*	40
p.I241S	p.I272S	c.815T>G	5	ep	*Chinese, Taiwanese*	35,97,107,108
p.R246W	p.R277W	c.829C>T	5	ep	*Caucasian, Chinese, USA*	23,35,38,108,109
p.R246Q	p.R277Q	c.830G>A	5	ep	*Bahamian, Italian, Taiwanese*	10,38,100,107
skip exon 5	**skip exon 5**	IVS5+4A>G	intron 6	ep	*Japanese*	11
p.M261V	p.M292V	c.874A>G	6	ep-AR	*Korean*	36
p.M265T	p.M296T	c.887T>C	6	ep-AR	*European*	102
p.R277C	p.R308C	c.922C>T	6	ep-AR	*French*	110
p.P283S	p.P314S	c.940C>T	6	ep-AR	*Japanese*	98
p.H293R	p.H324R	c.971A>G	6	ep-AR	*Indian*	24
p.G295D	p.G326D	c.977G>A	6	ep-AR	*Japanese*	40
p.I298T	p.I329T	c.986T>C	6	ep-AR	*Asian, Chinese, Indian*	38,97
p.N300K	p.N331K	c.993C>A	6	ep-AR	*Italian*	25
p.C303V	p.C334V	c.1000-1001TG>GT	6	ep-AR	*Japanese*	57
p.C303X	**p.C334X**	c.1002T>A	6	ep	*Indian*	10
p.R306Q	p.R337Q	c.1010G>A	6	ep	*Japanese*	11
p.A310P	p.A341P	c.1021G>C	6	ep	*Chinese*	108
p.V315M	p.V346M	c.1036G>A	6	ep	*European*	102
p.N317D	p.N347D	c.1021A>G	6	ep	*European*	102
p.R321C	p.R352C	c.1054C>T	6	ep	*Japanese*	40
p.V331A	p.V362A	c.1085T>C	7	ep	*Japanese*	11
p.H333R	p.H364R	c.1091A>G	7	ep	*Caucasian*	29
p.R335W	p.R366W	c.1096C>T	7	ep	*Caucasian*	38,111
p.L347del;	p.L378del;	c.1132_1134	7	ep	*Caucasian*	111

Amino Acid Substitution[1]		Nucleotide Substitution[2]	*GNE* Exon[2]	GNE Protein Domain[3]	Ethnicity	Refs
hGNE1 ***NP_005467***	**hGNE2** ***NP_001121699***	***mRNA variant 1*** ***NM_001128227***				
H348N	H379N	del;c.1135C>A				
p.352fsX15; p.Y355_C357 del	**p.383fsX15; p.Y386_C388 del**	IVS7+2dupT c.1163+2dupT	intron 8	ep	*European, Italian*	100,102
p.Y361X	**p.Y392X**	c.1176T>G	8	ep	*Caucasian*	29
p.V367I	p.V398I	c.1192G>A	8	ep	*Iranian*	112
p.I377fsX16	**p.I408fsX16**	c.1223delT	8	ep	*Italian*	100
p.D378Y	p.D409Y	c.1276G>T	8	ep	*European, Irish, Japanese, USA*	11,56,102
p.L379H	p.L410H	c.1229T>A	8	UF	*Tunisian*	21
p.R420X	**p.R451X**	c.1351C>T	8	kin	*Japanese*	98
p.V421A	p.V452A	c.1355T>C	8	kin	*Japanese*	98
p.K432fsX17	**p.K463fsX17**	c.1388delA	9	kin	*Indian*	34
p.Q436X	**p.Q467X**	c.1399C>T	9	kin	*Taiwanese*	38
p.A460V	p.A491V	c.1472C>T	9	kin	*Japanese*	113
p.I472T	p.I503T	c.1508T>C	10	kin	*Japanese*	11,114
p.W495X	**p.W526X**	c.1577G>A	10	kin	*Caucasian*	102
p.L508S	p.L539S	c.1616T>C	10	kin	*Chinese*	35,97
p.H509Y	p.H540Y	c.1618C>T	10	kin	*Chinese*	97
p.P511H	p.P542H	c.1625C>A	10	kin	*Japanese*	105
p.P511L	p.P542L	c.1625C>T	10	kin	*Thai*	101
p.W513X	**p.W544X**	c.1632G>A	10	kin	*Chinese, Taiwanese*	35,108
p.N519S	p.N550S	c.1649A>G	10	kin	*Italian*	100
p.A524V	p.A555V	c.1664C>T	10	kin	*French, Mexican, South American, Thai*	101,109,110
p.F528C	p.F559C	c.1676T>G	10	kin	*German*	56
p.G545EfsX9	**p.G576EfsX9**	c.1727delG	11	kin	*Korean*	36
p.L556S	p.L587S	c.1760T>C	11	kin	*Caucasian*	38
p.I557T	p.I588T	c.1763T>C	11	kin	*Italian, Japanese*	56,98
p.G559R	p.G590R	c.1768G>C	11	kin	*Greek*	76
p.G568S	p.G599S	c.1795G>A	11	kin	*Japanese*	40

Amino Acid Substitution[1]		Nucleotide Substitution[2]	*GNE* Exon[2]	GNE Protein Domain[3]	Ethnicity	Refs
hGNE1 *NP_005467*	**hGNE2** *NP_001121699*	*mRNA variant 1* *NM_001128227*				
p.V572L	p.V603L	c.1807G>C	11	kin	*Asian, Chinese, Japanese, Korean*	35,36,57,99,113
p.G576E	p.G607E	c.1820G>A	11	kin	*USA*	10
p.C586X	**p.C617X**	c.1850delG	11	kin	*Japanese*	40
p.I587T	p.I618T	c.1853T>C	11	kin	*Algerian, Chinese, Italian, USA*	35,41,57,110
p.A591T	p.A622T	c.1864G>A	11	kin	*Chinese, Korean*	97,99
p.A600T	p.A631T	c.1891G>A	11	kin	*Italian*	100
p.A600E	p.A631E	c.1892C>A	11	kin	*Japanese*	40
p.L603F	p.L634F	c.1900C>T	11	kin	*Japanese*	40
skip exon 11	**skip exon 11**	IVS12+5G>A c.1909+5G>A	intron 12	kin	*Indian*	22
p.S615X	**p.S646X**	c.1937C>G	12	kin	*Caucasian*	38
p.A630T	p.A661T	c.1981G>A	12	kin	*Japanese*	11
p.A631T	p.A662T	c.1984G>A	12	kin	*Caucasian, Senegalese, USA*	10,102,110
p.A631V	p.A662V	c.1985C>T	12	kin	*Caucasian, Chinese, German, Irish, Japanese, Korean, USA*	11,29,35,36,38,56,57,106
p.I656N	p.I687N	c.2060T>A	13	kin	*Thai*	101
p.G669R	p.G700R	c.2098G>C	13	kin	*Asian, Indian*	102
p.Y675H	p.Y706H	c.2116T>C	13	kin	*Caucasian, Mexican, South American*	38,109
p.V679G	p.V710G	c.2129T>G	13	kin	*French*	110
p.V696M	p.V727M	c.2179G>A	13	kin	*Algerian, Asian, Chinese, Middle-Eastern, Indian, Pakistani, Thai*	10,22,34,38,76,97,101,102,110
p.S699L	p.S730L	c.2189C>T	13	kin	*Middle-Eastern*	102

Amino Acid Substitution[1]		Nucleotide Substitution[2]	*GNE* Exon[2]	GNE Protein Domain[3]	Ethnicity	Refs
hGNE1 *NP_005467*	**hGNE2** *NP_001121699*	*mRNA variant 1* *NM_001128227*				
p.G708S	p.G739S	c.2215G>A	13	kin	*Japanese*	98
p.M712T	p.M743T	c.2228T>C	13	kin	*Egyptian-Muslim, Persian Jewish*	10,21,41,98,104,109
large deletion	**large deletion**	del ex 4-ex 10 (>35.7kb)	4-10	ep+kin	*Italian*	103

[1]Amino acid mutation nomenclature for both hGNE1 (previous terminology; before 2011) and hGNE2 (current terminology; after 2011) proteins are listed.

[2]Nucleotide mutation nomenclature and exon numbering are according to the longest GNE mRNA variant 1 (current terminology; NM_001128227), encoding the hGNE2 protein. Published mutation nomenclatures and exon numbers were converted to current terminology and exon numbering.

[3]GNE protein domain functions: ep = epimerase; NES = putative nuclear export signal; AS = experimental allosteric region; UF = unknown function; kin = kinase. See text and Figure 258-4 for details.

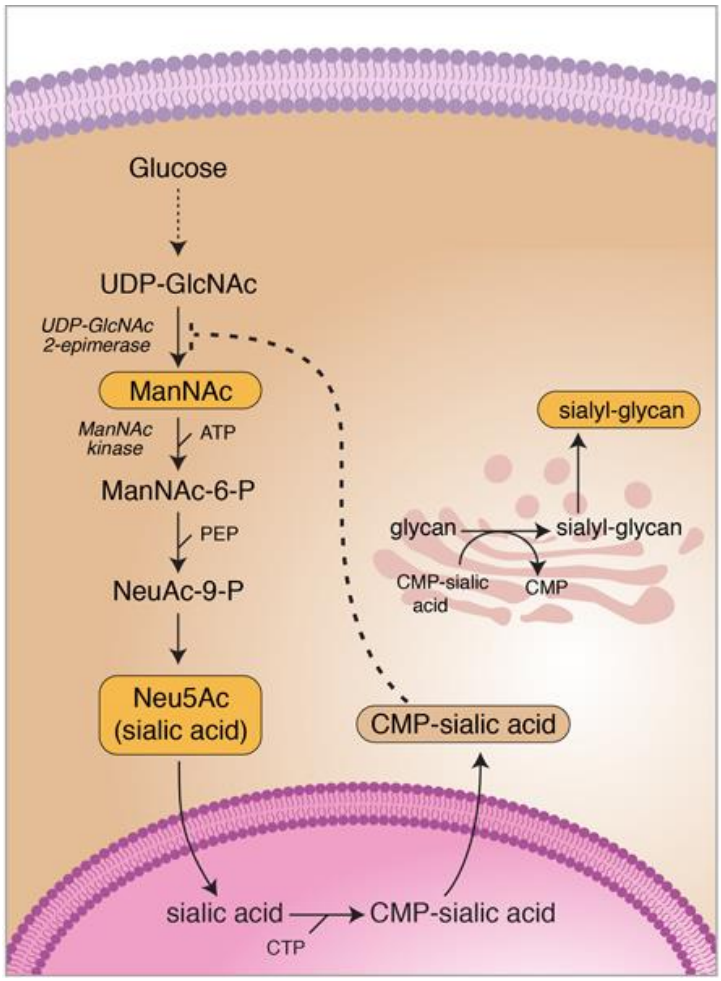
[4]Bold print = severe mutations, likely resulting in nonsense mediated mRNA decay and limited GNE protein expression.

The multiple historic names of the disorder could potentially be confusing for clinicians, patients, and researchers. Therefore, the disorder has recently been renamed, substituting all previous disease denotations, to the profound name *GNE myopathy*, to which we henceforth refer.

Sialic Acid Metabolism and GNE Function

GNE mRNA is translated into the 722 amino acid bifunctional enzyme uridine diphospho (UDP)-N-acetylglucosamine (GlcNAc) 2-epimerase/N-acetylmannosamine (ManNAc) kinase (GNE/MNK, henceforth referred to as GNE).[10,12,13] GNE catalyzes the first 2 committed, rate-limiting steps in the biosynthesis of 5-N-acetylneuraminic acid (Neu5Ac) (Figure 258-1). GNE is a soluble protein that localizes in the cytoplasm, the Golgi region, and the cell nucleus. GNE is not predicted to be glycosylated, and its role in the nucleus is not yet determined.

Figure 258-1



Sialic acid synthesis pathway in mammalian cells Schematic of *N*-acetylneuraminic acid (Neu5Ac, sialic acid) enzymatic synthesis and attachment to glycan structures. The biosynthesis of sialic acid in cells begins with glucose in the cytoplasm. Newly synthesized sialic acid moves into the nucleus, where it is activated to CMP-sialic acid, which then moves back into the cytoplasm. The subsequent transport of CMP-sialic acid into the Golgi provides a substrate pool to be added to newly synthesized glycoconjugates. See text for further details. (Courtesy of Darryl Leja and Julia Fekecs, NHGRI, NIH, Bethesda, MD)

Sialic acids are the most abundant terminal monosaccharides on glycoconjugates in eukaryotic cells and can have more than 50 different modifications of their carboxylated amino groups on their scaffold of 9 carbon atoms.[14] Neu5Ac (henceforth referred to as sialic acid) is the most abundant mammalian sialic acid and the precursor of most other sialic acids.[14] It is typically found as the terminal sugar on glycoconjugates, where it plays a role in protein turnover, and various cell-cell interactions.[15–17]

Figure 258-1 depicts the sialic acid synthesis pathway. UDP-GlcNAc, the initial substrate for the synthesis of sialic acid, is derived from glucose. UDP-GlcNAc is acted upon in the cytoplasm by the 2 functional domains of GNE, an epimerase domain and a kinase domain. Initially, the UDP-GlcNAc 2-epimerase domain synthesizes ManNAc from UDP-GlcNAc. ManNAc is then

phosphorylated using phosphoenolpyruvate by the ManNAc kinase domain of GNE to generate ManNAc-6-phosphate (ManNAc-6-P).12 ManNAc-6-P is condensed further to sialic acid, which can then be activated using cytidine triphosphate (CTP) to form cytidine monophosphate (CMP)-sialic acid in the nucleus of the cell. CMP-sialic acid returns to the cytoplasm, from which it can be transported into the lumen of the Golgi compartment, where it is utilized in the synthesis of oligosaccharides. Cytosolic CMP-sialic acid regulates the GNE epimerase catalytic activity through a negative feedback mechanism at its allosteric site.18–20

Clinical Basis and Natural History

GNE myopathy is a disorder characterized by slowly progressive atrophy and weakness, primarily of skeletal muscle, with onset in early adulthood. It has worldwide occurrence; although initially identified in Persian Jewish and Japanese populations, patients from Italian, English, Irish, Egyptian, Tunisian, Indian, Chinese, and other backgrounds have been identified (Table 258-1).10,21–25 The prevalence in the Persian Jewish community is estimated to be 1 in 1500,26 and the worldwide prevalence is estimated at 1 in 1,000,000.

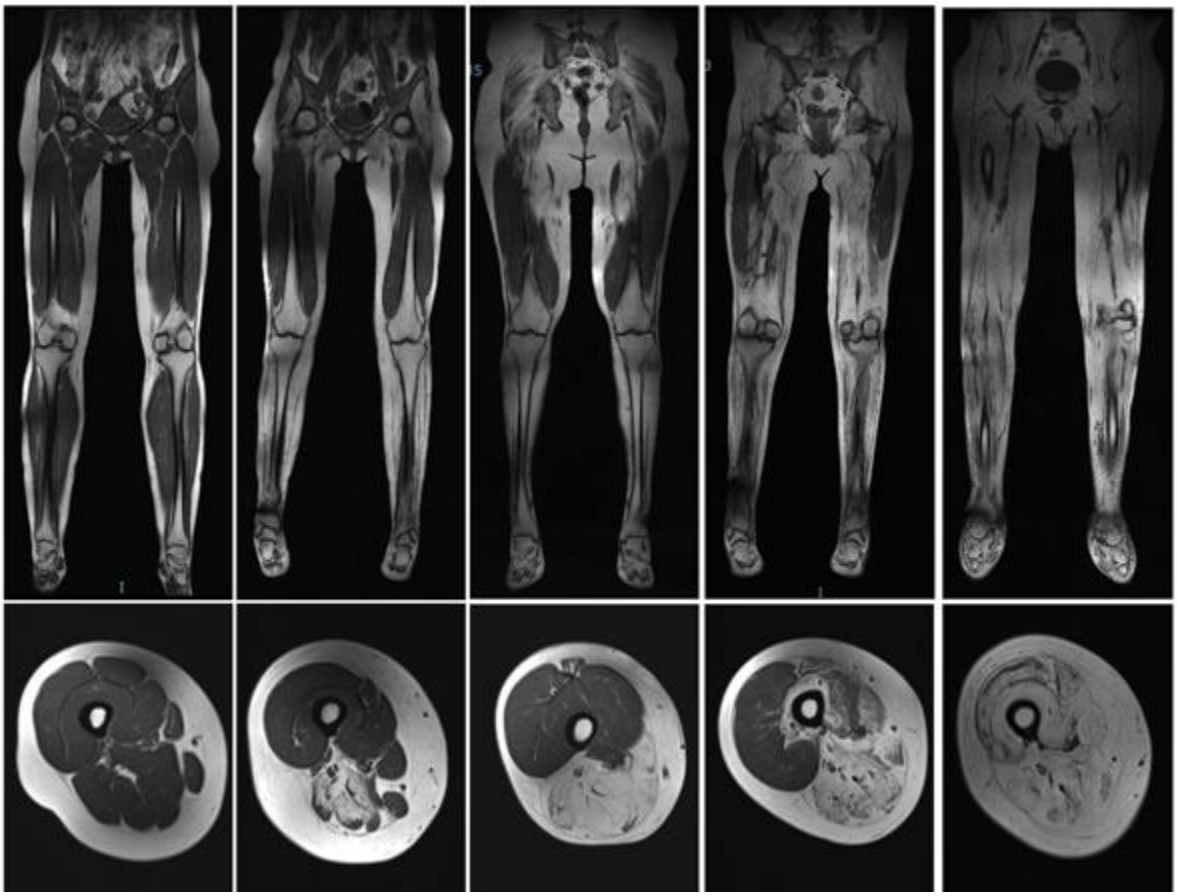
Presentation

GNE myopathy typically presents in young adults between 20 to 40 years of age, although earlier and later presentations are described. Initial manifestations are usually secondary to weakness of the tibialis anterior muscle (ankle dorsiflexion), which manifests as tripping, inability to stand on toes, or gait disturbance—usually detected by observers. When patients are evaluated in this early stage of the disease, distal lower extremity muscle weakness presented by foot drop may be the only manifestation of the disease. Patients with GNE myopathy thus present to neurologists, physical therapists, or orthopedists, all of whom should be familiar with the disease. The typical presentation may be modified by extreme muscle overuse and the weakness being apparent on the "overused" muscle, for example hand weakness in work-related overuse.27

Natural History

Muscle weakness and atrophy in GNE myopathy typically progresses slowly first in the lower extremities and subsequently in the upper extremities, moving from distal to proximal muscle groups. After involvement of the tibialis anterior muscle, muscles of the lower extremities become progressively affected over the next decade: muscles of the anterior compartment of the leg followed by the posterior compartment of the leg, hamstrings, and gluteal muscles (Figure 258-2). The quadriceps remains unaffected until late in the disease5 and its preserved strength compensates for weakness in other muscles, allowing patients to stand and walk with assistance until late in the disease, when all other lower extremity muscles have been affected (Figure 258-2). The functional deficits associated with muscle weakness manifest slowly and allow patients to adapt to muscle weakness. The gait becomes progressively affected, resulting in steppage gait, frequent falls, and decrease in balance confidence that require the use of a cane or walker. Patients require wheelchairs approximately 20 years after the onset of symptoms (see Table 258-2).

Figure 258-2



GNE myopathy muscle MRI Representative muscle MRI images of patients with GNE myopathy at different stages of the disease, with early stages on the left progressing to late stages on the right. Coronal T1-weighted muscle MRI images (upper panels) represent the progressive atrophy and fatty replacement of muscles in the lower extremities. Axial T1-weighted images of the mid-femoral thigh (lower panels) show progressive atrophy noted initially in the posterior compartment (hamstrings) and followed by involvement of muscles in the anterior compartment; the vastus lateralis is the last portion of the quadriceps to be affected.

Table 258-2 Clinical Manifestations of GNE Myopathy

Lower Extremity Weakness
Difficulty walking on toes and heels
Foot drop
Progressive gait abnormalities:
Waddling gait
Steppage gait
Ankle-foot orthotic use
Cane use
Wheelchair use
Falls
Difficulty standing from squatting position
Difficulty climbing steps
Difficulty standing from sitting
Muscle cramps
Upper Extremity Weakness
Grip and pinch weakness
Elbow flexion and extension
Shoulder girdle weakness
Scapular winging
Neck muscle weakness
Other
Pulmonary
Pulmonary dysfunction (advanced cases)[30]
Heart
Cardiomyopathy[31]
Cardiac conduction abnormalities[32]
Sudden death[33]

Approximately 5 to 15 years after the onset of lower extremity symptoms, weakness of the upper extremities becomes apparent. Grip is affected first and atrophy of the dorsal interosseous is apparent on exam. As weakness progresses from distal to proximal, flexors and extensors are involved, and the muscles of the shoulder girdle are affected late in the disease. Scapular winging is seen in some patients. A significant loss of grip and upper extremity strength heralds the need for full-time care. Loss of neck flexors and extensor strength are late clinical manifestations. Muscles innervated by cranial nerves, cognition, sensation, and swallowing are generally unaffected.

The rate of progression is variable among patients, even within the same family. Usually, earlier presentation heralds a more rapidly progressive disease. The genetic factors that affect progression are under investigation, but environmental factors such as fractures, prolonged inactivity, or muscle overuse may affect disease progression.

Although initial studies suggested that there was no respiratory muscle involvement associated with GNE myopathy[11,28] recent publications suggest that respiratory muscle weakness is a manifestation of GNE myopathy, particularly in advanced stages of the disease.[29,30] A recent retrospective study of patients with advanced GNE myopathy found that 30 percent of patients had abnormal respiratory function tests (forced vital capacity [FVC] less than 80 percent); 92 percent of these patients were wheelchair-dependent. A subset of 4 patients (approximately 13 percent) had an FVC under 50 percent and had clinical manifestations of respiratory dysfunction.[30] Patients with GNE myopathy, particularly those in advanced stages, should be monitored for respiratory dysfunction.

Dilated cardiomyopathy has been described in association with GNE myopathy in 2 siblings harboring compound heterozygote mutations (p.F528C/p.A631V in hGNE1 nomenclature) of *GNE*. Patients presented with clinical manifestations of cardiomyopathy 20-26 years after disease onset.[31] A small percentage of patients also exhibit cardiac conduction abnormalities, and there are case reports of sudden death presumably due to fatal arrythmia in patients with GNE myopathy.[32–34]

Laboratory and Imaging Findings

No routine laboratory test suggests the presence of GNE myopathy. The serum creatine phosphokinase (CPK) levels in patients may be normal or elevated usually <1000 mcg/L depending on the stage of the disease.[35,36] Electromyograms (EMGs) may show mixed or myopathic features.[37,38] Nerve conduction studies are nonspecific or normal.[34] T1-weighted muscle magnetic resonance imaging (MRI) shows fatty-fibrous replacement of affected muscles and identifies the vastus lateralis as the last portion spared in the quadriceps femoris muscle (Figure 258-2).[25]

Diagnosis

Clinical Suspicion

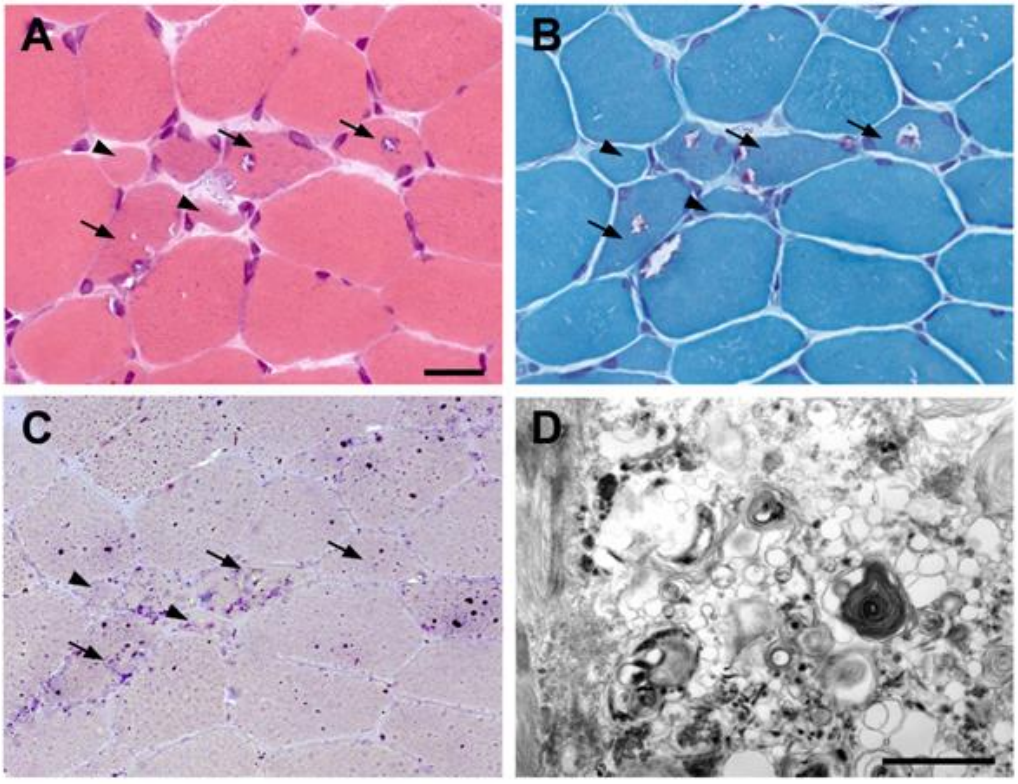
The diagnosis of GNE myopathy should be considered in any patient presenting in early adulthood with distal muscle weakness of the lower extremities. The diagnosis should not be delayed until the characteristic clinical finding, sparing of the quadriceps, becomes evident because that occurs late in the disease.

Currently, the diagnosis relies on muscle biopsy and genetic testing. As mentioned, CPK levels are variable and nerve conduction studies and electromyograms are unspecific and not helpful in obtaining a specific diagnosis.

Muscle Biopsy

In most muscle biopsies, myopathic changes are evident with variation in size in both type 1 and 2 muscle fibers and lack of inflammation or necrosis. Characteristic findings on the muscle biopsy include atrophic fibers, which are occasionally aggregated, forming small groups (Figure 258-3A), and the presence of rimmed vacuoles and inclusions. The characteristic inclusions may be missed on hematoxylin and eosin staining, but the use of modified Gomori trichrome staining facilitates the visualization of (red) rimmed vacuoles (RVs) (Figure 258-3B). The RVs in GNE myopathy muscle are thought to represent clusters of lysosomal/autophagic vacuoles, as acid phosphatase (a lysosomal enzyme) staining is increased within the fibers with RVs (Figure 258-3C). On electron microscopy, these correspond to clusters of autophagic vacuoles and multilamellar (myeloid) bodies (Figure 258-3D).[36,39] The rimmed vacuoles and inclusions do not run all throughout the muscle fiber, thus in some muscle sections these could be missed. The reliability of muscle biopsy for the diagnosis of GNE myopathy is variable. Depending on the muscle biopsied, abnormalities may be focal or absent.[22]

Figure 258-3



GNE myopathy muscle histology Representative images of stained sections of a biceps brachii muscle biopsy from a GNE myopathy patient. (**A**) to (**C**) are 8-μm serial cryosections. Hematoxylin and eosin (H&E) staining (**A**) shows moderate to marked variation in muscle fiber size, presence of atrophic fibers (arrowheads), and the presence of the characteristic fibers with rimmed vacuoles (RVs, arrows). These RVs are relatively more evident in modified Gomori

trichrome staining (**B**), appearing as vacuoles rimmed by red granules. The presence of intense staining of these affected fibers with acid phosphatase (a lysosomal enzyme) staining (**C**), which correlates to increased lysosomal staining, implies that these areas with RVs may be clusters of autophagic vacuoles. Electron microscopy findings (**D**) confirm that these cytoplasmic areas in the myofibers are indeed composed of autophagic vacuoles and multilamellar (myeloid) bodies, in addition to excessive cellular debris. Scale bar in (**A**) represents 50 μm in section images shown in (**A**), (**B**), and (**C**). Scale bar in (**D**) denotes 1 nm.

Genetic Testing

Identification of 2 allelic disease-causing mutations in the *GNE* gene confirms the diagnosis (Table 258-1). Once the diagnosis is made, genetic counseling by a certified genetic counselor should be provided to the patient and family. Genotype-phenotype correlations in patients with GNE myopathy are difficult to study because of the rare nature of the disease, the allelic heterogeneity, and the variability observed even within families. Studies suggest that patients with p.D176V (hGNE1 nomenclature) mutations in the epimerase domain may be asymptomatic and in the heterozygote state are associated with less severe disease.[11,36] This was also noted in a study of 71 individuals with GNE myopathy in which patients harboring homozygous p.V572L mutations in the kinase domain had a more severe phenotype than those with compound heterozygote mutations in the epimerase and kinase domains; p.D176V accounted for 68 percent of the epimerase alleles.[40] A patient who was a compound heterozygote for missense mutations in the epimerase and kinase domains (p.L179F/I587T; hGNE1 nomenclature) had a very early presentation and rapid progression.[41] Unaffected individuals with 2 disease-causing mutations have also been described.[11,28]

Diagnostic Delay

Major barriers to the diagnosis of GNE myopathy have been the rare nature of the disease and the lack of an inexpensive and noninvasive diagnostic test. The typical clinical characteristic, sparing of the quadriceps, is evident until late in the disease and is present in other myopathies.[37] Genetic testing is expensive and unlikely to be performed unless there is supportive diagnostic evidence. Together, the lack of sensitivity and specificity of muscle biopsy histology and the rare nature of the disease have contributed to misdiagnosis and a significant diagnostic delay in patients with GNE myopathy. Patients may be diagnosed with other conditions, such as distal myopathy, spinal muscular atrophy, limb-girdle muscular dystrophy,[22] Charcot-Marie-Tooth or autoimmune polymyositis.

The delay in diagnosis is unfortunate because it leads to anxiety and unnecessary testing. Novel blood-based diagnostic tests are needed to narrow the diagnostic gap in patients with GNE myopathy. Potential blood-based biomarkers are being explored.[42] ApoCIII isofocusing may be reduced or abnormal.[34]

Molecular and Cellular Basis

GNE Gene

The human *GNE* gene (RefSeqGene NG_008246) is located on chromosome 9p13.3 and consists of 13 exons. Alternative splicing events of mainly the N-terminal exons result in 8 human mRNA transcripts, translated in human GNE (hGNE) isoforms 1-8. hGNE1 is the ubiquitously expressed major isoform, while hGNE2-8 are differentially expressed and may act as tissue-specific regulators of sialylation.[43,44] Overall *GNE* transcription can be regulated by CpG promotor methylation[45] and likely contributes to tissue-specific adjustment of *GNE* expression. The 3′ untranslated regions (3′UTR) of h*GNE* mRNA transcripts are extremely long, consisting of more than 2000 base pairs, while other organisms have much shorter *GNE* 3′UTRs. Other organisms also appear to have fewer *GNE* mRNA transcripts; mice, for example, have only 2 transcripts, encoding mGne1 and mGne2, which have high homologies of 98.5 percent and 96.8 percent to hGNE1 and hGNE2, respectively.[44,46] These features imply that GNE is subject to evolutionary mechanisms to increase the number of cellular functions without increasing the number of genes.

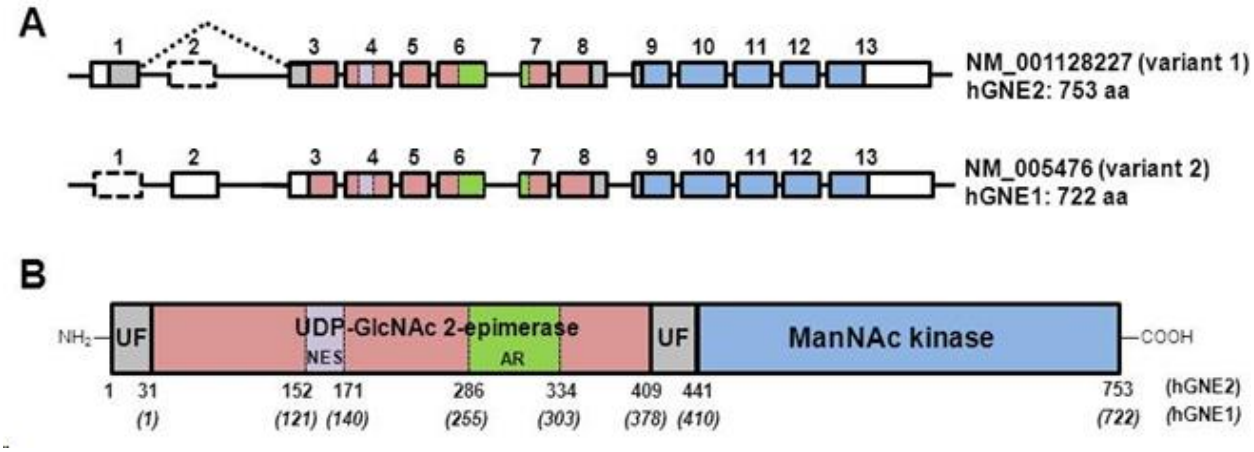
Expression of h*GNE* mRNA is highest in liver and placenta.[13,43] Liver is the major organ of sialic synthesis and has high GNE enzymatic activities, while high h*GNE* expression in placenta may be related to an essential role of sialic acid during development.[13] Skeletal muscle has low h*GNE* mRNA expression, which may help explain why skeletal muscle is affected in GNE myopathy patients.

GNE Protein

GNE is a highly conserved protein and localized mainly to the cytosol, where it functions in sialic acid synthesis (Figure 258-1). GNE protein expression was also detected in the nucleus and associated with Golgi membranes,[47] suggesting an additional GNE regulatory mechanism via differential subcellular localization.

The GNE protein consists of an N-terminal part responsible for the UDP-GlcNAc 2-epimerase activity and a C-terminal part covering the ManNAc kinase activity (Figure 258-4). A three-dimensional (crystal) structure of the complete bifunctional GNE enzyme is lacking. The ManNAc kinase domain of human GNE in its ligand-free state has been solved,[48] and comparisons of both the GNE epimerase and kinase domains to crystal structures of related (mostly prokaryotic) enzymes were made.[49,50] These sequence comparisons together with in vitro enzyme activity measurements specified the GNE epimerase domain to amino acids 1 to 378 (using the hGNE1 protein nomenclature), and the kinase domain covers at least amino acids 410 to 722 (hGNE1 nomenclature).[49,51,52] The exact allosteric site of GNE for CMP-sialic acid binding is not well defined, but clustering of sialuria mutations in codons 263 and 266[19,20] (hGNE1 nomenclature, see also Chapter 200) and experimental evidence of reduced feedback-inhibition by CMP-sialic acid with mutations of other codons between 255 and 303 suggest that this region is part of the allosteric site.[53] Furthermore, GNE forms oligomeric structures; a tetrameric GNE complex has both epimerase and kinase activity, while a GNE dimer only displays kinase activity. Intracellularly, a dynamic interplay between dimers and tetramers is suggested to occur, which can be influenced by different GNE ligands, including UDP-*N*-acetylglucosamine and CMP-sialic acid.[54]

Figure 258-4



GNE gene and protein structure

A. Exon (boxes)-intron (lines) structures of the 2 major human *GNE* mRNA transcripts, formed by alternative splicing of N-terminal exons. mRNA variant 1 (the longest splice form) encodes the hGNE2 protein, while mRNA variant 2 encodes the hGNE1 protein (traditionally known and studied as the sole translated GNE protein). GenBank accession numbers and predicted number of translated amino acids (aa) are indicated. Solid colored boxes represent the open reading frame (colors correspond with translated protein domains in B). White boxes represent untranslated regions (UTRs) and white dotted lined boxes the skipped exons of each transcript.

B. hGNE2 protein structure. Gray UF, unknown function; pink, UDP-GlcNAc 2-epimerase enzymatic activity encoding domain; purple, putative nuclear export signal (NES); green, experimental allosteric region (AR) for CMP-sialic acid binding, which results in feedback inhibition of epimerase enzymatic function; blue, ManNAc kinase enzymatic activity encoding domain. Amino acid numbering of both hGNE1 and hGNE2 protein isoforms are indicated below the structure.

The 8 different splice variants of the *GNE* mRNA altogether encode (at least theoretically) for 8 different protein isoforms. Whether these isoforms exist as a protein in vivo remains unknown, partly because functional antibodies and extensive protein expression studies of GNE have been lacking.[43,44,55] The originally described GNE protein is also called hGNE1 (illogically encoded by *GNE* mRNA transcript variant 2, NM_005476), which covers 722 amino acids with a calculated molecular mass of about 79 kDa. hGNE2 is an isoform with 31 additional amino acids at the N-terminus, which extends the N-terminal epimerase domain for about 3 kDa. hGNE2 is encoded by the longest *GNE* mRNA transcript (variant 1, NM_001128227). The discovery of the additional N-terminal sequence (and novel exon 1) encoding hGNE2 is potentially confusing, since all previous biochemical and mutation analysis studies (before 2011) refer to the nomenclature of the hGNE1 isoform, while according to universally adapted gene/protein nomenclature rules, the longest mRNA splice form ought to be used for annotating mutations and functional domains (http://www.hgvs.org/mutnomen/refseq.html). Hence, amino acid numbering of previously reported GNE studies, including patient mutation reports, should be supplemented with 31 amino acids to yield the current (hGNE2) nomenclature. To accommodate the adaption to this new terminology, we list both hGNE1 and hGNE2

nomenclatures in Figure 258-4 and Table 258-1. Human isoforms hGNE3-8 are encoded by shorter mRNA splice variants, and it is unclear whether these isoforms are expressed as proteins. If so, they likely lack either epimerase (hGNE3, 6,7,8) or kinase (hGNE4) enzymatic activities because of partial (splice) deletions within these respective domains.43

GNE Gene Mutations Associated with GNE Myopathy

Homozygosity mapping in several affected families of Persian Jewish and Kurdish-Iranian Jewish origin aided identification of *GNE* as the gene responsible for GNE myopathy.10 All patients of Middle Eastern origin have since been found to harbor a p.M712T (hGNE1 nomenclature) *GNE* founder mutation.10,56 Two *GNE* founder mutations are recognized within the Japanese population, p.D176V and p.V572L (hGNE1 nomenclature).11,57 At present, more than 100 other *GNE* mutations have been reported in patients worldwide (Table 258-1).

GNE myopathy-associated *GNE* mutations are predominantly missense (82 percent), and *GNE* null mutations have never been identified on both alleles in a patient; this would most likely be lethal as a *Gne* "knock-out" mouse model did not survive past the embryonic stage.58 The majority of *GNE* mutations are scattered throughout the UDP-GlcNAc 2-epimerase and ManNAc kinase coding domains. So far, there are no mutations reported in the recently discovered N-terminal 31 amino acids extension of hGNE2 isoform (UF, amino acids 1-31 in hGNE2, Figure 258-4). Three missense variants are reported to be located in the putative nuclear export signal (ep-NES, amino acids 121-140, hGNE1 nomenclature, Figure 258-4 and Table 258-1), which may play a role in nuclear localization of GNE.47 No GNE myopathy-associated mutations are reported in the allosteric site defined by human sialuria mutations (amino acids 263 and 266, hGNE1 nomenclature). However, 9 mutations are located in the "experimental" allosteric region (ep-AR, hGNE1 amino acids 255-303, Figure 258-4 and Table 258-1) and may need further research regarding their effect on allosteric feedback inhibition of CMP-sialic acid.

Secondary structure predictions are described for a large number of *GNE* mutations.49,50 UDP-GlcNAc 2-epimerase and ManNAc kinase enzymatic activities were also determined for selected GNE mutations both in cellular and in cell free systems.23,49,59–61 These enzymatic activities were reduced, but never absent, and mutations in one enzymatic domain affect not only that domain's enzyme activity, but also the activity of the other domain. In addition, compared with enzyme activities in a cell-free system, fibroblasts exhibited higher residual activities of both UDP-GlcNAc 2-epimerase and ManNAc kinase, suggesting the presence of additional sugar epimerases and kinases with overlapping substrate specificity.23

GNE Myopathy Disease Models

Several genetic strategies to manipulate the *Gne* gene have been pursued to generate mouse models for GNE myopathy. Simple knock-out mice presented with embryonic lethality by embryonic day 9.5, suggesting the importance of sialic acid in early embryogenesis.58 The *Gne*-deficient embryonic stem (ES) cells of this model showed affected polysialylation of the neural cell adhesion molecule (NCAM), which could be restored with supplementation of ManNAc to the growth medium.58 Further studies of the *Gne*-deficient ES-cell showed that Gne, apart from its role in sialic acid synthesis, may also play a role in cell proliferation, gene expression, and

cell differentiation.[62] Heterozygous *Gne*-deficient mice were found to be vital and did not show a significant phenotype, although their overall sialylation was reduced by 25 percent.[63]

A *Gne* myopathy knock-in mouse model was created through homologous recombination, introducing the Persian Jewish founder mutation, p.M712T (hGNE1 nomenclature), into the ManNAc kinase domain encoding region of the endogenous mouse *Gne* gene. This model showed a renal phenotype, including proteinuria, hematuria, effacement of the podocyte foot processes, and segmental splitting of the glomerular basement membrane so severe that most homozygous mice could not survive beyond 3 days after birth (P3).[64] Biochemical analysis of mutant mice kidneys revealed decreased UDP-GlcNAc 2-epimerase activity, deficient overall glomerular sialylation, and poor sialylation of the major podocyte proteins, podocalyxin and nephrin, suggesting that decreased renal sialic acid production led to lethality in these mice.[64,65] Oral supplementation of ManNAc to pregnant and nursing mothers resulted in increased survival of mutant pups beyond P3. Mutant survivors displayed improved kidney histology, increased overall sialylation as well as podocalyxin and nephrin sialylation, increased GNE protein expression, and UDP-GlcNAc 2-epimerase activities.[64,65] GNE myopathy patients, however, have no indications of renal abnormalities. The importance of sialic acid to the kidney as well as the need for sialic acid, at least during development, may differ between humans and mice, and protein glycosylation patterns also vary. It is known that O- and N-linked glycosylation patterns of podoxalyxin differ among species, including different types of sialic acids.[15,66] Mutant p.M712T knock-in pups did not live long enough to assess the development of a muscle phenotype. However, in mutant pups rescued from neonatal lethality by ManNAc administration, and not receiving further ManNAc after weaning (onward of age 3 weeks), hyposialylation of muscle tissue can be detected by lectin staining.[67] Further studies are pending regarding development of muscle pathology.

A third *Gne* myopathy mouse model is a transgenic model created through overexpressing the human *GNE* cDNA through a transgene with the p.D176V (hGNE1 nomenclature) epimerase domain mutation, common among Japanese patients, on a mouse background with a disrupted mouse *Gne* gene. This model created a scenario in which only mutated GNE proteins are highly expressed and the endogenous *Gne* gene was disrupted.[68] The transgenic mutant mice were born at almost Mendelian rate with normal appearance. As expected, blood and several organs including skeletal muscle exhibited hyposialylation. With age, these mice reproduced several myopathic phenotypes seen in muscles of human GNE myopathy patients. After 20 weeks of age, the GNE myopathy mice showed physiologic muscle weakness, seen as impaired motor performance of the mouse and reduced force generation of skeletal muscle.[69] This reduction of the force can be attributed to muscle atrophy, as specific twitch and tetanic forces per cross-section area are maintained at normal values. The reduction in gross size of skeletal muscle is accompanied by increase in the number of small angular fibers on muscle cross-sections. Serum creatine kinase is moderately elevated at this age. After 30 weeks of age, specific force generation in gastrocnemius and tibialis anterior muscles was notably reduced, while in muscle pathology, variation in muscle fiber size was more remarkable and intracellular deposition of amyloid and other various proteins was noted in the gastrocnemius muscle. After 40 weeks, the muscle force generation increasingly worsened, as reflected by increased twitch/tetanic ratio, which could likely be due to the appearance of the characteristic rimmed vacuole and accumulation of autophagic vacuoles[39] that can impair the contractile system of the muscle.

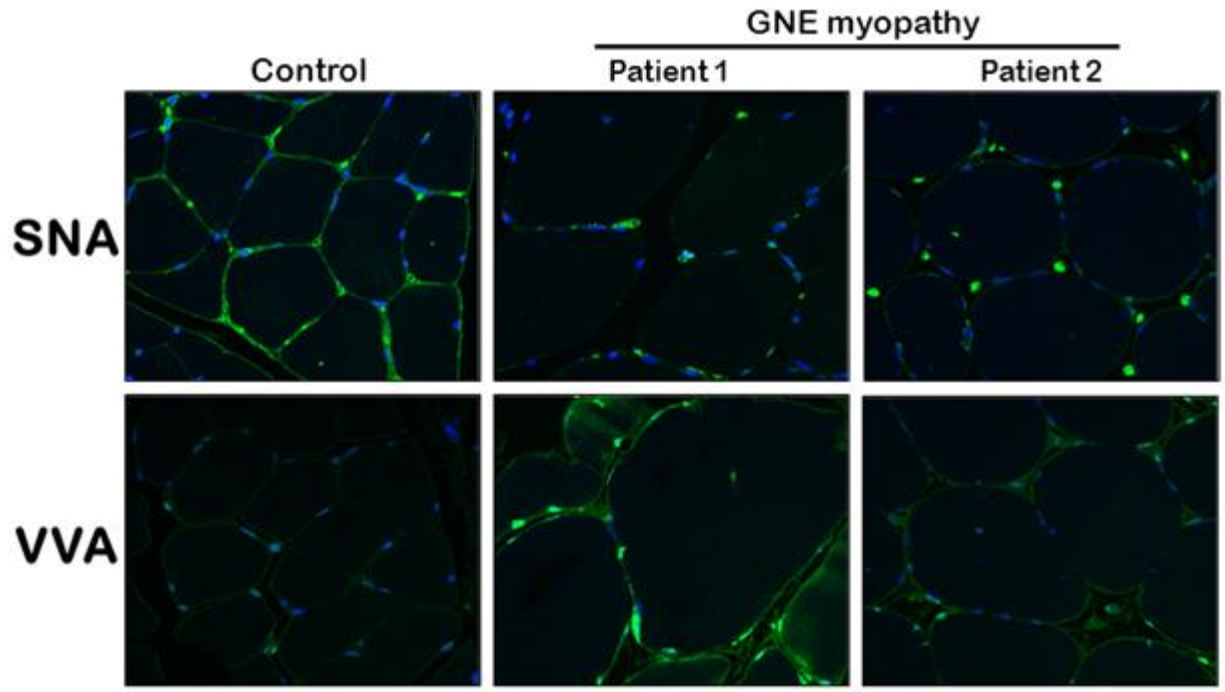
Importantly, oral prophylactic treatment (starting at week 10-20) of ManNAc, sialic acid or the sialic acid conjugate sialyllactose (containing approximately 45 percent of sialic acid) rescued the muscle phenotype in these transgenic mice. Compared to an untreated group, mutant mice in all treatment groups at 54-57 weeks of age showed higher survival rates, increased body weight and muscle mass, increased sialic acid levels in serum, muscle and other organs, decreased serum creatine kinase, better overall motor performance (treadmill and hanging wire test), and a marked improvement in pathology (decreased number of rimmed vacuoles and congophilic, amyloid-positive and tau-positive inclusions).[70] These results strongly support consideration of ManNAc, sialic acid, or sialic acid conjugates as a treatment for GNE myopathy. Future research may elucidate phenotypic differences between the transgenic and the knock-in *Gne* mouse models.

To our knowledge, no other multiple cell organisms are reported as models for GNE myopathy. Some cell lines with *GNE* mutations or decreased *GNE* expression have been used for in vitro studies. Lec3 Chinese hamster ovary (CHO) cell lines with *GNE* mutations showed loss of UDP-GlcNAc 2-epimerase enzymatic activity and had reduced/absent sialylation of glycans, including NCAM, which was rescued by exogenously added ManNAc or mannosamine.[71] Similar results were found in studies of cultured myotubes from GNE myopathy patients[59] or human lymphoid or hematopoietic cell lines with no detectable UDP-GlcNAc 2-epimerase activity.[17] Although ManNAc kinase activity was also severely decreased or absent in these mutant cells, it was demonstrated that ancillary kinases (eg, GlcNAc kinase) exist that can convert ManNAc to ManNAc-6-P and aid sialic acid synthesis.[72]

Cellular Findings and Pathologic Basis

The exact pathophysiology of GNE myopathy remains unknown, but mutated GNE suggests involvement of impaired sialylation. Several studies have shown that overall sialylation of tissues appears to be normal in GNE myopathy, but muscle tissue appears to be hyposialylated. For example, total sialic acid content in serum, cells, and nonmuscle tissues of GNE myopathy patients was not decreased,[59–61,73] but skeletal muscle tissue showed a significant decrease of overall sialylation.[59,74] Lectin staining studies of GNE myopathy muscle specimens suggest that membrane-associated O-linked glycans may be predominantly hyposialylated (Figure 258-5).[59,75] More evidence that hyposialylation is a key factor in the pathomechanism of GNE myopathy came from the transgenic mouse model, in which muscle atrophy and weakness could be prevented by treatment with sialic acid metabolites.[70]

Figure 258-5



Muscle lectin histochemistry Paraffin-embedded muscle sections from biceps (control and patient 1) and gastrocnemius (patient 2) were stained with 2 lectins (green). SNA (*Sambucus nigra* agglutinin), which predominantly binds terminal α(2,6)-linked Neu5Ac (sialic acid) on all glycans, and VVA (*Vicia villosa* agglutinin), which predominantly binds terminal GalNAc, without Neu5Ac attached, O-linked to serine or threonine residues of glycoproteins. Costaining was done with the nuclear dye DAPI (blue) on all slides. GNE myopathy muscle specimens show hyposialylation, demonstrated by decreased SNA and increased VVA staining, compared to control muscle. All images are 1D projections of confocal Z-stacks. All imaging was performed at the same microscope intensity settings per lectin (with a 63× objective). (Courtesy of Dr. Petcharat Leoyklang, NHGRI, NIH, Bethesda, MD)

Since GNE myopathy is an adult-onset disease, and patients have residual UDP-GlcNAc 2-epimerase and ManNAc kinase enzymatic activities, the effects of sialic acid deficiency may appear gradually. Some glycoconjugates, such as N-linked glycans, might be more readily sialylated than others, for example O-linked or polysialylatedglycans. Furthermore, a preference for the glycosidic linkage of sialic acids (α2,3, α2,6 and α2,8) is likely. It was suggested that when a shortage of sialic acid occurs, specific (muscle) proteins may be inadequately glycosylated/sialylated, contributing to the pathology of GNE myopathy, and specific muscle glycoproteins and glycolipids were found to be hyposialylated, including alpha-dystroglycan,[76] polysialic acid on neural crest adhesion molecule (PSA-NCAM),[37] neprilysin,[77] and the GM3 ganglioside.[78]

Apart from hyposialylation, other hypotheses exist for a role of mutated GNE in the pathology of GNE myopathy. These include the unusual subcellular distribution (nuclear versus cytoplasmic) of the GNE protein in cells,[47,79] existence of different GNE isoforms with tissue-specific expression,[43,55] involvement of mutated GNE in apoptotic pathways,[80] and mitochondrial

processes.[81,82] Other intriguing findings that may contribute to disease pathology are that GNE may control sialyltransferase expression, ganglioside production, and modulation of proliferation and apoptosis, independent of sialic acid production.[83] Different interactors of GNE may also contribute to disease pathology, including alpha-actinin-1 (an actin binding and crosslinking protein),[84] the transcription factor PLZF, the collapsin response mediator protein CRMP1 (involved in growth cone collapse and F-actin depolymerization), receptor interacting factor 1, and with KIAA 1549 (a protein of unknown function).[85]

Treatment

Understanding the biochemical and molecular basis of GNE myopathy allowed the development of therapeutic strategies for the disease based on (A) supplementation of precursors or end products of the sialic acid biosynthetic pathway (Figure 258-1) or (B) restoring UDP-GlcNAc 2-epimerase and ManNAc kinase enzymatic activities by gene or cell therapy approaches. Although no therapies are currently approved for GNE myopathy, studies on murine models and preliminary substrate supplementation or gene therapy applications in human subjects have provided promising data, and definitive therapeutic trials are currently underway.

Based on the hypothesis that, with supplementation of sialic acid, patients' hyposialylated muscle glycoproteins might regain structure or function or be protected from continuing damage, a pilot study was performed using intravenous immunoglobulin G (IVIG) as a source of exogenous sialic acid (http://clinicaltrials.gov/ identifier: NCT00195637).[86] IgG contains approximately 8 μmol of sialic acid per gram, providing approximately 1.8 mmol of sialic acid (Neu5Ac), which translates into roughly 5 days' worth of normal sialic acid production (0.3 mmol per 24 hours) for an average subject weighing 70 kg. Four GNE myopathy subjects were loaded with 1g/kg IVIG on 2 consecutive days followed by 3 doses of 400 mg/kg at weekly intervals. This study showed improvement in strength of different muscle groups both after loading and at the end of the study. Immunohistochemical staining of muscle biopsies for α-dystroglycan and NCAM did not show evidence of significantly increased sialylation after IVIG treatment, despite the notable subjective improvement reported by the patients. It is possible that changes in glycosylation require longer than symptomatic improvement, similar to what is described with mannose treatment in patients with congenital disorder of glycosylation type 1b.[87] However, the improvement after IVIG suggested that provision of sialic acid holds therapeutic promise and set precedence for the development of further strategies to restore sialylation in individuals with GNE myopathy.

Oral free sialic acid (Neu5Ac) is a negatively charged sugar and is almost totally absorbed by the gastrointestinal tract of rats.[88] It is mainly excreted in its free form via the kidneys (80 percent), while the remaining 20 percent is found in the feces. The negative charge of sialic acid makes uptake by cells in culture inefficient.[89] The first pass effect and inefficient uptake in cells may require administration of high concentrations of oral free sialic acid to obtain sufficient uptake. The sialic acid precursor, N-acetylmannosamine (ManNAc), on the other hand, is uncharged and crosses membranes more easily. In vitro assays using radioactive tracers demonstrated that the cellular uptake of ManNAc is a low-efficiency process, which is linear and nonsaturable up to 20mM and is not influenced by the concentrations of glucose, serum, amino acids, vitamins, or glutamine in the culture media.[90]

ManNAc is an uncharged physiological monosaccharide, and is the first committed precursor for sialic acid biosynthesis (Figure 258-1). ManNAc is formed fromUDP-GlcNAc by the action of UDP-GlcNAc 2-epimerase activity of GNE, hence, administration of exogenous ManNAc bypasses the rate-limiting GNE-epimerase step. ManNAc is then phosphorylated by ManNAc kinase activity of GNE. There is evidence that, besides ManNAc kinase, ancillary sugar kinases (including GlcNAc kinase) can contribute to this enzymatic step.[23,72] Another enzyme that can compete for externally supplemented ManNAc is GlcNAc 2-epimerase, which catalyzes the formation of GlcNAc from ManNAc.[91] However, GlcNAc 2-epimerase has a Km value for ManNAc in the mM range, while ManNAc kinase has a Km for ManNAc in the μM range. Hence, if GNE is present, ManNAc is phosphorylated by ManNAc kinase and is used solely for the biosynthesis of Neu5Ac (sialic acid). Residual ManNAc kinase activity in GNE myopathy patients,[23] or ancillary kinases such as GlcNAc kinase, and other nonspecific sugar kinases, might convert ManNAc into ManNAc-6P for subsequent synthesis of sialic acid.

Supplementation with ManNAc or Neu5Ac restored sialylation of primary human GNE myopathy patients' myotubes,[59] and ManNAc supplementation restored sialylation of *GNE*-deficient lymphoid (B lymphoma cell line BJA-B) or hematopoietic (HL-60 myeloid leukemia) cells,[17] as well as *Gne*-deficient murine embryonic stem cells.[58] Addition of unnatural ManNAc derivatives (ManLev, N-levulinoylmannosamine or ManNAz, N-azidoacetylmannosamine) to terminally differentiated human neurons resulted in the incorporation of the resulting sialic acid analogs, SiaLev (N-levulinoyl sialic acid) or SiaNAz (N-azidoacetyl sialic acid), into cell surface glycoconjugates.[92] Most importantly, preclinical studies in 2 different murine models of GNE myopathy provided the ultimate evidence for efficacy of sialic acid or precursor supplementation as a therapeutic approach for this disease: (1) oral ManNAc supplementation had a significant effect on survival from the neonatal onset lethal glomerulopathy and sialylation status of key glycoproteins in a *Gne* knock-in mouse model,[64] and (2) oral ManNAc, sialic acid, or sialyllactose supplementation resulted in amelioration of the myopathic phenotype observed in a GNE myopathy transgenic mouse model.[70] These studies served as a strong basis for the development of human clinical trials for this disease and phase 1 and 2 studies are currently ongoing (http://clinicaltrials.gov/ identifiers: NCT01634750 "Phase I Clinical Trial of ManNAc in Patients With GNE Myopathy or Hereditary Inclusion Body Myopathy [HIBM]"; NCT01236898 "Pharmacokinetic Study on N-acetylneuraminic Acid"; and NCT01517880 "A Phase 2 Study to Evaluate the Dose and Pharmacodynamic Efficacy of Sialic Acid-Extended Release [SA-ER] Tablets in Patients With GNE Myopathy or Hereditary Inclusion Body Myopathy").

In addition to substrate supplementation, gene and cell therapies are increasingly being explored for the treatment of GNE myopathy. Preliminary data have been obtained in compassionate investigational new drug trials on a patient with an advanced stage of the disease. Intramuscular[93] and systemic (intravenous)[74] administration of a cytomegalovirus (CMV) promotor-driven wild type human *GNE* cDNA (mRNA variant 2) vector complexed with a cationic liposome for delivery (*GNE*-lipoplex) resulted in significant muscle mRNA expression of the delivered *GNE*, increased sialylation of glycoproteins and stabilization of the decline in muscle strength, without any overt side effects. Cell-based therapy in the form of allogeneic bone marrow transplantation in the GNE myopathy transgenic murine model resulted in a marked improvement in lifespan and motor performance, associated with an increased sialylation of

muscle glycoproteins.[94] This study suggests that hematopoietic cells may provide a lasting supply of sialic acid, not subject to the challenging pharmacokinetic properties of the oral supplement. Lastly, a number of muscle-targeted cell therapy strategies are being explored for various myopathies,[95,96] which could be translated to GNE myopathy therapy if proven successful.

Until disease-specific treatments become available, conservative management should focus on preserving strength and optimizing mobility, while at the same time ensuring the safety of the patients. Working closely with a rehabilitation medicine team will facilitate the development of a patient-focused plan for physical therapy and the appropriate adaptive equipment, including ankle foot orthoses (AFOs) to provide support and maximize safety for the patients while walking. Lastly, regular screenings for respiratory difficulties, sleep apnea, cardiac conduction abnormalities, and cardiomyopathy should be part of the routine follow-up of patients with GNE myopathy to allow for the early detection and management of these disease complications.

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