発表者名	論文タイトル	発表誌	巻・号	ページ	出版年
Nonaka R, Iesaki T, de Vega S, Daida H, Okada T, Sasaki T, and Arikawa-Hirasawa E	Perlecan deficiency causes endothelial dysfunction by reducing the expression of endothelial nitric oxide synthase.	27;3. pii: e12272, 2015	27;3. pii:	e12272,	2015
Kerever A, Kamagata K, Yokosawa S, Otake Y, Ochi H, Yamada T, Hori M, Kamiya K, Nishikori A, Aoki S, Arikawa-Hirasawa E.	See-through Brains and Diffusion Tensor MRI Clarified Fiber Connections:	A Magnetic Resonance in Medical Sciences .	14	159-162	2015
Iwata S, Ito M, Nakata T, Noguchi Y, Okuno T, Ohkawara B, Masuda A, Goto T, Adachi M, Osaka H, Nonaka R, Arikawa-Hirasawa E, Ohno K	A missense mutation in domain III in HSPG2 in Schwartz-Jampel syndrome compromises secretion of perlecan into the extracellular space	Neuromuscul Disord.	8	00153-00154	2015

発表者名	論文タイトル	発表誌	巻・号	ページ	出版年
Aurelien Kerevera, Taihei Yamadaa, Yuji Suzukia, Frederic Mercierb, Eri Arikawa-Hirasawa	Fractone aging in the subventricular zone of the lateral ventricle	Journal of Chemical Neuroanatomy.	66-67	52-60	2015
Ning L, Xu Z, Furuya N, Nonaka R, Yamada Y, Arikawa-Hirasawa E.	Perlecan inhibits autophagy to maintain muscle homeostasis in mouse soleus muscle.	Matrix Biol.	Oct;48	26-35	2015
de Vega S, Hozumi K, Suzuki N, Nonaka R, Seo E, Takeda A, Ikeuchi, T Nomizu, M Yamada Y, Arikawa-Hirasawa E	Identification of Peptides Derived from the C-terminal Domain of Fibulin-7 Active for Endothelial Cell Adhesion and Tube Formation Disruption.	Peptide Science	In press		

発表者名	論文タイトル	発表誌	巻・号	ページ	出版年
Azuma Y, Nakata T, Tanaka M, Shen XM, Ito M, Iwata S, Okuno T, Nomura Y, Ando N, Ishigaki K, Ohkawara B, Masuda A, Natsume J, Kojima S, Sokabe M, Ohno K.	Congenital myasthenic syndrome in Japan: Ethnically unique mutations in muscle nicotinic acetylcholine receptor subunits	Neuromuscul Disord	25	60-69	2015
Masuda A, Takeda J, Okuno T, Okamoto T, Ohkawara B, Ito M, Ishigaki S, Sobue G, Ohno K.	Position-specific binding of FUS to nascent RNA regulates mRNA length	Genes Dev	29	1045-1057	2015
Selcen D, Ohkawara B, Shen XM, McEvoy K, Ohno K, Engel AG.	Impaired Synaptic Development, Maintenance, and Neuromuscular Transmission in LRP4-Related Myasthenia	JAMA Neurol	72	889-896	2015
Iwata S, Ito M, Nakata T, Noguchi Y, Okuno T, Ohkawara B, Masuda A, Goto T, Adachi M, Osaka H, Nonaka R, Arikawa-Hirasawa E, Ohno K.	A missense mutation in domain III in HSPG2 in Schwartz-Jampel syndrome compromises secretion of perlecan into the extracellular space	Neuromuscul Disord	25	667-671	2015

発表者名	論文タイトル	発表誌	巻・号	ページ	出版年
Rahman MA, Azuma Y, Nasrin F, Takeda J, Nazim M, Ahsan KB, Masuda A, Engel AG, Ohno K.	SRSF1 and hnRNP H antagonistically regulate splicing of COLQ exon 16 in a congenital myasthenic syndrome	Sci Rep	5	13208	2015
Otsuka K, Ito M, Ohkawara B, Masuda A, Kawakami Y, Sahashi K, Nishida H, Mabuchi N, Takano A, Engel AG, Ohno K.	Collagen Q and anti-MuSK autoantibody competitively suppress agrin/LRP4/MuSK signaling	Sci Rep	5	13928	2015
Ito M, Ohno K.	A hereditary mutation in Schwartz-Jampel syndrome	Atlas of Science AoS Nordic AB, Stockholm		http://atlasofscience.org/a-hereditary-mutation-in-schwartz-jampel-syndrome/ (査読有)	2015
Rahman MA, Ohno K.	Splicing aberrations in congenital myasthenic syndromes	J Investig Genomics Ed. by Lynn C Yeoman.		in press (査読有)	
Sugie K, Sugie M, Taoka T, Tonomura Y, Kumazawa A, Izumi T, Kichikawa K, Ueno S.	Characteristic MRI Findings of upper Limb Muscle Involvement in Myotonic Dystrophy Type 1.	PLoS One	10(4)	e0125051	2015

発表者名	論文タイトル	発表誌	巻・号	ページ	出版年
Sugie K, Kumazawa A, Ueno S.	Sporadic inclusion body myositis presenting with Beevor's sign	Intern Med	54(21)	2793-2794	2015
杉江和馬.	VIII.自己貪食空胞性ミオパチー:Danon 病. 骨格筋症候群(第 2 版)－その他の神経筋疾患を含めて－.(下)	別冊日本臨床新領域別症候群シリーズ	33	253-257	2015
杉江和馬.	VIII.自己貪食空胞性ミオパチー:過剰自己貪食を伴うX連鎖性ミオパチー. 骨格筋症候群(第 2 版)－その他の神経筋疾患を含めて－.(下)	別冊日本臨床新領域別症候群シリーズ	33	258-261	2015
Nishikawa A, Mori-Yoshimura M,Segawa K,Hayashi YK, et al	Respiratory and cardiac function in Japanese patients with dysferlinopathy.	Neuromuscul Disord	53(3)	394-401	2016
Iida S, Nakamura M, Wate R, Kaneko S, Kusaka H	Successful treatment of paroxysmal tonic spasms with topiramate in a patient with neuromyelitis optica.	Multiple sclerosis and related disorders	4(5)	457-459	2015
Oki M, Hori S, Asayama S, Wate R, Kaneko S, Kusaka H	Early-onset parkinson's disease associated with chromosome 22q11.2 deletion syndrome.	Internal medicine	55(3)	303-305	2016

発表者名	論文タイトル	発表誌	巻・号	ページ	出版年
WatanabeY, Suzuki S, Nishimura H, Murata K ら	Statins and myotoxic effects associated with anti-3-hydroxy-3-methyl glutaryl-coenzyme A reductase autoantibodies: an observational study in Japan	Medicine	94	e416	2015
Nakane S, Higuchi O, Koga M, Kanda T, Murata K	Clinical features of autoimmune autonomic ganglionopathy and the detection of subunit-specific autoantibodies to the ganglionic acetylcholine receptor in Japanese patients	PLos one	10(3)	e0118312	2015
Murata K, Maeba A, Yamanegi M, Nakanishi I, Ito H	Methotrexate myelopathy after intrathecal chemotherapy: a case report	Journal of Medical Case Reports	9	135	2015
Honjo Y, Ayaki T ら	Increased GADD34 in oligodendrocytes in Alzheimer's disease.	Neurosci Lett.	602	50-5	2015
村田顕也	封入体筋炎	免疫性神経疾患，日本臨床社		574-584	2015
村田顕也	増殖性筋炎	領域別症候群シリーズ，骨格筋症候群	上(第2版)	389-390	2015

発表者名	論文タイトル	発表誌	巻・号	ページ	出版年
村田顕也	巣状筋炎	領域別症候群シリーズ，骨格筋症候群	上（第2版）	391-393	2015
村田顕也	微小管障害性ミオパチー	領域別症候群シリーズ，骨格筋症候群	下	300-303	2015
Yamashita S., Mori A., et al.	Clinicopathological features of the first Asian family having vocal cord and pharyngeal weakness with distal myopathy due to a *MATR3* mutation.	Neuropathol. Appl. Neurobiol.	41	391-398	2015

Ⅴ．研究成果に関する刊行物

REVIEW

GNE myopathy: current update and future therapy

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ABSTRACT

GNE myopathy is an autosomal recessive muscle disease caused by biallelic mutations in *GNE*, a gene encoding for a single protein with key enzymatic activities, UDP-N-acetylglucosamine 2-epimerase and N-acetylmannosamine kinase, in sialic acid biosynthetic pathway. The diagnosis should be considered primarily in patients presenting with distal weakness (foot drop) in early adulthood (other onset symptoms are possible too). The disease slowly progresses to involve other lower and upper extremities' muscles, with marked sparing of the quadriceps. Characteristic findings on biopsies of affected muscles include 'rimmed' (autophagic) vacuoles, aggregation of various proteins and fibre size variation. The diagnosis is confirmed by sequencing of the *GNE* gene. Note that we use a new mutation nomenclature based on the longest transcript (GenBank: NM_001128227), which encodes a 31-amino acid longer protein than the originally described one (GenBank: NM_005476), which has been used previously in most papers. Based upon the pathophysiology of the disease, recent clinical trials as well as early gene therapy trials have evaluated the use of sialic acid or *N*-acetylmannosamine (a precursor of sialic acid) in patients with GNE myopathy. Now that therapies are under investigation, it is critical that a timely and accurate diagnosis is made in patients with GNE myopathy.

INTRODUCTION

GNE myopathy is a progressive muscle disease caused by mutations in the *GNE* gene, which encodes for a key enzyme in the sialic acid biosynthesis pathway (figure 1). In 2001, the gene defect associated with hereditary inclusion body myopathy (HIBM) was identified in Iranian Jews and other ethnicities.[1] Several mutations in the gene encoding sialic acid synthesis, called *GNE*, were identified. Soon afterwards, it became clear that distal myopathy with rimmed vacuoles (DMRV), first described in Japan by Nonaka and collegues,[2] is also caused by defects in the same gene. More than a decade afterwards, numerous patients with GNE defects were described worldwide. Other names such as inclusion body myopathy type 2 and quadriceps-sparing myopathy have been used to describe this disease. To avoid confusion, a group of international experts working in the field of GNE myopathy recently met and decided to unify the nomenclature to GNE myopathy (name of disease and its mutations).[3]

In the passing decade, much progress has been achieved in clarifying some biochemical, genetic and phenotypic variations of this myopathy, but enigmas still persist about its pathogenesis.[4] Importantly, formal therapeutic trials have been initiated in the past 2 years. This timely review of the current knowledge about this unique myopathy also contains information presented at the recent third meeting of the GNE Consortium (San Francisco, September 2013).

CLINICAL FEATURES

GNE myopathy is a relatively rare muscle disease with some typical clinical and pathological characteristics that may be very important for its correct identification. This is especially true in regions where the disease is probably less prevalent or under-recognised (see Demographics section). GNE myopathy is an adult onset muscle disorder with signs typically appearing in the third decade of life. However, onset at teenage has been reported, the earliest probably around 12 years of age. The commonest presentation is weakness of the distal muscle of the leg (foot drop), thus GNE myopathy is still classified in the group of distal myopathies. Less common presentations include asymmetric foot drop or manifestations initially appearing in upper extremities and in the proximal leg musculature. The disease does not remain limited to the distal musculature but slowly progresses to involve more proximal leg muscles and the upper limbs. A very unique feature of this myopathy is the relative or full sparing of the quadriceps, even in advanced stages of the disease. This pattern, when recognised in a patient, is probably diagnostic and can be visualised by muscle imaging, which will also help differential diagnosis and selecting the biopsy site (see Diagnosis section). However, the unique pattern of involvement becomes evident only after the proximal leg musculature becomes affected. It is of note that about 5%[5] of patients may have marked early quadriceps involvement making diagnosis more difficult. The pattern of muscle weakness in the upper limbs is more variable and can include scapular weakness (mimicking scapuloperoneal syndrome) or distal weakness of the hands with varying degrees of involvement. There are patients with onset in proximal leg muscles only mimicking an unusual pattern of limb girdle muscular dystrophy[6]; such onset may delay diagnosis, but in retrospect, clinical and imaging features show that the posterior thigh muscles become markedly affected while the quadriceps is spared.

Cardiac involvement is not a classical feature of GNE myopathy. However, some patients with histological or electrophysiological evidence for heart disease have been reported.[7] Although its association with GNE myopathy needs to be further defined, ECG may need to be performed every few years. Respiratory muscles are usually

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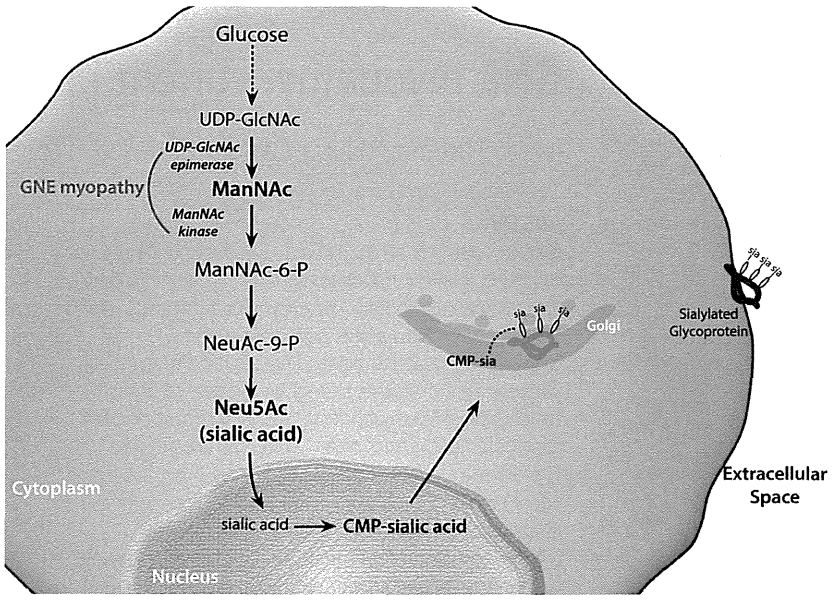

Figure 1 Sialic acid biosynthesis pathway. The biosynthesis of sialic acid (5-*N*-acetylneuraminic acid (Neu5Ac)) occurs in the cytoplasm. The initial substrate for this pathway (UDP-*N*-acetylglucosamine (GlcNAc)) is derived from glucose. In the rate-limiting step of the pathway, UDP-GlcNAc is epimerised into *N*-acetylmannosamine kinase (ManNAc) by GlcNAc 2-epimerase, encoded by the epimerase domain of *GNE*. ManNAc is phosphorylated by ManNAc kinase encoded by 'kinase' domain of *GNE*. Once Neu5Ac acid is synthesised, it becomes 'activated' by the effect of cytidine monophosphate (CMP)-sialic acid synthetase in the nucleus. CMP-sialic acid, the active form of Neu5Ac is used as a donor of sialic acid to nascent proteins in the golgi for the generation of glycoproteins. CMP-sialic acid also acts as a feedback inhibitor of the UDP-GlcNAc 2-epimerase enzyme by binding to its allosteric site.

not clinically affected during the course of the disease until the later stages when a proportion of wheelchair users have reduced respiratory function.[8] It is very rare to have a patient with a need of respiratory support even in the final stage of the disease, but this may occur.[9]

The course is slowly progressive with variable pace. In many patients, especially those of Persian Jewish ancestry, walking is still maintained (at least on flat ground) for 15–20 years (and even more) after the onset of the disease.[5] However, a study from a large cohort of patients in Japan noted an average 10 years until the need to use wheelchair. In this cohort, there was a suggestion that patients with a homozygous kinase mutation do better than those with a compound heterozygous mutation for such GNE mutation.[10] The progression of GNE myopathy and the contribution of genetic and environmental factors to its variability need to be further delineated.

PATHOLOGICAL FEATURES

Pathological features of GNE myopathy include 'rimmed' vacuoles, aggregation of various proteins and fibre size variation. 'Rimmed' vacuoles are recognised as small empty spaces surrounded by tiny red granules in the cytoplasm of muscle fibres typically on modified Gomori trichrome (mGT) staining. Although this empty space is called 'vacuole', this is a space artificially produced during staining procedures. The area was originally occupied mostly by red-coloured granules, but they become detached from the slide glass. On electron microscopy (EM), clusters of autophagic vacuoles are seen and each autophagic vacuole corresponds to a red-coloured granule on mGT.

Rimmed vacuoles are probably the most prominent finding on routine muscle histochemistry as protein aggregates are often hardly visualised without immunohistochemical staining. Aggregated proteins include β-amyloid, phosphorylated τ, TAR DNA-binding protein 43 kDa (TDP-43) and α-synuclein. β-Amyloid is supposed to be detected on Congo red stain but in reality often needs immunostaining for visualisation.

Most of the aggregated proteins are ubiquitinated and are believed to be targeted for autophagy clearance through p62-dependent aggresome formation, which is sometimes termed 'aggrephagy'.[11] However, these proteins cannot be digested; thereby autophagy buildup occurs, which is detected as rimmed vacuoles on histochemistry. Therefore, protein aggregation should be upstream in the pathological cascade that produces rimmed vacuoles. In support of this notion, aggregation of β-amyloid is observed prior to the development of rimmed vacuoles in GNE myopathy model mouse. On EM, autophagic vacuoles are often present next to the filamentous inclusions, also suggesting a close relationship between autophagy and protein aggregation. In the nucleus as well as the cytoplasm, tubulofilamentous inclusions 18–21 nm in diameter are observed. Of note, this protein aggregation-rimmed vacuole pathology is not an exclusively specific feature of GNE myopathy but is rather commonly seen in other hereditary and acquired myopathies, including sporadic inclusion body myositis (IBM).

Fibre size variation is mainly due to the presence of atrophied fibres, which are often angular in shape. For unknown reasons, atrophic fibres tend to cluster in GNE myopathy, sometimes giving a false impression of neurogenic atrophy. In the mouse model, muscle fibre atrophy starts earlier than protein aggregation and rimmed vacuole formation, indicating that, at least in part, the mechanism of muscle fibre atrophy is independent from that of aggrephagy-related degenerative pathway.

Although inflammatory change is usually not a feature of GNE myopathy, there are reports of rare cases with lymphocyte infiltration into the endomysium[5 12 13] that could potentially mislead to a diagnosis of sporadic IBM. Nevertheless, the pattern of muscle involvement and the age of disease onset are different. Of note, a recent study showed upregulation of proinflammatory cell stress response with overexpression of αB-crystallin and inducible nitric oxide synthase (iNOS), which seems to precede muscle degeneration with accumulation of β-amyloid, suggesting that inflammation may play a role in the early stages of the pathological cascade of GNE myopathy although cellular response is absent.[14]

Another pitfall is the selection of biopsy site. As mentioned earlier, one of the most characteristic clinical features is

quadriceps sparing. Therefore, biopsy of quadriceps muscle, which is one of the most frequently biopsied muscles, often gives a minimal or even completely normal histology. A significant number of cases may thus be undiagnosed or misdiagnosed because of quadriceps biopsy. When available, muscle imaging is highly recommended for choosing an appropriate biopsy site.

GENETIC CAUSE AND POSSIBLE MOLECULAR MECHANISM

GNE myopathy is an autosomal recessive disease caused by biallelic *GNE* gene mutations[1 2 15] (figure 2). Missense mutations account for the majority of alleles and no patient with biallelic null mutations has ever been found, suggesting that probably only 'mildly deleterious' mutations that are not associated with complete loss of GNE protein are necessary to cause this adult-onset myopathy. In fact, knocking out the *Gne* gene in mice results in embryonic lethality.[16] It is possible that in humans biallelic null mutations are either lethal too or associated with a different, currently unrecognised disorder.

In humans, at least six different *GNE* transcripts have been described.[3 17] The originally described transcript (GenBank: NM_005476; Ensembl: ENST00000377902; UCSC: uc010mlh.3) encodes 722 amino acids, while the longest transcript (GenBank: NM_001128227; Ensembl: ENST00000396594; UCSC: uc010mli.3) encodes 753 amino acids. Both transcripts are encoded in 12 exons and the difference between the two transcripts is in alternative first exons. NM_005476 has a non-coding first exon and initial codon starts in the 43rd nucleotide in the second exon. In contrast, the longer NM_001128227 uses a different, 17-amino acid coding exon 1. The second exon is the same as NM_005476 but the first 42 nucleotides before NM_005476's initial codon are also transcribed in NM_001128227, making the NM_001128227 transcript 31 amino acid longer than NM_005476. As this 31-amino acid coding sequence is added in the 5′ part of NM_005476, description of the mutation position will be changed depending on which transcript is used as the standard sequence. Since so far no pathogenic mutation has been found in NM_001128227 specific region, it is still unknown which transcript is crucial for causing GNE myopathy. We adopt the mutation nomenclature based on NM_001128227 throughout this manuscript, following the guidelines of the Human Genome Variation Society (http://www.hgvs.org). Furthermore, as the NM_001128227's first exon resides before the NM_005476's first exon, now the former is named exon 1 and the latter exon 2, and the remaining exons are labelled exons 3–13 (figure 2).

GNE encodes a single protein with two enzymatic activities in the biosynthetic pathway of 5-*N*-acetylneuraminic acid (Neu5Ac): UDP-*N*-acetylglucosamine 2-epimerase (GlcNAc 2-epimerase) and *N*-acetylmannosamine kinase (ManNAc) (figure 1). Sialic acids are monosaccharides and Neu5Ac is the most abundant sialic acid in mammals. Neu5Ac is usually present in the terminal portion of sugar chains in glycoproteins and glycolipids where they mediate several biological processes.[18]

Owing to recessive mutations in the *GNE* gene, sialic acid production is decreased and consequently, sialylation, that is, incorporation of sialic acid to glycoproteins and glycolipids, is also decreased.[19 20] Hyposialylation appears to be a major cause of this myopathy as administration of sialic acid or its precursor ManNAc prevents or arrests the development of disease in the mouse models of GNE myopathy.[21] This is the rationale behind current therapeutic trials (see below). However, the exact mechanism by which GNE defects lead to the human disease is still not fully understood and additional processes may contribute to it.

DIAGNOSIS

Currently, the diagnosis of GNE myopathy relies on identifying characteristic clinical manifestations and histopathological findings on muscle biopsy and is confirmed by the identification of biallelic *GNE* mutations.[22]

The diagnosis should be considered in patients presenting in young adulthood with foot drop, although the identification of the disease may be done at more advanced stages of the disease, when more proximal lower extremity or upper extremity muscles are affected. Clinically, the diagnosis may be confused with other conditions, such as other distal myopathies, limb girdle muscular dystrophy,[23] spinal muscular atrophy or Charcot-Marie-Tooth disease. The reliability of muscle biopsy for the diagnosis of GNE myopathy appears to depend on the technical skill and diagnostic expertise of those handling and evaluating the specimen (see above). GNE protein is present in the diseased muscle; thus, immunohistology may not identify the defect and furthermore no specific GNE antibody that could be used for diagnostics has yet been synthesised.

The use of muscle imaging can guide the choice of muscle for biopsy and can help establish disease severity. Muscle MRI of the affected muscles initially shows increased hyperintensity on T2 STIR sequences followed by fatty-fibrous replacement evident on T1-weighted images.[24]

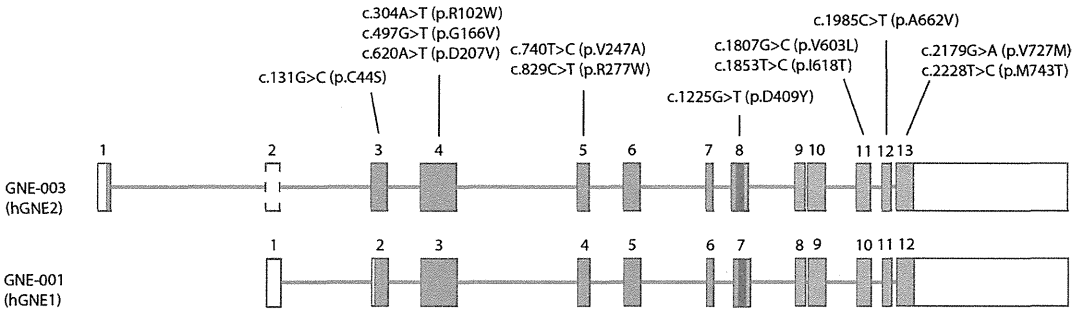

Figure 2 Schematic illustration of GNE gene structure. Gene structure for the two most representative transcripts is shown. The longest transcript (NM_001128227) encodes 753 amino acids, including 17 amino acid encoded by exon 1. The originally described transcript shown at the bottom (GenBank: NM_005476) uses an alternative first exon which is non-coding and the initial codon resides in the 43rd–45th nucleotides in the second exon, which makes the protein shorter by 31 amino acids. Note exon 8 encodes the last part of epimerase domain, junctional region and initial part of kinase domain. The size of exons is to scale but that of introns is not. Boxes indicate exons. Open box means non-coding region. Blue and pink, respectively, indicate epimerase and kinase encoding regions. Mutations mentioned in the text are included for reference.

The identification of biallelic mutations in *GNE* is the only definite diagnostic tool. As there are 147 known *GNE* mutations associated with GNE myopathy to date (based on HGMD Professional V.2013.4), sequencing of *GNE* is necessary when considering the diagnosis. In regions where one mutation is very prevalent (eg, p.M743T in the Middle East), testing for it may suffice. Patients with typical clinical and histological manifestations and only one heterozygous *GNE* mutation identified by sequencing have been encountered. Such patients may have deletions[25] not identified by sequencing or mutations in non-coding regions of *GNE* on the other allele. Alternatively, they may have a genetically different disorder. In such cases, next generation sequencing could be considered in the further diagnostic effort. Heterozygous carriers have no phenotype, although heterozygous mice have decreased sialylation.[19]

Owing to the rarity of this disease and the diagnostic difficulties aforementioned, patients may remain undiagnosed for a long period of time. In one cohort of patients followed at the National Institutes of Health (NIH), the diagnosis was delayed by an average of 10 years (NCC, unpublished).

DEMOGRAPHICS

GNE myopathy is a disorder found worldwide; however, until recently, it was mostly recognised in patients of Japanese and Persian Jewish ethnicity, where founder mutations are prevalent and different names, namely DMRV and HIBM, are used. However, after the identification of the genetic defect,[1] it is now clear that this is a worldwide disorder with an estimated prevalence of about 1/1 000 000 (higher prevalence is seen in Middle-Eastern Jews and Japanese; figure 3). In the past decade, there have been a plethora of reports from Europe, many Asian countries and North America. Interestingly, no patients were reported from South America, apart from two families of Persian Jewish ancestry residing in Argentina (ZA's personal observations). The lack of report from South America may be due to a decreased recognition of the condition.

Japan and Asian Oceanian region

Among all patients whose muscle biopsy was examined at the National Center of Neurology and Psychiatry (NCNP) in Tokyo between 1978 and 2005, 42 had GNE myopathy. During the same period of time, 502 had Duchenne muscular dystrophy (DMD), suggesting that the prevalence of GNE myopathy is roughly one log lower than that of DMD. In Japan, the prevalence of DMD ranges roughly from 1500 to 4000, indicating that 150–400 patients may be present in Japan. The cumulative number of Japanese patients who have been diagnosed to have biallelic GNE mutations at NCNP since 1978 is 237 at the time of writing. Although some patients may not be alive by now, it is of note that the estimated number of patients and actual number of genetically diagnosed patients are in a similar range.

Among all mutations identified, 95% are missense, as aforementioned. Three most frequent mutations are p.V603L, p.D207V and p.C44S, with allele frequency of 46.8%, 21.9% and 3.2%, respectively.[2 26 27] The p.V603L and p.C44S mutations were also identified in Korea and northern part of China, probably being compatible with a hypothesis of historical migration of people from the continent to Japan through Korean peninsula.[28 29]

In other parts of Asia, much fewer patients have been reported. Nevertheless, p.A662V and p.V727M seem to be common in the South-East Asian region: the former in Vietnam and Malaysia while the latter in Thailand and Malaysia, in addition to India.[29–31] The former has also been found in the USA and Australia. However, ethnically, they appear to originate from Vietnam.

Israel and Middle East

The largest cluster of GNE myopathy is that of Jews originating from Iran and neighbouring countries (Uzbekistan, Afghanistan, Iraq and Syria). They are all homozygous for the kinase mutation p.M743T, which is the commonest *GNE* mutation worldwide. About 150 such patients were identified in Israel over the years, and the estimated carrier frequency is 1 in 20 in this ethnic group.[1] A survey in the large Persian (Iranian) Jewish community residing in southern California suggested an even higher carrier rate of 1 in 11.[32]

Interestingly, the p.M743T mutation has been identified not only in Middle-Eastern Jews but also in Muslim Arabs in Israel (of Bedouin and Palestinian origins) who all (five families) carry it in a homozygous genotype. Furthermore, this homozygous mutation has been reported in Muslim patients from North

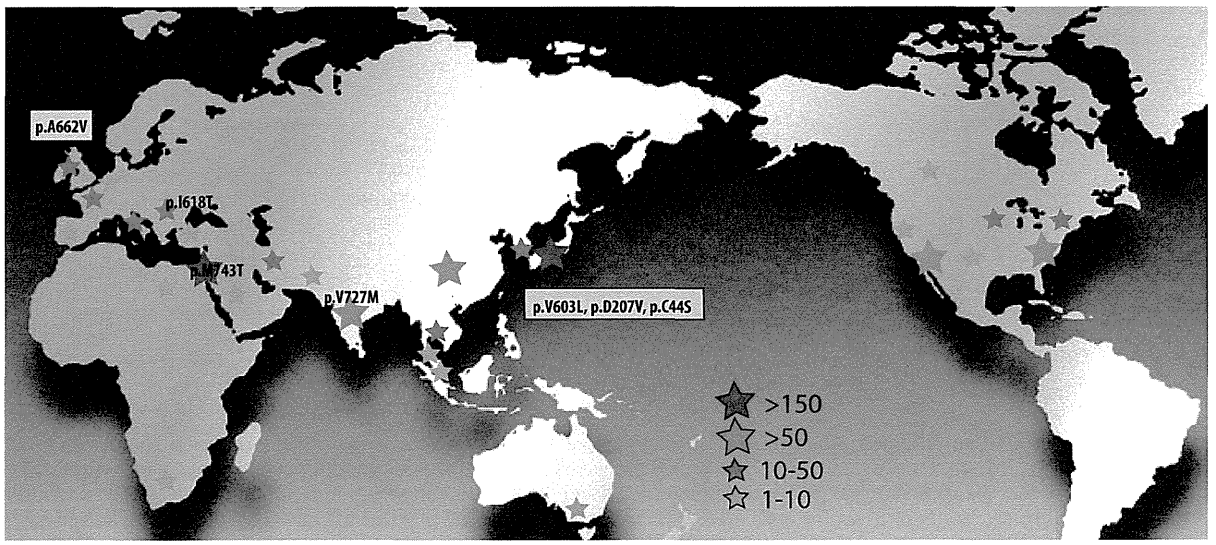

Figure 3 The worldwide prevalence of GNE myopathy is estimated at 1/1 000 000.

Africa (Egypt and Tunisia).[33] Thus, a regional founder mutation is strongly suggested and unpublished data suggest this mutation to be about 2500 years old. The origin of this high-frequency p.M743T GNE mutation in Persian Jews coming from various regions of Iran is unclear, as no data on general population testing in Iran are available. However, a cluster of patients with GNE myopathy due to p.M743T mutation were identified in a small town (Sangesar) in northern Iran. They all belonged to the Bahai religion (a relatively new religion originating in Persia during the 19th century), and a carrier rate of 1 in 25 was estimated.[34] It is unclear if this cluster is due to 'spread' of mutation from neighbouring Jewish residents.

Knowledge about this common mutation is important for easy diagnosis in patients originating from the Middle East residing outside this region. However, one should be cautious since although for more than a decade no patient with GNE myopathy having other mutations was identified in Israel, three families with different mutations were identified in 2013. One of those is a Jewish family from Mumbai, India. Both patients were homozygous to a mutation not reported in patients from other regions of India. This fact emphasises the need for pattern recognition of the clinical features of GNE myopathy in order not to delay correct diagnosis.

North America

Many patients in North America have been identified as having GNE myopathy, mostly in the USA and Canada. A significant portion of these patients are homozygous for the p.M743T mutation and are of Middle-Eastern background. The remainder is comprised mostly by patients who are compound heterozygotes for private mutations of *GNE*, reflecting the mixed ethnic background in the USA. Mutations in these patients have been traced to various ethnic backgrounds such as German p.V247A, p.D409Y and p.F559C; British p.G166V and p.R277W; Irish p.A662V and p.D409Y; Indian p.V727M and Cajun p.I618T.[1 35–37] Other mutations, such as p.R102W, have only been described in America.[37]

The only description of GNE myopathy in Hispanics is of a compound heterozygote patient (p.A555V/Y706H) whose ethnic background included Mexico.[38]

Europe

Since the identification of the causative gene, patients with novel *GNE* mutations were identified in numerous European countries (eg, Italy, Germany, the Netherlands, France and Belgium). However, because many European countries have large immigrant communities, including Asian, the recognition of the clinical pattern of GNE myopathy is critical for neuromuscular practice in this continent. Special attention should be given to mutations with possible founder effect. One such cluster was identified in Gypsies/Roma patients who are all homozygous for the kinase mutation p.I618T.[39] The mutation was not new when identified, however, at least 27 patients shared it. Two unusual features were mentioned: atrophy of thenar muscles and cardiac arrhythmias. Another region with relatively high GNE myopathy prevalence was recently identified in northern UK and Ireland. Point prevalence was estimated to be 0.19–0.44 in 100 000 for Scotland and northern Ireland. Two mutations were the most frequent: p.A662V, which is a mutation described in other regions of the world, and p.A409T, which seemed to be of northern British origin.[40]

NATURAL HISTORY AND PATIENT REGISTRY

Patient monitoring programme (Ultragenyx/TREAT-NMD)

The rate of progression of GNE myopathy has been variable over a few decades. There is a need for more accurate assessment of the clinical variability as well as identifying markers of progression that will optimise the design and interpretation of therapeutic trials. In addition, there is a need for patients' registry that will identify patients worldwide and serve as a source for patients' information. Such a programme was developed by TREAT-NMD and Ultragenyx (HIBM patient monitoring programme). There are two components of this programme: the first is patients' registry that will be open to all patients worldwide based on their willingness to add their data. This programme will combine the physician's reported information with the patient's personal report and will be conducted under the auspice of TREAD-NMD complying with Good Clinical Practice guidelines. This module has already been initiated (http://gnem-dmp.com/). The second part of this programme looking at the natural history of GNE myopathy will be conducted in several sites with large cohorts of patients. These will be different from the sites running therapeutic trials and will have larger distribution in Europe and North America. This second module of the programme is currently in progress as a sponsored clinical trial.

NIH study

In 2011, a longitudinal, prospective, single-centre natural history study of patients with GNE myopathy was initiated at the NIH (NIH study 11-HG-0218; ClinicalTrials.gov: NCT01417533). The objectives of the study are to delineate the natural history of GNE myopathy in a genetically diverse cohort by characterising the pattern and rate of progression of muscle weakness, its effect on patients' function and their quality of life and its correlation with genotype and environmental factors; to identify ideal outcome measures to be used in clinical trials and to discover blood biomarkers that would allow for diagnosis and monitoring of patients. Patients are evaluated every 6–12 months during an inpatient visit that lasts 3–4 days at the NIH Clinical Center. Evaluations include confirmation of *GNE* mutations, blood and urine laboratory tests, ECG, echocardiogram, pulmonary function tests, muscle MRI and measures of strength, function and quality of life.

Remudy (Japanese registry)

Remudy (Registry of Muscular Dystrophy) is a national patient registry for muscle diseases in Japan that was originally established for dystrophinopathy[41] (http://remudy.jp). GNE myopathy patient registration began in June 2012. By the end of 2013, 146 patients with GNE myopathy had been registered. Registered items include personal information, family history, diagnostic information and current clinical status. The registration form is filled and signed by patients themselves and their physicians. This registry will be harmonised with the international registry, which is run by TREAT-NMD and Ultragenyx as part of the patient monitoring programme (see above).

MOUSE MODEL AND THERAPEUTIC DEVELOPMENT

As mentioned, the *Gne* knock-out mouse model is embryonic lethal.[16]

The NIH-USA group established a mouse model by knocking-in the p.M743T mutation. However, most mice died with 72 h after birth due to renal disease and showed no myopathic phenotype; ManNAc administration rescued the neonatal

lethal phenotype in these mice.[42] Similar results were obtained in other laboratories.[43] Interestingly, the *Gne* M712T knock-in model developed by the Jerusalem group had a different phenotype. In some animals, no renal disease was observed and animals survived more than 1 year without any therapy.[43] Those that died at a later age did not show muscle abnormalities. The explanation for these variations in the model remains unclear but may be due to genetic background differences. A group in Kanazawa University in Japan developed *Gne* V603L knock-in model mouse. Their mice also showed a renal phenotype with shorter lifespan but without myopathy, which was rescued by the administration of NeuAc.[44]

The Tokyo group cross-mated heterozygote mice with a transgenic mouse model expressing human p.D207V mutant *GNE*, eventually obtaining mice overexpressing human mutant GNE protein and disrupting the production of their own *Gne*. This transgenic mouse model recapitulated the phenotype GNE myopathy clinically, pathologically and biochemically. Mice developed muscle atrophy and weakness after 20 weeks of age, β-amyloid after 30 weeks and rimmed vacuoles after 40 weeks while their sialic acid level was persistently low.[45] NeuAc, ManNAc and sialyllactose were administered presymptomatically to these mice and continued for 54–57 weeks, when all the clinicopathological features are supposed to have already developed. Treated mice showed improved survival, body weight, muscle pathology and muscle mass and strength comparable to that of their unaffected littermates.[21] Sialic acid content in muscle was increased but was still considerably lower than in littermates, indicating that even mild increase of muscle sialic acid level is efficacious at least in mice, and that we could expect even better efficacy if sialic acid level could be further increased. Overall, these results provided a proof-of-concept evidence supportive of initiating clinical trials in humans.

CLINICAL TRIALS

Metabolic supplementation with ManNAc, sialic acid and intravenous immunoglobulin (IVIG; as a source of sialic acid) has been evaluated (Table 1). It is not clear the extent to which metabolic supplementation can correct the defect or modify the course of the disease. Given the slow progression in GNE myopathy, significant changes in muscle strength may not be observed after a relatively short-term metabolic treatment. As muscle is replaced by fibrofatty tissue over time in GNE myopathy, stopping or slowing the progression of the disease is realistic, and can have a considerable impact in patients with this chronic debilitating myopathy.

IVIG trial (NIH)

In 2005, IVIG was used to investigate the effects of sialic acid (Neu5Ac) in four patients with GNE myopathy at the NIH (ClinicalTrials.gov: NCT00195637), since IgG contains 8 μmol of Neu5Ac/g. IVIG was infused as a loading dose of 1 g/kg on two consecutive days followed by three doses of 400 mg/kg at weekly intervals, providing a total of 1.8 mmol (0.55 g) of Neu5Ac for an average participant weighing 70 kg, that is, roughly 6 days worth of normal Neu5Ac production (0.3 mmol/24 h). IVIG administration improved objective measures of muscle strength (by 35% in the quadriceps and 46% in the shoulders), as well as function in patients with GNE myopathy.[46] Patients lost the benefit of IVIG and its sialic acid contribution about 2 weeks after stopping its administration. The clinical improvements were not accompanied by demonstrable histological changes or increased sialylation of target glycoproteins (using available methods at that time), possibly because such changes require longer term treatment or muscle regeneration. However, the finding of definitive improvements after IVIG treatment suggests that provision of sialic acid holds therapeutic promise.

NeuAc (Japan)

Phase 1 clinical trial was conducted at Tohoku University from November 2010 to June 2011 (ClinicalTrials.gov: NCT01236898). Three genetically confirmed patients were recruited and were given 800 mg of NeuAc three times a day up to five consecutive days. No significant adverse effects were observed.

SA-ER (Ultragenyx)

Since regular sialic acid is rapidly excreted after oral administration, a slow release product (sialic acid extended release (SA-ER)) was developed by Ultragenyx, a company involved in developing metabolic treatments for rare diseases. A trial of 47 recruited patients for oral supplementation using this investigational new drug was started in 2012 (ClinicalTrials.gov: NCT01517880). Baseline serum sialic acid levels were reduced in patients and this highly correlated to their performance in several muscle functional measurements.[47] The trial design was 24 weeks of double-blind administration of two doses of SA-ER at a dose of 3 or 6 g/day and a placebo-control group. This was followed by continued administration of either the high or the low dose for an additional 24 weeks. Results of the first phase of the trial gave a modest positive sign in the upper limb functional measurements, compared with a decline in the placebo group (unpublished data presented at the GNE myopathy Consortium meeting, September 2013). Patients with greater walking ability at baseline had a

Table 1 Clinical trials for the development of therapy in GNE myopathy

Clinical trial ID	Sponsor	Drug	Phase	Number of Patients	Status	Outcomes
NCT00195637	NHGRI	Immune globulin	1	4	Completed	
NCT01236898	Tohoku University	NeuAc	1	6	Completed	Safe, no ADE
UMIN000011532	Tohoku University	SA-ER tablet	1	9	Active	
NCT01359319	Ultragenyx Pharmaceutical Inc	SA-ER tablet	1	46	Completed	
NCT01517880	Ultragenyx Pharmaceutical Inc	SA-ER tablet	2	46	Completed	
NCT01830972	Ultragenyx Pharmaceutical Inc	SA-ER/SA-IR capsule	2	56	Active, not recruiting	
NCT01634750	TRND/NHGRI	ManNAc	1	22	Completed	Safe

ADE, adverse drug event; ManNAc, *N*-acetylmannosamine kinase; NeuAc, *N*-acetylneuraminic acid; NHGRI, National Human Genome Research Institute; SA-ER, sialic acid extended release; SA-IR, sialic acid immediate release; TRND, Therapeutics for Rare and Neglected Diseases.

better effect, suggesting that the degree of advancement of this myopathy may be a factor in the observed response. As expected, the serum sialic acid levels rose significantly. There were no serious side effects, and minimal adverse events were not dose related. Results of phase 2 are pending. All 46 of the continuing patients are now on an open-label, high-dose SA-ER for additional 48 weeks.

ManNAc (NIH)

ManNAc is a naturally occurring uncharged monosaccharide and is the first committed precursor for the biosynthesis of Neu5Ac and a substrate of the GNE enzyme. Oral administration of ManNAc in two independent GNE myopathy mouse models improved muscle pathology and hyposialylation.[21 42]

There is an anecdotal evidence of patients with GNE myopathy using ManNAc from a non-pharmaceutical source and without medical supervision in doses up to approximately 12 g/day and ranging from a period of 2 months to several years. The most common reported symptoms are gastrointestinal symptoms, such as abdominal cramps and diarrhoea.

A first-in-human phase 1a, randomised, placebo-controlled, double-blind, single-dose study (ClinicalTrials.gov NCT01634750; IND No.78 091) was conducted at the NIH in 2012–2013. The purpose of this study was to evaluate the safety, pharmacokinetics and pharmacodynamics of ManNAc in participants with GNE myopathy. A total of 22 participants were enrolled in three cohorts. Cohort A included six participants who were randomly assigned in a 2:1 ratio to receive ManNAc (n=4) or placebo (n=2) orally as a liquid solution. Cohorts B and C included eight participants randomly assigned in a 3:1 ratio to receive ManNAc (n=6) or placebo (n=2). The dose levels investigated were 3000, 6000 and 10 000 mg. ManNAc was safe and well tolerated in all participants who participated in this study.

A phase 1b escalating multiple-dose study and a phase 2 efficacy study of ManNAc in participants with GNE myopathy are being planned.

Liposomal systemic GNE delivery

A single patient with GNE myopathy due to two missense mutations (one in the kinase and one in the epimerase domains) was given seven intravenous injections of incremental doses of wild-type GNE over a period of 13 months.[48] The DNA vector was coupled to a human cytomegalovirus immediate early enhancer and promoter (CMV promoter) and delivered systemically in a liposomal package (lipoplex). The effect on muscle function was minimal, but the patient was in an advanced phase of the disease and much strength recovery could not be expected. However, 72 h after the highest dose, expression of wild-type *GNE* and increased sialylation in muscle could be demonstrated. This single-patient trial for compassionate use showed proof-of-principle for this delivery method, although it is expected that infusions will have to be intermittently repeated, as the delivered gene is not expected to persist in the cell cytoplasm.

Future therapeutic development

While metabolic supplementation as therapy for GNE myopathy seems promising, there are still other strategies including developments of: (1) better GNE metabolites or sialic acid compounds,[49] (2) drugs to block or modify degenerative process and (3) gene-based or cell-based therapy. These may be combined with supplementation therapy in the future. Approaches should be explored as they may better correct all deleterious effects of decreased *GNE* function, although safety and feasibility will need to be established. The GNE research laboratory in Jerusalem (under S Mitrani Rosenbaum) with collaboration of other laboratories is trying to develop an AAV-mediated gene vector for systemic administration of *GNE*. Initial results of this approach in animals are promising,[50] but the final proof-of-principle of this approach will be only when human trials are started.

CONCLUSIVE REMARK

Much progress towards understanding and treating GNE myopathy has been achieved, but the final target of developing an efficacious therapy is still underway. However, this is one of the first human hereditary myopathies where a logical metabolic therapy is currently being evaluated and a gene therapy is actively developed.

As clinical trials for potential therapies for GNE myopathy are underway, it is necessary to provide a timely diagnosis for patients with GNE myopathy. An early diagnosis has the potential of maximising the effect of such therapies and reducing anxiety and unnecessary testing in these patients.

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REFERENCES

1 Eisenberg I, Avidan N, Potikha T, *et al*. The UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase gene is mutated in recessive hereditary inclusion body myopathy. *Nat Genet* 2001;29:83–7.
2 Nishino I, Noguchi S, Murayama K, *et al*. Distal myopathy with rimmed vacuoles is allelic to hereditary inclusion body myopathy. *Neurology* 2002;59:1689–93.
3 Huizing M, Carrillo-Carrasco N, Malicdan MC, *et al*. GNE myopathy: new name and new mutation nomenclature. *Neuromuscul Disord* 2014;24:387–9.
4 Argov Z, Mitrani-Rosenbaum S. The hereditary inclusion body myopathy enigma and its future therapy. *Neurotherapeutics* 2008;5:633–7.
5 Argov Z, Eisenberg I, Grabov-Nardini G, *et al*. Hereditary inclusion body myopathy: the Middle Eastern genetic cluster. *Neurology* 2003;60:1519–23.
6 Park YE, Kim HS, Choi ES, *et al*. Limb-girdle phenotype is frequent in patients with myopathy associated with GNE mutations. *J Neurol Sci* 2012;321:77–81.
7 Chai Y, Bertorini TE, McGrew FA. Hereditary inclusion-body myopathy associated with cardiomyopathy: report of two siblings. *Muscle Nerve* 2011;43:133–6.
8 Mori-Yoshimura M, Oya Y, Hayashi YK, *et al*. Respiratory dysfunction in patients severely affected by GNE myopathy (distal myopathy with rimmed vacuoles). *Neuromuscul Disord* 2013;23:84–8.
9 Weihl CC, Miller SE, Zaidman CM, *et al*. Novel GNE mutations in two phenotypically distinct HIBM2 patients. *Neuromuscul Disord* 2011;21:102–5.
10 Mori-Yoshimura M, Monma K, Suzuki N, *et al*. Heterozygous UDP-GlcNAc 2-epimerase and N-acetylmannosamine kinase domain mutations in the GNE gene result in a less severe GNE myopathy phenotype compared to homozygous N-acetylmannosamine kinase domain mutations. *J Neurol Sci* 2012;318:100–5.
11 Lamark T, Johansen T. Aggrephagy: selective disposal of protein aggregates by macroautophagy. *Int J Cell Biol* 2012;2012:736905.
12 Krause S, Schlotter-Weigel B, Walter MC, *et al*. A novel homozygous missense mutation in the GNE gene of a patient with quadriceps-sparing hereditary inclusion body myopathy associated with muscle inflammation. *Neuromuscul Disord* 2003;13:830–4.
13 Kannan MA, Challa S, Urtizberea AJ, *et al*. Distal myopathy with rimmed vacuoles and inflammation: a genetically proven case. *Neurol India* 2012;60:631–4.
14 Fischer C, Kleinschnitz K, Wrede A, *et al*. Cell stress molecules in the skeletal muscle of GNE myopathy. *BMC Neurol* 2013;13:24.
15 Mitrani-Rosenbaum S, Yakovlev L, Becker Cohen M, *et al*. Sustained expression and safety of human GNE in normal mice after gene transfer based on AAV8 systemic delivery. *Neuromuscul Disord* 2012;22:1015–24.

16 Schwarzkopf M, Knobeloch KP, Rohde E, *et al*. Sialylation is essential for early development in mice. *Proc Natl Acad Sci USA* 2002;99:5267–70.
17 Yardeni T, Choekyi T, Jacobs K, *et al*. Identification, tissue distribution, and molecular modeling of novel human isoforms of the key enzyme in sialic acid synthesis, UDP-GlcNAc 2-epimerase/ManNAc kinase. *Biochemistry* 2011;50:8914–25.
18 Schauer R. Sialic acids as regulators of molecular and cellular interactions. *Curr Opin Struct Biol* 2009;19:507–14.
19 Gagiannis D, Orthmann A, Danssmann I, *et al*. Reduced sialylation status in UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (GNE)-deficient mice. *Glycoconj J* 2007;24:125–30.
20 Salama I, Hinderlich S, Shlomai Z, *et al*. No overall hyposialylation in hereditary inclusion body myopathy myoblasts carrying the homozygous M712T GNE mutation. *Biochem Biophys Res Commun* 2005;328:221–6.
21 Malicdan MC, Noguchi S, Hayashi YK, *et al*. Prophylactic treatment with sialic acid metabolites precludes the development of the myopathic phenotype in the DMRV-hIBM mouse model. *Nat Med* 2009;15:690–5.
22 Huizing M, Krasnewich DM. Hereditary inclusion body myopathy: a decade of progress. *Biochim Biophys Acta* 2009;1792:881–7.
23 Boyden SE, Duncan AR, Estrella EA, *et al*. Molecular diagnosis of hereditary inclusion body myopathy by linkage analysis and identification of a novel splice site mutation in GNE. *BMC Med Genet* 2011;12:87.
24 Tasca G, Ricci E, Monforte M, *et al*. Muscle imaging findings in GNE myopathy. *J Neurol* 2012;259:1358–65.
25 Del Bo R, Baron P, Prelle A, *et al*. Novel missense mutation and large deletion of GNE gene in autosomal-recessive inclusion-body myopathy. *Muscle Nerve* 2003;28:113–17.
26 Tomimitsu H, Shimizu J, Ishikawa K, *et al*. Distal myopathy with rimmed vacuoles (DMRV): new GNE mutations and splice variant. *Neurology* 2004;62:1607–10.
27 Cho A, Hayashi YK, Monma K, *et al*. Mutation profile of the GNE gene in Japanese patients with distal myopathy with rimmed vacuoles (GNE myopathy). *J Neurol Neurosurg Psychiatry* 2014;85:914–7.
28 Kim BJ, Ki CS, Kim JW, *et al*. Mutation analysis of the GNE gene in Korean patients with distal myopathy with rimmed vacuoles. *J Hum Genet* 2006;51:137–40.
29 Lu X, Pu C, Huang X, *et al*. Distal myopathy with rimmed vacuoles: clinical and muscle morphological characteristics and spectrum of GNE gene mutations in 53 Chinese patients. *Neurol Res* 2011;33:1025–31.
30 Liewluck T, Pho-Iam T, Limwongse C, *et al*. Mutation analysis of the GNE gene in distal myopathy with rimmed vacuoles (DMRV) patients in Thailand. *Muscle Nerve* 2006;34:775–8.
31 Nalini A, Gayathri N, Nishino I, *et al*. GNE myopathy in India. *Neurol India* 2013;61:371–4.
32 Kaback M, Lopatequi J, Portuges AR, *et al*. Genetic screening in the Persian Jewish community: a pilot study. *Genet Med* 2010;12:628–33.
33 Amouri R, Driss A, Murayama K, *et al*. Allelic heterogeneity of GNE gene mutation in two Tunisian families with autosomal recessive inclusion body myopathy. *Neuromuscul Disord* 2005;15:361–3.
34 Khademian H, Mehravar E, Urtizberea J, *et al*. Prevalence of GNE p.M712T and hereditary inclusion body myopathy (HIBM) in Sangesar population of Northern Iran. *Clin Genet* 2013;84:589–92.
35 Eisenberg I, Grabov-Nardini G, Hochner H, *et al*. Mutations spectrum of GNE in hereditary inclusion body myopathy sparing the quadriceps. *Hum Mutat* 2003;21:99.
36 Vasconcelos OM, Raju R, Dalakas MC. GNE mutations in an American family with quadriceps-sparing IBM and lack of mutations in s-IBM. *Neurology* 2002;59:1776–9.
37 Saechao C, Valles-Ayoub Y, Esfandiarifard S, *et al*. Novel GNE mutations in hereditary inclusion body myopathy patients of non-Middle Eastern descent. *Genet Test Mol Biomarkers* 2010;14:157–62.
38 Darvish D, Vahedifar P, Huo Y. Four novel mutations associated with autosomal recessive inclusion body myopathy (MIM: 600737). *Mol Genet Metab* 2002;77:252–6.
39 Kalaydjieva L, Lochmuller H, Tournev I, *et al*. 125th ENMC International Workshop: neuromuscular disorders in the Roma (Gypsy) population, 23–25 April 2004, Naarden, The Netherlands. *Neuromuscul Disord* 2005;15:65–71.
40 Chaouch A, Brennan KM, Hudson J, *et al*. Two recurrent mutations are associated with GNE myopathy in the North of Britain. *J Neurol Neurosurg Psychiatry* 2014;85:1359–65.
41 Nakamura H, Kimura E, Mori-Yoshimura M, *et al*. Characteristics of Japanese Duchenne and Becker muscular dystrophy patients in a novel Japanese national registry of muscular dystrophy (Remudy). *Orphanet J Rare Dis* 2013;8:60.
42 Galeano B, Klootwijk R, Manoli I, *et al*. Mutation in the key enzyme of sialic acid biosynthesis causes severe glomerular proteinuria and is rescued by N-acetylmannosamine. *J Clin Invest* 2007;117:1585–94.
43 Sela I, Yakovlev L, Becker Cohen M, *et al*. Variable phenotypes of knockin mice carrying the M712T Gne mutation. *Neuromolecular Med* 2013;15:180–91.
44 Ito M, Sugihara K, Asaka T, *et al*. Glycoprotein hyposialylation gives rise to a nephrotic-like syndrome that is prevented by sialic acid administration in GNE V572L point-mutant mice. *PLoS ONE* 2012;7:e29873.
45 Malicdan MC, Noguchi S, Hayashi YK, *et al*. Muscle weakness correlates with muscle atrophy and precedes the development of inclusion body or rimmed vacuoles in the mouse model of DMRV/hIBM. *Physiol Genomics* 2008;35:106–15.
46 Sparks S, Rakocevic G, Joe G, *et al*. Intravenous immune globulin in hereditary inclusion body myopathy: a pilot study. *BMC Neurol* 2007;7:3.
47 Mayhew JE, Skrinar AM, Bronstein F, *et al*. *Characterization of strength and function in adults with inclusion body myopathy (HIBM)/GNE myopathy. 18th International Congress of The World Muscle Society*. Asilomar, CA: Neuromuscular Disorders, 2013:755.
48 Nemunaitis G, Jay CM, Maples PB, *et al*. Hereditary inclusion body myopathy: single patient response to intravenous dosing of GNE gene lipoplex. *Hum Gene Ther* 2011;22:1331–41.
49 Malicdan MC, Noguchi S, Tokutomi T, *et al*. Peracetylated N-acetylmannosamine, a synthetic sugar molecule, efficiently rescues muscle phenotype and biochemical defects in mouse model of sialic acid-deficient myopathy. *J Biol Chem* 2012;287:2689–705.
50 Tal-Goldberg T, Lorain S, Mitrani-Rosenbaum S. Correction of the Middle Eastern M712T mutation causing GNE myopathy by trans-splicing. *Neuromolecular Med* 2013;16:322–31.

GNE myopathy: current update and future therapy

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Hepatitis C virus infection in inclusion body myositis

A case-control study

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ABSTRACT

Objective: To clarify whether there is any association between inclusion body myositis (IBM) and hepatitis C virus (HCV) infection.

Methods: We assessed the prevalence of HCV infection in 114 patients with IBM whose muscle biopsies were analyzed pathologically for diagnostic purpose from 2002 to 2012 and in 44 age-matched patients with polymyositis diagnosed in the same period as a control by administering a questionnaire survey to the physicians in charge. We also compared clinicopathologic features including the duration from onset to development of representative symptoms of IBM and the extent of representative pathologic changes between patients with IBM with and without HCV infection.

Results: A significantly higher number of patients with IBM (28%) had anti-HCV antibodies as compared with patients with polymyositis (4.5%; odds ratio 8.2, 95% confidence interval 1.9–36) and the general Japanese population in their 60s (3.4%). Furthermore, between patients with IBM with and without HCV infection, we did not find any significant difference in the clinicopathologic features, indicating that the 2 groups have essentially the same disease regardless of HCV infection.

Conclusion: Our results provide the statistical evidence for an association between IBM and HCV infection, suggesting a possible pathomechanistic link between the 2 conditions. ***Neurology*®** **2016;86:211–217**

GLOSSARY

Ab = antibodies; **CI** = confidence interval; **HBV** = hepatitis B virus; **HCV** = hepatitis C virus; **HTLV-1** = human T-lymphotropic virus type 1; **IBM** = inclusion body myositis; **IFN** = interferon; **MHC** = major histocompatibility complex; **NCNP** = National Center of Neurology and Psychiatry; **OR** = odds ratio; **PM** = polymyositis.

Inclusion body myositis (IBM) is the most common form of idiopathic inflammatory myopathy among individuals aged over 50 years, and its pathomechanism is not fully understood.[1] While the influence of genetic backgrounds on disease susceptibility and clinical phenotype has been suggested,[2] environmental factors might be involved with the pathogenesis as well. For instance, our recent study has shown that the number of patients with IBM is increasing in Japan, suggesting that westernization of dietary habits may influence the occurrence of the disease.[3] To elucidate such environmental factors that can trigger IBM pathogenesis would lead to better understanding of the enigmatic pathomechanism.

Chronic infection with hepatitis C virus (HCV) in patients with IBM has been reported in several articles,[4–9] suggesting a possible association of HCV infection with IBM. Nevertheless, no statistical evidence for the association has been provided and it is not known whether any etiologic link exists between the 2 conditions or it is a coincidence. We therefore conducted a retrospective case-control study to clarify whether HCV infection is associated with IBM.

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Supplemental data at Neurology.org

METHODS Epidemiologic analyses. The National Center of Neurology and Psychiatry (NCNP) functions as a referral center for muscle pathology and collects muscle biopsy samples from all over Japan. We re-evaluated muscle biopsy samples collected at NCNP

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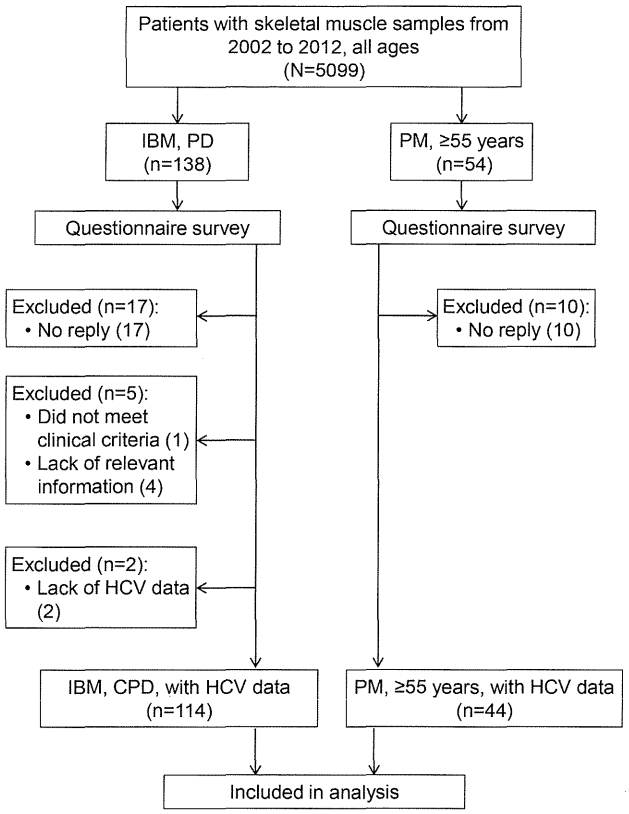
Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Figure 1 Study flow diagram



CPD = clinicopathologically defined; HCV = hepatitis C virus; IBM = inclusion body myositis; PD = pathologically defined; PM = polymyositis.

over 10 years from May 2002 to April 2012 and found 138 cases fulfilling pathologic criteria to define clinicopathologic IBM from the 2011 European Neuromuscular Centre (ENMC) IBM Research Diagnostic Criteria: (1) endomysial inflammatory infiltrate, (2) rimmed vacuoles (RV), and (3) aberrant protein accumulation (p62 or congophilic deposits) in muscle fibers.[10] We sent a questionnaire to the physicians in charge of the patients (appendix e-1 on the *Neurology*® Web site at Neurology.org) inquiring into (1) serum anti-HCV antibodies (Ab), hepatitis B virus (HBV) antigens (if any, the types of the antigens), anti-HIV Ab, and anti–human T-lymphotropic virus type 1 (HTLV-1) Ab; (2) distribution of affected muscles; (3) age at onset; and (4) duration from onset to the development of representative symptoms of IBM: (1) incapability of opening a plastic bottle, which reflects finger flexion weakness; (2) incapability of standing up from a squatting position, which reflects quadriceps femoris weakness; and (3) loss of ambulation. As a disease control, we also investigated 54 consecutive age-matched (>55 years old at muscle biopsy) patients with polymyositis (PM) whose muscle pathology was evaluated at NCNP in the same period and diagnosed as definite PM using the diagnostic criteria of Dalakas and Hohlfeld.[11]

We received replies from physicians of 121 patients with IBM and 44 patients with PM. Among the patients with IBM, the following 7 patients were excluded from further study: (1) one patient whose age at onset was 42 years, not fulfilling the ENMC criteria; (2) 4 patients whose relevant clinical information was lacking and thus we could not judge whether they met the clinical criteria; and (3) 2 patients who did not receive a test for anti-HCV Ab. Consequently, we analyzed 114 patients with IBM who met criteria for clinicopathologically defined IBM in the ENMC criteria and 44 age-matched patients with PM (mean ages at muscle biopsy were 69.0 ± 7.9 years and 69.0 ± 7.5 years, respectively) (figure 1).

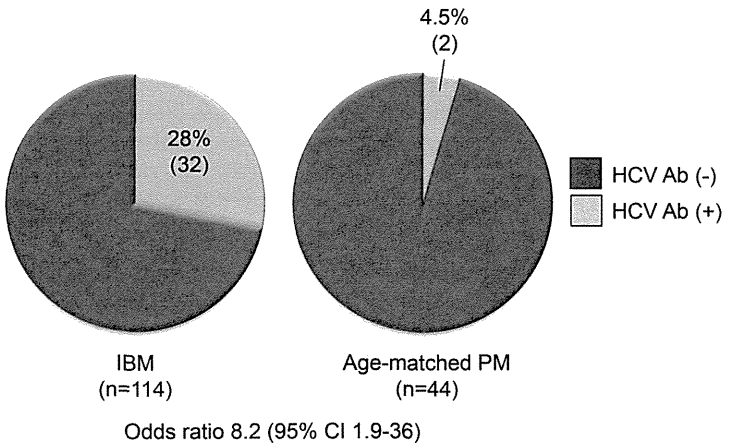
Pathologic analyses. Frequencies of fibers with RV, which is known as a pathologic hallmark of IBM, were assessed in more than 300 fibers on frozen muscle sections stained for modified Gomori trichrome. Major histocompatibility complex (MHC) class 1 and 2 expressions in muscle fibers are often seen in idiopathic inflammatory myopathies including IBM.[12] We performed immunohistochemical analyses for class 1 and 2 using 6-μm-thick frozen sections and monoclonal Ab (HLA-ABC, W6/32, 1:5,000 dilution, Thermo Fisher Scientific, Waltham, MA; and HLA-DR, B308, 1:5,000, Affinity BioReagents, Golden, CO) with the Ventana immunohistochemistry detection system (Ventana Medical Systems, Tucson, AZ). We assessed proportions of cases with a particular staining pattern of MHC 1 and 2: expression of MHC class 1 and 2 was graded as follows: − meant no positively stained fibers, and 1+, 2+, 3+, and 4+ were defined according to frequency of positively stained fibers, corresponding to 1%–10%, 11%–25%, 26%–50%, and 51%–100%, respectively. We judged as positive only when the cytoplasm of non-necrotic fibers was diffusely stained and negative if only sarcolemma were stained.

For immunohistochemical analyses of HCV peptides, we prepared 7-μm-thick frozen sections and fixed them as described previously.[13] The primary antibodies were as follows: HCV-core (C7-50; 1:500; Thermo Fisher Scientific), HCV-NS1 (polyclonal; 1:500; Abbiotec, San Diego, CA), HCV-NS5a (7-D4; 1:500; Virogen, Watertown, MA), CD68 (H-255; 1:200; Santa Cruz Biotechnology, Dallas, TX), and dystrophin (Dy8/6C5; 1:200; Novocastra Laboratories, Newcastle upon Tyne, UK). We labeled the antibodies with fluorophores by using APEX Antibody Labelling Kits (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. The antibodies for HCV-core, -NS1, and -NS5a were labeled with the same fluorophore. After 2-hour incubation at room temperature, we observed the specimens with BZ-X710 fluorescence microscope (Keyence, Osaka, Japan).

RT-PCR for HCV-RNA. RNA was extracted from frozen skeletal muscle using PureLink RNA Mini Kit (Life Technologies, Gaithersburg, MD) and reverse transcribed into cDNA with SuperScript VILO cDNA synthesis Kit (Life Technologies). We amplified 260-bp products of the cDNA to verify the presence of HCV-RNA, using the following primers, which had been developed to detect HCV-RNA in liver and serum: sense, 5′ GCC ATG GCG TTA GTA TGA GT 3′, and antisense, 5′ TGC ACG GTC TAC GAG ACC TC 3′.[14] Control muscle samples were derived from subjects with anti-HCV Ab, whose clinicopathologic diagnoses were PM (n = 3), acquired necrotizing myopathies (n = 3), mitochondrial diseases (n = 3), neuropathies (n = 3), myofibrillar myopathies (n = 2), Becker muscular dystrophy (n = 1), primary amyloidosis (n = 1), polymyalgia rheumatica (n = 1), and normal or nonspecific pathologies (n = 4).

Statistical analyses. All statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA). We used Fisher exact test to compare qualitative variables, parametric unpaired *t* test and nonparametric Mann-Whitney tests to compare continuous variables, Kaplan-Meyer curves and Gehan-Breslow-Wilcoxon test to compare the speed of development of symptoms, and χ^2 test for trend to estimate the relationship between patterns of staining of MHC class 1 or 2 and presence of HCV infection. We considered p values <0.05 statistically significant.

Figure 2 Prevalence of HCV infection



Positive rates of anti-hepatitis C virus antibodies (HCV Ab) in the inclusion body myositis (IBM) and polymyositis (PM) groups are shown. The numbers of positive subjects are shown inside parentheses. CI = confidence interval.

Standard protocol approvals, registrations, and patient consents. The study was approved by the NCNP Ethical Committee (A2013-003). All patients gave written informed consents for the use of materials for neuromuscular research.

RESULTS High prevalence of HCV infection in patients with IBM. We found that 32 (28%) out of 114 patients with IBM had anti-HCV Ab (figure 2). This prevalence was significantly higher than the age-matched PM group (2/44 [4.5%]).

In contrast, positive rates of anti-HTLV-1 Ab, anti-HIV Ab, and HBV antigens did not show significant differences between the IBM and PM groups. Positive rates of anti-HTLV-1 Ab were 7.8% (4/51) in the IBM group and 25% (4/16) in the PM group (odds ratio [OR] 0.26, 95% confidence interval [CI] 0.056–1.2). None had anti-HIV Ab in our cohort (data were available in 44 patients with IBM and 14 patients with PM). HBV antigens were positive in 1.8% (2/109) of the patients with IBM and 2.3%

Table 1 Clinical and pathologic features of the patients with inclusion body myositis

	Anti-HCV Ab		
	Positive	Negative	*p* Value
Male:female	1.3:1	1.4:1	0.84
Age at onset, y, mean ± SD	65.7 ± 7.8	64.1 ± 8.6	0.37
Concurrent autoimmune disease, % (n)[a]	11 (3/28)	18 (13/72)	0.55
Serum CK level, IU/L, median (range)	455 (84-3,085)	487 (75-2,400)	0.43
Positive ANA, % (n)	26 (7/27)	29 (21/72)	0.81
Positive anti-SS-A Ab, % (n)	16 (4/25)	15 (8/52)	1.00
Positive anti-SS-B Ab, % (n)	4 (1/24)	6 (3/52)	1.00
Frequency of fibers with RV, % (range)[b]	1.6 (0.2-8.1)	2.3 (0.2-14)	0.24
Pattern of staining of MHC class 1, % (n)[c]			0.92
–	0	0	
1+	6 (2/32)	10 (8/82)	
2+	34 (11/32)	28 (23/82)	
3+	25 (8/32)	29 (24/82)	
4+	34 (11/32)	33 (27/82)	
Pattern of staining of MHC class 2, % (n)[c]			0.85
–	6 (2/32)	15 (12/82)	
1+	38 (12/32)	33 (27/82)	
2+	38 (12/32)	27 (22/82)	
3+	13 (4/32)	18 (15/82)	
4+	6 (2/32)	7 (6/82)	

Abbreviations: Ab = antibodies; ANA = antinuclear antibodies; CK = creatine kinase; HCV = hepatitis C virus; MHC = major histocompatibility complex; RV = rimmed vacuoles.

[a] In the 3 anti-HCV Ab-positive patients who had autoimmune diseases, 2 had autoimmune thyroid disease (Hashimoto thyroiditis) and one had Sjögren syndrome. In the 13 negative patients, the following diseases were described: autoimmune thyroid disease (n = 5: Hashimoto thyroiditis, n = 3, and Graves disease, n = 2), Sjögren syndrome (n = 5), primary biliary cirrhosis (n = 2), rheumatoid arthritis (n = 2), autoimmune hepatitis (n = 1), and idiopathic thrombocytopenic purpura (n = 1). Four of the negative patients had multiple autoimmune diseases.

[b] Cases with more than 300 muscle fibers were analyzed. The number of anti-HCV Ab-positive patients analyzed was 31 and that of negative ones was 74.

[c] – to 4+ = see Methods.