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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

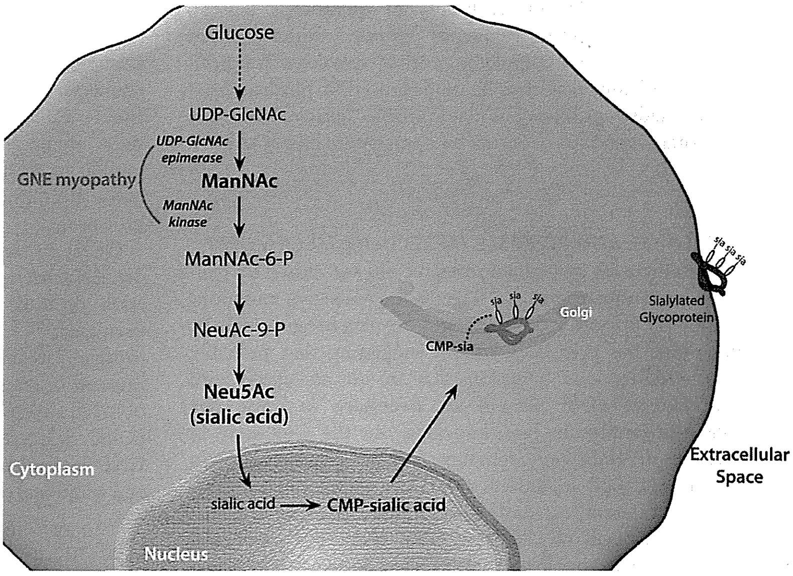

Figure 1 Sialic acid biosynthesis pathway. The biosynthesis of sialic acid (5-*N*-acetylneuraminic acid (Neu5Ac)) occurs in the cytoplasm. The initial substrate for this pathway (UDP-*N*-acetylglucosamine (GlcNAc)) is derived from glucose. In the rate-limiting step of the pathway, UDP-GlcNAc is epimerised 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]

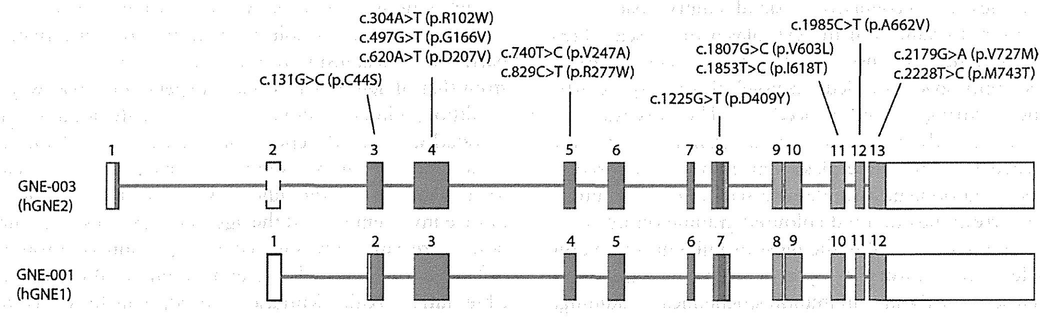

Figure 2 Schematic illustration of GNE gene structure. Gene structure for the two most representative transcripts is shown. The longest transcript (NM_001128227) encodes 753 *amino acids, including* 17 *amino acid encoded by* exon 1. *The originally described transcript shown at the bottom* (GenBank: 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

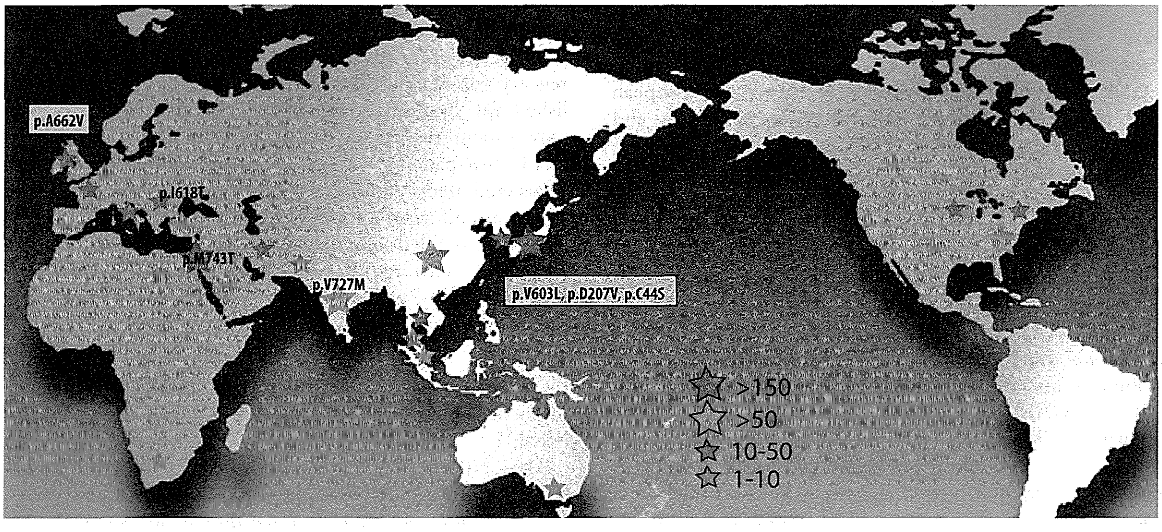

Figure 3 The worldwide prevalence of GNE myopathy is estimated at 1/1 000 000.

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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GNE myopathy: current update and future therapy

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Notes

RESEARCH PAPER

Mutation profile of the *GNE* gene in Japanese patients with distal myopathy with rimmed vacuoles (GNE myopathy)

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ABSTRACT

Background GNE myopathy (also called distal myopathy with rimmed vacuoles or hereditary inclusion body myopathy) is an autosomal recessive myopathy characterised by skeletal muscle atrophy and weakness that preferentially involve the distal muscles. It is caused by mutations in the gene encoding a key enzyme in sialic acid biosynthesis, UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (GNE).

Methods We analysed the *GNE* gene in 212 Japanese GNE myopathy patients. A retrospective medical record review was carried out to explore genotype–phenotype correlation.

Results Sixty-three different mutations including 25 novel mutations were identified: 50 missense mutations, 2 *nonsense mutations*, 1 insertion, 4 *deletions*, 5 *intronic* mutations and 1 single exon deletion. The most frequent mutation in the Japanese population is c.1714G>C (p.Val572Leu), which accounts for 48.3% of total alleles. Homozygosity for this mutation results in more severe phenotypes with earlier onset and faster progression of the disease. In contrast, the second most common mutation, c.527A>T (p.Asp176Val), seems to be a mild mutation as the onset of the disease is much later in the compound heterozygotes with this mutation and c.1714G>C than the patients homozygous for c.1714G>C. Although the allele frequency is 22.4%, there are only three homozygotes for c.527A>T, raising a possibility that a significant number of c.527A>T homozygotes may not develop an apparent disease.

Conclusions Here, we report the mutation profile of the *GNE* gene in 212 Japanese GNE myopathy patients, which is the largest single-ethnic cohort for this ultra-orphan disease. We confirmed the clinical difference between mutation groups. However, we should note that the statistical summary cannot predict clinical course of every patient.

INTRODUCTION

GNE myopathy, which is also known as distal myopathy with rimmed vacuoles,[1] quadriceps sparing myopathy[2] or hereditary inclusion body myopathy (hIBM),[3] is an autosomal recessive myopathy characterised by skeletal muscle atrophy and weakness that preferentially involve the distal muscles such as the tibialis anterior. It is a progressive disease, whereby the symptoms of muscle weakness start to affect the patient from the second or third decade of life, and most of the patients become wheelchair-bound between twenties and sixties.[4] The characteristic histopathological features in muscle biopsy include muscle fibre atrophy with the presence of rimmed vacuoles and intracellular congophilic deposits.[4,5] GNE myopathy is caused by mutations in the gene encoding a key enzyme in sialic acid biosynthesis, UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (GNE).[6–8] Genetically confirmed GNE myopathy was initially recognised in Iranian Jews and Japanese,[7,9] but later appeared to be widely distributed throughout the world. More than 100 mutations in the *GNE* gene have been described up to date.

During the last decade, there has been extensive experimental work to elucidate the pathogenesis and to develop therapeutic strategies of GNE myopathy.[6,10–12] Better knowledge on the basis of those research achievements have currently enabled us to enter the era of clinical trial for human patients. At this moment, the identification of new GNE myopathy patients with precise genetic diagnosis and the expansion of global spectrum of *GNE* mutations are timely and important. Here, we report the molecular profile of Japanese GNE myopathy patients with a brief discussion of genotype–phenotype correlations.

METHODS

Patients

Two hundred and twelve patients from 201 unrelated Japanese families were included in this study. There were 117 female and 95 male patients. All cases were genetically confirmed as GNE myopathy. A retrospective medical record review was carried out to explore genotype–phenotype correlation. Informed consent was obtained for the collection of clinical data and extraction of DNA to perform mutation analysis.

Genetic analysis

DNA was extracted from peripheral blood leukocytes or skeletal muscle tissue. We used the previously described sequencing method to describe mutations at cDNA level.[7] All exons and splice regions of the *GNE* gene were sequenced. NM_005476.5 was used as a reference sequence. We screened 100 alleles from normal Japanese individuals to determine the significance of novel variations.

Pathological analysis

To evaluate histopathological phenotype according to genotype, we analysed muscle biopsies from two most common genotype groups in Japanese population. Each of the three age-matched and biopsy site-matched samples from c.1714G>C homozygous group and c.1714G>C/c.527A>T compound heterozygous group was compared. Muscle samples were taken from biceps brachii and frozen with isopentane cooled in liquid nitrogen. Serial frozen sections of 10 μm were stained using a set of histochemical methods including haematoxylin-eosin and modified Gomori trichrome.

Statistical analysis

Statistics were calculated using GraphPad Prism 5 software (GraphPad Software, La Jolla, California, USA). Between-group comparison for clinical data was performed using one-way analysis of variance with Dunnett's post-test. All values are expressed as means±SD. We performed two-sided tests with a $p<0.05$ level of significance.

RESULTS

Mutation profile

We identified homozygous or compound heterozygous *GNE* mutations in all 212 patients (see online supplement 1). In total, 63 different mutations were found including 50 missense mutations, 2 nonsense mutations, 1 insertion, 4 deletions, 5 intronic mutations and 1 single exon deletion (figure 1). Twenty-five novel mutations were identified including 17 missense mutations, 4 small deletions, 3 intronic mutations and 1 single exon deletion (figure 1, see online supplement).

Twenty-one mutations were found to be shared between two or more unrelated families. The three mutations occurring most frequently in the Japanese population were c.1714G>C (p.Val572Leu), c.527A>T (p.Asp176Val) and c.38G>C (p.Cys13Ser); these comprised 48.3%, 22.4% and 3.5%, respectively, of the total number of alleles examined (table 1).

Table 1 Allele frequency for GNE mutations in 212 Japanese GNE myopathy patients

	Allele frequency
Mutation type	
Missense	402 (94.8%)
Nonsense	3 (0.7%)
Insertion	1 (0.2%)
Small deletion	4 (0.9%)
Single exon deletion	2 (0.5%)
Intron	12 (2.8%)
Three most common mutations	
c.1765G>C (p.Val572Leu)	205 (48.3%)
c.578A>T (p.Asp176Val)	95 (22.4%)
c.38G>C (p.Cys13Ser)	15 (3.5%)
Total alleles	424

Genotype–phenotype correlations

The mean age of genetic analysis was 41.6±14.1 years (n=212), and the mean age of symptom onset based on the data available was 28.4±10.2 years (n=195). The earliest onset age was 10 and the latest was 61 years old in our cohort. Thirty-six among 154 patients (23.4%) were full-time wheelchair users at the point of genetic diagnosis with the average age at loss of ambulation being 36.8±11.3 years (n=36). The youngest wheelchair-bound age was 19, and the oldest ambulant age was 78. To investigate genotype–phenotype correlations in the major *GNE* mutations of Japanese population, we compared the age at symptom onset and loss of ambulation between the patients groups carrying either of the two most frequent mutations, c.1714G>C and c.527A>T (table 2). As with a previous report,[13] homozygous c.1714G>C mutations resulted in earlier symptom onset (23.9±7.1 years, $p<0.01$) and the majority of full-time wheelchair users were in this group. On the other hand, c.1714G>C/c.527A>T compound heterozygous patients first developed symptoms at a later age (37.6±12.6 years, $p<0.01$), and there were no wheelchair-bound patients at the time of genetic analysis in this group. Only three homozygous c.527A>T mutation patients were identified, and their average onset age (32.3±5.7 years) was also higher among total patients (28.4±10.2 years). All three patients were ambulant until the last follow-up visits (29, 40 and 44 years).

Among 212 cases, 80 patients underwent muscle biopsies. Overall pathological findings in our series were compatible with GNE myopathy. The characteristic rimmed vacuoles were observed in the majority (76/80, 95.0%) of the cases. Through the analysis of muscle biopsies from age-matched and biopsy site-matched samples, we found that the histopathological phenotypes were in line with these genotype–phenotype correlations (figure 2). Homozygous c.1714G>C mutations have led to much more advanced pathological changes with severe myofibre atrophy and increased numbers of rimmed vacuoles. Marked adipose tissue replacement was appreciated in a case with reflecting very advanced stage of muscle degeneration.

DISCUSSION

As shown in figure 1, mutations were located throughout the whole open reading frame of the *GNE* gene. The majority (94.8%, 402/424 alleles) of the mutations in our series were missense mutations (table 1), and there were no homozygous null mutations. These results are in accordance with previous reports[7] [9] signifying that total loss of GNE function might be

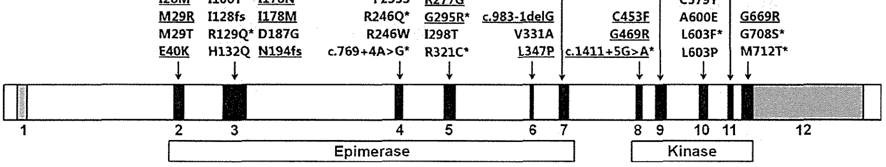

Figure 1 Mutation spectrum of *GNE* in the Japanese population. The mutations are located throughout the whole open reading frame. Twenty-five novel mutations are underlined, and 21 shared mutations are indicated with asterisks.

Table 2 Comparison of clinical course between two most frequent GNE mutations in Japanese population

Mutations	Age at exam (years)		Age at onset (years)		Age at WB (years)		Ambulant
c.1714G>C/c.1714G>C	38.6±13.4	(n=71)	23.9±7.1	(n=65)**	35.4±10.6	(n=28)	n=22
c.1714G>C/other	32.3±13.2	(n=25)	21.9±6.8	(n=22)*	37.0±8.6	(n=4)	n=16
c.1714G>C/c.527A>T	48.9±14.1	(n=38)	37.6±12.6	(n=35)**		(n=0)	n=29
c.527A>T/c.527A>T	37.7±7.7	(n=3)	32.3±5.7	(n=3)		(n=0)	n=3
c.527A>T/other	41.3±11.1	(n=51)	30.6±8.0	(n=46)		(n=2)	n=33
other/other	49.8±14.7	(n=24)	28.8±9.5	(n=24)		(n=2)	n=16
Total	41.6±14.1	(n=212)	28.4±10.2	(n=195)	36.8±11.3	(n=36)	n=118

Dunnett's multiple comparison test (control: total patients) $^*p<0.05$, $^{**}p<0.01$.
Other: a mutation other than c.1714G>C and c.527A>T; WB, wheelchair-bound.

lethal in human beings. The embryonic lethality of null mutation in *GNE* had also been proved in the mouse model.[14] Only three of total 212 patients carried a nonsense mutation; clinical data were available for two of them. Interestingly, one patient with compound heterozygous c.22C>T (p.Arg8X)/c.1714G>C (p.Val572Leu) mutations developed his first symptoms at the age of 15, while the other patient with c.1258C>T (p.Arg420X)/c.527A>T (p.Asp176Val) mutations developed her symptoms much later, at the age of 45. The similar difference was also observed in the phenotypes of patients with frame-shift mutations. A patient carrying c.383insT (p.I128fs) and c.1714G>C (p.Val572Leu) mutations developed his first symptom at the age of 13, whereas another two patients with c.1541-4del4 (p.Val514fs)/c.527A>T (p.Asp176Val) and c.581delA (p.N194fs)/c.527A>T (p.Asp176Val) mutations had later symptom onset, at the age of 30 and 32 years, respectively. This clinical variation can be explained as it reflects alternative missense mutations, because the two patients with very early onset shared the same missense mutation c.1714G>C, while the patients with the milder phenotype shared c.527A>T.

Among five intronic mutations identified in our series, c.617−4A>G and c.769+4A>G were previously reported as pathological mutations.[7] [15] Three novel variants were located at splice junction of exon 6 (c.983–1delG), exon 8 (c.1411+5G>A) and exon 9 (c.1505–4G>A), raising the high possibility of relevant exons skipping. These variants were not detected in 200 alleles from normal Japanese individuals and also in the single nucleotide polymorphism (SNP) database.

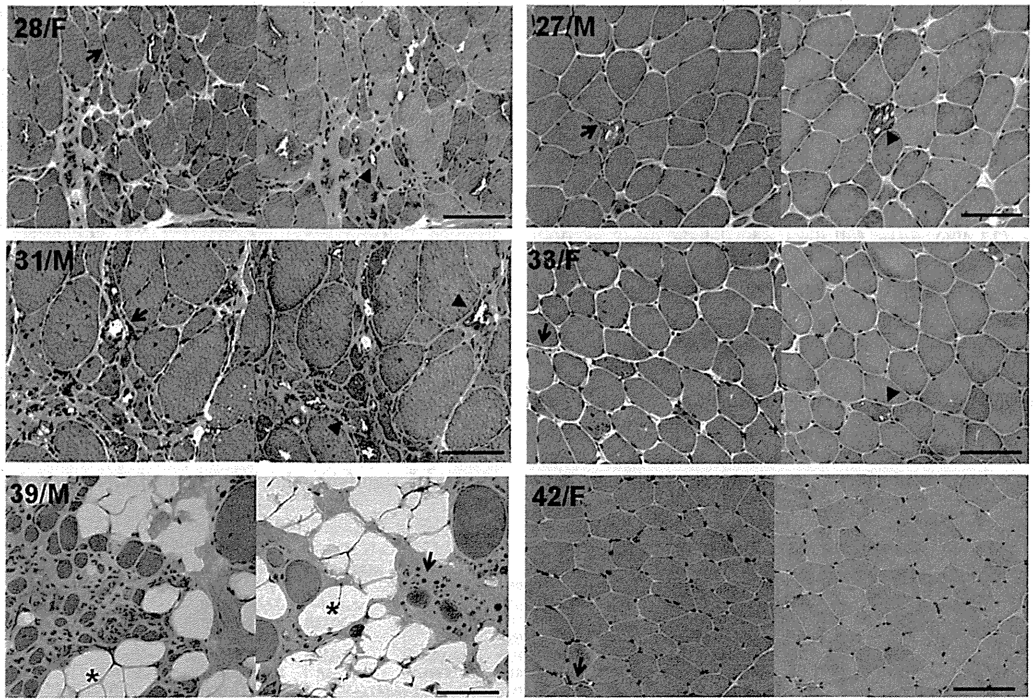

Figure 2 Comparison of muscle pathology between patients with homozygous c.1714G>C (p.Val572Leu) and with compound heterozygous c.1714G>C (p.Val572Leu)/c.527A>T (p.Asp176Val) mutations. Homozygous c.1714G>C (p.Val572Leu) mutations have led to much more advanced histopathological changes compared with compound heterozygous c.1714G>C (p.Val572Leu)/c.527A>T (p.Asp176Val) mutations. Haematoxylin-eosin (left) and modified Gomori trichrome (right) stains of muscle sections from age (c.1714G>C/c.1714G>C: 28, 31 and 39 years, c.1714G>C/c.527A>T: 27, 33 and 42 years) and biopsy site (biceps brachii muscles) matched samples. Bar=100μm; triangles: rimmed vacuoles; arrows: atrophic fibres; asterisks: adipose tissue.

As there are ethnic differences in *GNE* mutation frequencies,[9 16–19] establishing the mutation spectrum and defining predominant mutations in a certain population may be helpful for the diagnosis. Three most common mutations in the Japanese population and their allele frequencies (table 1) were in agreement with previous data.[7 13] The allele frequencies of top two mutations (c.1714G>C and c.527A>T) comprise more than two-third of the total number of alleles suggesting that founder effects are involved in the relatively higher incidence of GNE myopathy in Japan.

Although most of patients showed characteristic pathological features, the existence of exceptional cases with atypical biopsy findings implies that GNE myopathy cannot be totally excluded from the absence of rimmed vacuoles in muscle biopsies. On the other hand, we found 94 patients who were pathologically or clinically suspected but not had mutations in *GNE*. Several cases of VCP myopathy mutations in (*VCP*), myofibrillar myopathy mutations in (*DES*) and reducing body myopathy (*FHL1*) were later identified in this group, suggesting these diseases should be included as differential diagnosis of GNE myopathy.[20]

In terms of genotype–phenotype correlations, we confirmed that homozygosity for c.1714G>C (p.Val572Leu) mutation resulted in more severe phenotypes in clinical and histopathological aspects. In contrast, the second most common mutation, c.527A>T (p.Asp176Val), seems to be a mild mutation as the onset of the disease is much later in the compound heterozygotes with this mutation and c.1714G>C. Several evidences further strengthened the link between the more severe phenotype and c.1714G>C, and between the milder phenotype and c.527A>T. Compound heterozygosity for c.1714G>C and non-c.527A>T mutations resulted in earlier symptom onset (22.9±6.8 years, $p<0.05$) compared with the average onset age of the total group, whereas c.527A>T, both presented as homozygous and as compound heterozygous mutations, lead to slower disease progression (table 2). In addition, only three patients carrying this second most common mutation c.527A>T in homozygous mode were identified, which is much fewer than the number expected from high allele frequency (22.4%), raising a possibility that considerable number of c.527A>T homozygotes may not even develop a disease. In fact, we ever identified an asymptomatic c.527A>T homozygote at age 60 years.[7] Now he is at age 71 years and still healthy. Overall, these results indicate that different mutations lead to different spectra of severity. However, this is a result of a statistical summary that cannot predict clinical course of each individual patient.

Here, we presented the molecular bases of 212 Japanese GNE myopathy patients with 25 novel *GNE* mutations. Based on the current status of knowledge, sialic acid supplementation may lead to considerable changes in the natural course of GNE myopathy within near future. The ongoing identification of *GNE* mutations and further studies regarding the clinicopathological features of each mutation will provide better understanding of GNE myopathy and lead to accelerated development of treatment for this disease.

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Contributors AC had full access to all of the data in the study and wrote the manuscript; YKH supervised all aspects of this study including study design, data interpretation and manuscript preparation; KM and YO participated in collecting and analysing all the clinical and genetic data; SN, I Nonaka and I Nishino were involved in data analysis and interpretation and also supervised manuscript preparation.

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Competing interests None.

Ethics approval This study was approved by the ethics committee of National Center of Neurology and Psychiatry.

Provenance and peer review Not commissioned; externally peer reviewed.

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Mutation profile of the *GNE* gene in Japanese patients with distal myopathy with rimmed vacuoles (GNE myopathy)

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Short communication

GNE myopathy: New name and new mutation nomenclature

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The recessively inherited, adult onset, quadriceps sparing myopathy with a predilection for distal muscles has received multiple historic names. The disorder was described in 1981 in Japanese patients and termed Nonaka Distal Myopathy [1], later commonly referred to as Distal Myopathy with Rimmed Vacuoles (DMRV) (OMIM#605820). In 1984, the disorder was described as vacuolar myopathy sparing the quadriceps in Iranian-Jewish patients [2], later commonly referred to as Inclusion Body Myopathy 2 (IBM2) or Hereditary Inclusion Body Myopathy (HIBM) (OMIM#600737). Mapping of the causative gene to the same locus on chromosome 9 in different cohorts of patient [3,4], and ultimately identification of mutations in the causative gene *GNE* in all cohorts [5,6], confirmed that these myopathies are in fact the same condition.

However, since identification of *GNE* as the common causative gene, the multiple historic names for the disorder continue to be used by research groups worldwide. This disease nomenclature becomes increasingly confusing for clinicians, patients and researchers. Therefore, an international consortium (of which the authors are also members) has recently proposed to rename the disorder "GNE myopathy", substituting all previous disease definitions. We all are now using this new name and hope that it will become the only term worldwide.

After initial discovery of *GNE* gene defects to be causative for GNE myopathy, eight different *GNE* mRNA splice variants were identified, encoding (at least theoretically) eight protein isoforms [7]. The human *GNE* gene (GenBank Gene ID: 10020, NC_000009; ENSEMBL ENSG00000159921) consists of 13 exons, but each of the individual *GNE* mRNA splice variants consists of fewer exons. However, for mutation annotation purposes, only two major transcripts are relevant, which together span all 13 exons (Fig. 1) [7]. We provide NCBI GenBank accession numbers for the two major isoforms hGNE1 and hGNE2 in the text below, and provide their ENSEMBL IDs in Table 1.

hGNE1 (GenBank NP_005467) is the originally described GNE protein which covers 722 amino acids [5] and is, confusingly, encoded in GenBank by mRNA transcript variant 2 (NM_005476). The hGNE2 isoform (NP_001121699) covers 753 amino acids and is encoded by the longest *GNE* mRNA transcript, variant 1 (NM_001128227).

The discovery of the additional N-terminal sequence (and novel exon 1) [8] encoding hGNE2, is potentially confusing since most previous molecular and biochemical

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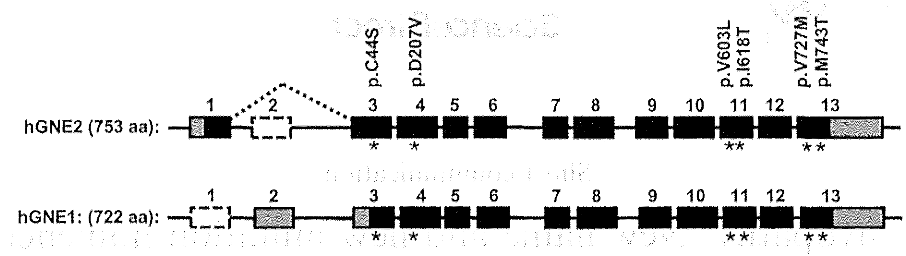

Fig. 1. Human *GNE* mRNA transcripts and isoforms. Structures of the two main human *GNE* mRNA transcripts (not to scale) and the human GNE isoforms (hGNE1 and hGNE2) are illustrated. Note that mRNA variant 1 (the longest splice form) encodes the hGNE2 protein, while mRNA variant 2 encodes the hGNE1 protein (traditionally known and studied as the sole translated GNE protein). Black boxes: open reading frame; Gray boxes: untranslated mRNA regions; White dotted lined boxes: skipped exons. Locations of selected GNE myopathy-associated mutations (see Table 1) are indicated by stars. GenBank Accession numbers and translated amino acids (aa) are provided. Modified and updated from [7].

Table 1
Most frequent GNE myopathy-associated *GNE* variants.

New nomenclature[a]			Previous nomenclature			
hGNE2 isoform	mRNA transcript	Exon	hGNE1 isoform	mRNA transcript	Exon	Ethnicity[b]
GenBank hGNE2	GenBank Variant 1	GenBank *GNE* gDNA	GenBank hGNE1	GenBank Variant 2		
NP_001121699	*NM_001128227*	*NC_000009*	*NP_005467*	*NM_005476*		
ENSEMBL	ENSEMBL	ENSEMBL	ENSEMBL	ENSEMBL		
GNE-003	GNE-003	*GNE* gDNA	GNE-001	GNE-001		
ENSP	ENST	*ENSG*	*ENSP*	*ENST*		
00000379839	00000396594	*00000159921*	*00000367134*	*00000377902*		
p.C44S	c.131G > C	3	p.C13S	c.38G > C	2	Japanese
p.D207V	c.620A > T	4	p.D176V	c.527A > T	3	Japanese
p.V603L	c.1807G > C	11	p.V572L	c.1714G > C	10	Japanese
p.I618T	c.1853T > C	11	p.I587T	c.1760T > C	10	Cajun, Roma Gypsies
p.V727M	c.2179G > A	13	p.V696M	c.2086G > A	12	Indian
p.M743T	c.2228T > C	13	p.M712T	c.2135T > C	12	Middle Eastern

[a] Nomenclature according to universally adapted gene/protein nomenclature rules.

[b] Ethnicity in which the variant is mostly reported.

studies (including all mutation reports) refer to the hGNE1 isoform, while according to universally adapted gene/protein nomenclature rules the longest mRNA splice form ought to be used for annotating nucleotide/amino acid locations (http://www.hgvs.org/mutnomen/refseq.html). Hence, amino acid numbering of previously reported GNE studies (based on hGNE1 nomenclature), including patient mutation reports, should be supplemented with 31 amino acids to adhere to the current (hGNE2) nomenclature guidelines, and nucleotide numbering should be supplemented with 93 bases. For exon numbering, the numbering according to the entire 13 exons *GNE* gDNA gene ought to be used, which means that exon numbering of previously reported GNE studies (based on hGNE1 nomenclature) have to be supplemented with one exon.

Adaptation to the hGNE2 nomenclature can initially be confusing; however, we strongly support adaptation of this ‘new’ nomenclature. Laboratories/researchers not familiar with the GNE myopathy field and disease/gene history will report patient mutations and research tools (antibodies, enzyme activities, siRNA, nextgen sequence databases, etc.) according to current universally adapted nomenclature rules. Moreover, although there are no variants reported yet in the additional 31 amino acids of hGNE2 (perhaps because this region has not been considered for mutation analysis in many patients), future variants in this region could not be accurately named using hGNE1 as a reference. To illustrate the new terminology, we list both hGNE2 (‘new’ nomenclature) and hGNE1 (‘previous’ nomenclature) classifications and up to date exon numbers of the most frequent *GNE* mutations associated with GNE myopathy in Table 1. However, since history will leave its tracks, we strongly suggest accompanying all future references to GNE with the appropriate GenBank accession numbers.

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