Neuromuscular

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).

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

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

Mutation type	Allele frequency
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

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
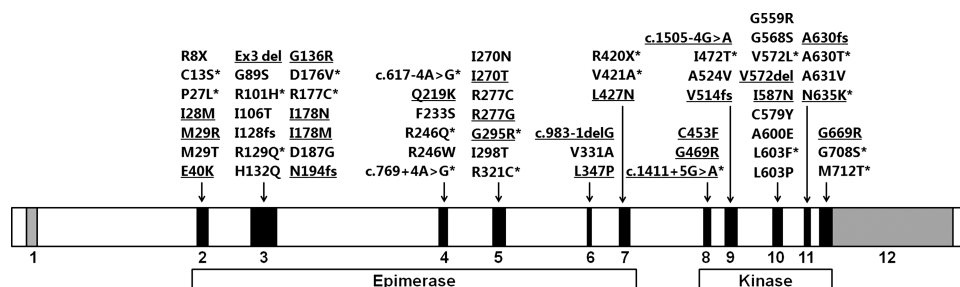

Figure 1 Mutation spectrum of GNE in the Japanese population. The mutations are located throughout the whole open reading frame. Twenty-five novel mutations are underlined, and 21 shared mutations are indicated with asterisks.

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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.
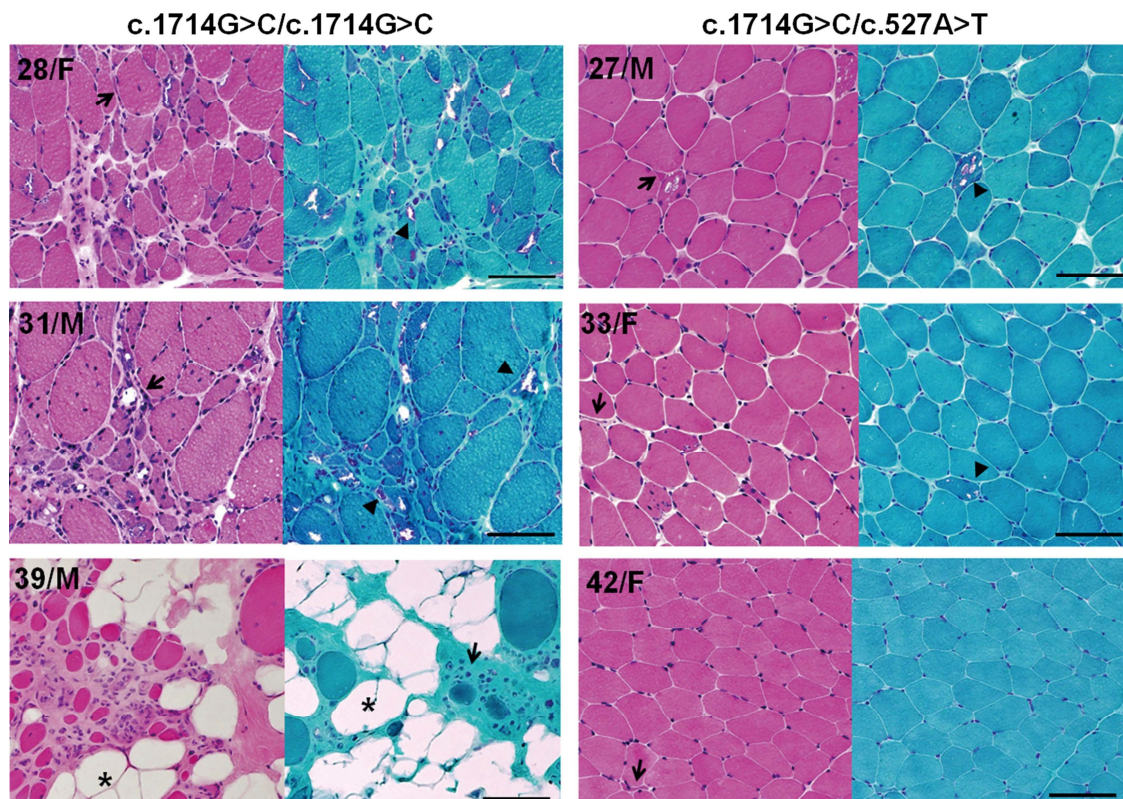

Figure 2 Comparison of muscle pathology between patients with homozygous c.1714G>C (p.Val572Leu) and with compound heterozygous c.1714G>C (p.Val572Leu)/c.527A>T (p.Asp176Val) mutations. Homozygous c.1714G>C (p.Val572Leu) mutations have led to much more advanced histopathological changes compared with 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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