Novel Mutations of the *GNE* Gene in Distal Myopathy with Rimmed Vacuoles Presenting with Very Slow Progression

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Abstract
We report novel compound heterozygous mutations of the UDP-N-acetylglucosamine-2-epimerase and N-acetylmannosamine kinase *(GNE)* gene, c.302G>A (p.R101H) and c.617-4A>G, in a Japanese family with distal myopathy with rimmed vacuoles (DMRV) presenting with slow progression. The three patients could stand and walk even 36, 34, and 39 years after onset, respectively, although affected individuals become wheelchair bound on average 12 years after onset of the disease. The clinical spectrum of DMRV seems to be wider than previously thought in terms of both the clinical course and the severity of the disease.

Introduction

Distal myopathy with rimmed vacuoles (DMRV), also called hereditary inclusion body myopathy (hIBM), is an juvenile-to-adult-onset autosomal-recessive disorder clinically characterized by preferential involvement of the lower leg muscles, especially the tibialis anterior muscle [1, 2]. Affected individuals become wheelchair bound on average 12 years after onset of the disease [1]. Muscle pathology is characterized by

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rimmed vacuoles, in addition to fiber size variation. The disease is caused by mutations in *GNE*, encoding a protein with activities of UDP-N-acetylglucosamine-2-epimerase and N-acetylmannosamine kinase (GNE), which is an essential enzyme in sialic acid biosynthesis [3–12]. We characterize novel mutations of the *GNE* gene in a Japanese family with DMRV presenting with slow progression.

Case Reports

Patient 1

A 64-year-old man had developed an insidious steppage gait at age 28. Within one year, he noticed weakness of the finger flexors/extensors. Muscle weakness and atrophy of the four limbs, which were more dominant in distal than proximal parts, gradually progressed. He became unable to write at age 62. He had a history of diabetes mellitus and liver dysfunction from alcoholism. His elder brother and sister had similar symptoms (fig. 1). He had no history of drinking excessive milk or eating swallow's nests, which are rich in sialic acid. On admission, his general physical examination was normal. On neurological examination, cranial nerves were intact. Motor examination revealed 0–1/5 strength in the distal part of the four limbs with muscle atrophy in bilateral digitorum extensor and flexor muscles, wrist extensor and flexor muscles, toe extensor and flexor muscles, tibialis anterior, and gastrocnemius, as delineated by the Medical Research Council of Great Britain (fig. 2a). Quadriceps and truncal muscles were spared. He could stand and walk unaided with a steppage gait. Biceps and triceps reflexes were diminished, although ankle and knee jerks were normal. Bilateral Babinski reflexes were absent. Vibration perception was mildly decreased in the distal part of the four limbs. The rest of the neurological examination was unremarkable. Serum creatine kinase (CK) and lactic dehydrogenase (LDH) were normal. Myoglobin was mildly elevated. Anti-Jo-1 antibody was undetected. Computed tomography (CT) scan revealed skeletal muscle atrophy in the extremities and fatty tissue infiltration in bilateral triceps brachii, posterior muscles of the thigh, and muscles of the lower legs. The quadriceps muscles were well preserved (fig. 2b–d). Electromyography of the upper and lower extremities demonstrated myogenic conversion with low amplitude motor unit potential. Muscle biopsy from the left biceps brachii revealed scattered fibers with rimmed vacuoles in addition to marked variation in fiber size (fig. 2e, f).

DNA was extracted from peripheral blood lymphocytes after informed consent was obtained. Each exon and flanking sequences of the *GNE* gene were amplified by polymerase chain reaction and the amplified fragments were directly sequenced. We identified a novel compound heterozygote of G-to-A transition at nucleotide position c.302 (c.302G>A) in exon 3, which is predicted to change an amino acid at codon 101 from arginine to histidine (p.R101H), and an A-to-G transition at nucleotide position c.617-4 (c.617-4A>G) in intron 3 (fig. 2g, h).

Patient 2

The patient was the elder brother of patient 1 (fig. 1). He complained of muscle weakness in both feet at age 36. He also developed muscle weakness in both finger flexors/extensors at age 43. Muscle weakness and atrophy of the four limbs, which were more dominant in distal than proximal parts, gradually progressed. Neurological examination at age 70 demonstrated muscle weakness and atrophy in the distal part of the four limbs and reduced deep tendon reflexes. He could stand and walk unaided with a steppage gait. Serum CK and myoglobin were normal. LDH was mildly elevated. CT scan revealed skeletal muscle atrophy in the extremities and fatty tissue infiltration in bilateral triceps brachii, posterior muscles of the thigh, and muscles of the lower legs. The quadriceps muscles were well preserved (table 1). The same mutations as in patient 1 were detected.

Patient 3

The patient was the elder sister of patient 1 (fig. 1). She complained of muscle weakness in both feet at age 29. She also developed muscle weakness in both finger flexors/extensors at age 40. Muscle weakness and atrophy of the four limbs, which were more dominant in distal than proximal parts, gradually progressed. Neurological examination at age 68 demonstrated muscle weakness and atrophy in the distal part of the four limbs and reduced deep tendon reflexes. Serum CK was mildly elevated. She could walk with a stick and steppage gait (table 1). The same mutations as in patient 1 were detected.

Discussion

We herein report novel compound heterozygous mutations of the *GNE* gene in a Japanese family with DMRV presenting with slow progression. To date, more than 50 different *GNE* mutations in DMRV have been reported to cause the disease. *GNE*, a rate-limiting enzyme that catalyses the initial two steps in the biosynthesis of sialic acid has two functional domains that work independently: the epimerase domain in the amino (N)-terminals, and the kinase domain in the carboxy (C)-terminus. Homozygous M712T is the most common mutation in Middle Eastern Jews, and homozygous V572L among Japanese patients [3–11]. In many other ethnic groups, compound heterozygous mutations have been reported [3–11]; however, the phenotypes are not fully described. The three patients could stand and walk even 36, 34, and 39 years after onset, respectively. Affected individuals become wheelchair bound within an average of 12 years after disease onset [1]. In our three patients, muscle CT revealed the typical distribution of muscle involvement previously reported in DMRV. The tibialis anterior, hamstrings, and adductors were severely affected, but the quadriceps muscles were well preserved. Although it was not clear why the quadriceps muscles of our patients were spared for so many years and there has been no correlation between genotype and phenotype with more than 50 mutations reported, our patients presented with the slowest progression of previously reported patients with DMRV in Japan. Thus, the clinical spectrum of DMRV seems to be wider than previously thought in terms of both the clinical course and the severity of the disease.

Nishino et al. [3] reported a healthy 67-year-old person of Persian Jewish origin carrying the common M712T *GNE* gene mutation in a homozygous form, suggesting incomplete penetrance of the disease. The results indicate that a modifier gene might be responsible for the phenotype differences, although no such modifier genes have been identified. Our finding of a novel mutant allele in a Japanese patient with DMRV expands on the genetic heterogeneity of this disease and allows for a broader phenotypic-genotypic assessment. The three patients had no history of drinking excessive milk or eating swallow's nests, which are rich in sialic acid. Although it is difficult to explain why this novel mutation leads to unusually slow progression of the disease, further investigation of the correlations among genotypes, enzyme activities and phenotypes is warranted.

Table 1. Clinical and laboratory findings of distal myopathy with rimmed vacuoles in three Japanese patients

	Patient 1	Patient 2	Patient 3
Sex	Male	Male	Female
Age, years	64	70	68
Age at onset, years	28	36	29
Initial presentation	Foot drop	Foot drop	Foot drop
Medical Research Council scales			
Neck flexor muscles	4	4	4
Upper limbs			
Deltoid	4	4	5
Biceps	4	4	4
Triceps	3	3	4
Intrinsic hand muscles	0	1	1
Lower limbs			
Quadriceps	4	4	4
Hamstrings	4	4	4
Tibialis anterior	0	0	0
Gastrocnemius	2	1	1
Quadriceps sparing	Yes	Yes	Yes
Creatine kinase (reference range: 62–287 IU/l)	90	184	293
CT imaging fatty infiltration			
Quadriceps	–	–	–
Hamstrings	++	+++	+++
Tibialis anterior	++++	++++	++++
Gastrocnemius	++++	++++	++++
Muscle biopsy	Myopathic changes with scattered fibers with rimmed vacuoles	not examined	not examined
Current status	Walk unaided	Walk unaided	Walk with assistance

+ = 0–25% of muscle fibers with fatty infiltration. ++ = 25–50% of muscle fibers with fatty infiltration. +++ = 50–75% of muscle fibers with fatty infiltration. ++++ = 75–100% of muscle fibers with fatty infiltration.
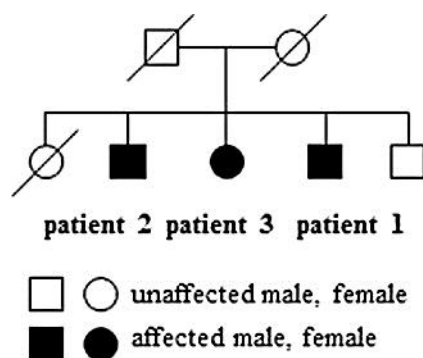

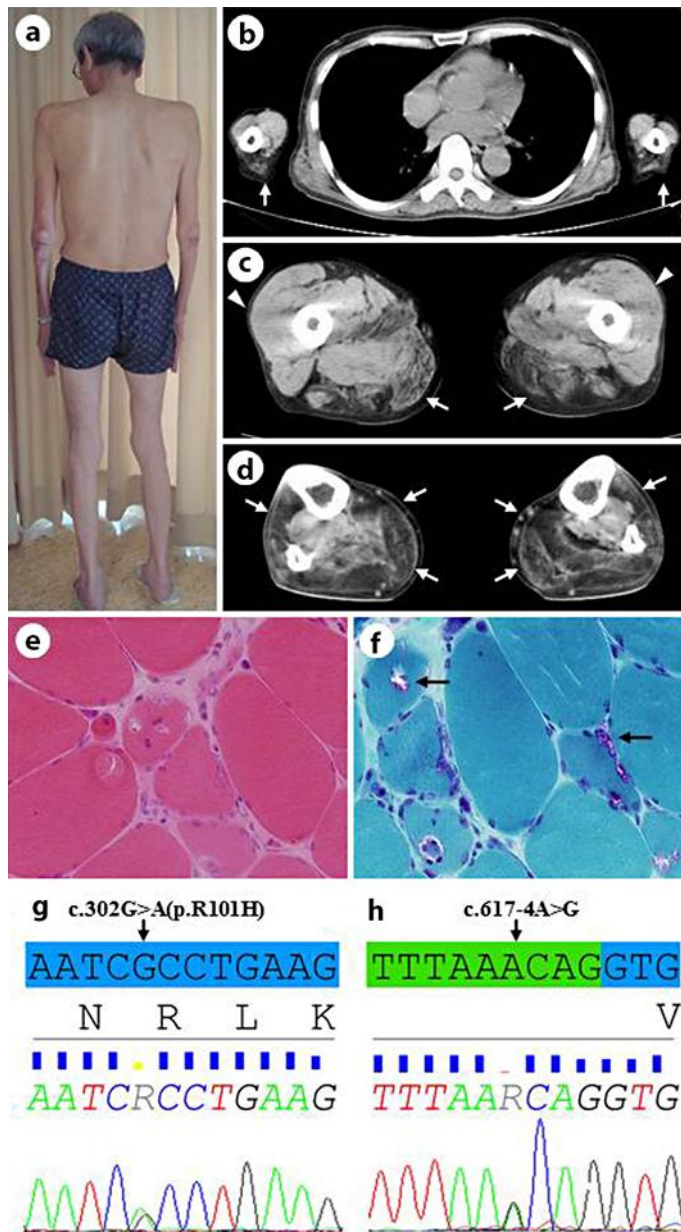**Fig. 1.** Pedigree of the patients' family.

Fig. 2. Image of patient 1 showing muscular atrophy in the distal part of the four limbs (**a**). CT scan showing skeletal muscle atrophy in the extremities and a low-density area in triceps brachii, posterior muscles of the thigh, and muscles of the distal leg, which showed fatty tissue infiltration of muscles (arrows, **b–d**). The quadriceps muscles were well preserved (arrowheads, **c**). In muscle biopsy of the biceps brachii, there is marked variation in fiber size with hypertrophic and small angular fibers, and mild interstitial fibrosis (**e**) (hematoxylin and eosin), rimmed vacuoles (arrow) are prominent in small fibers (**f**) (modified Gomori trichrome) (×100). Sequence chromatograms of the two heterozygous missense mutations. G-to-A transversion at nucleotide 302 in exon 3 results in a conservative amino acid change (R101H) (**g**), and A-to-G transition at nucleotide c.617-4 in intron 3 results in a missense mutation (**h**).

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