CASEREPORT

Novel GNE mutations in three Chinese patients with typical GNE myo-pathy

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Abstract

GNE myopathy is an adult-onset muscle disorder featuring distal muscle atrophy and weakness. Rimmed vacuoles found in the muscle biopsies and gene mutations lead to the diagnosis of GNE myopathy. We collected clinical information, performed muscle biopsies and genetic testing on three patients. These cases developed typical disease presentations with distal muscle weakness at the ages of 26, 23, and 37 years. Their muscle pathologies revealed rimmed vacuoles. Genetic analysis led to the findings which included, c.1543-1544delGA (p.D515QfsX2)/c.38G>C (p.C13S) compound heterozygous mutation, c.733A>G (p.K245E) homozygous mutation and c.527A>T (p.D176V)/c.1634-1G>C (splicing); in which c.1543-1544delGA (p.D515QfsX2), c.733A>G (p.K245E) and c.1634-1G>C (splicing) are three novel mutations that have never been reported before. In conclusion, this study broadens the mutational spectrum of the GNE gene. **Keywords:** Novel mutations, GNE myopathy, rimmed vacuoles. **https://doi.org/10.5455/JPMA.290893**

Introduction

GNE myopathy is an adult-onset muscle disorder (GNE is an abbreviation for the mutated gene Uridine diphosphate-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase, with an alternative name UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase) with signs typically appearing in the third decade of life.[1] It shows distal muscle atrophy and weakness, preferentially involving the tibialis anterior muscle. Characteristic histopathological findings in muscle biopsies include "rimmed" vacuoles, intracellular aggregation of various proteins and fiber size variation. Diagnosis is confirmed by sequencing of the GNE gene (9p13.3) which encodes a bi-functional protein with two enzymatic activities namely glucosamine (UDP-N-acetyl)-2-epimerase/N-acetyl mannosamine kinase.[2] To date, 165 missense/nonsense mutations, eight splicing mutations and 14 small deletion mutations have been reported (Human Gene Mutation Database, HGMD professional 2017.4). Here, we report three patients with GNE myopathy. Sequencing of the GNE gene identified three missense mutations, one deletion mutation, and one splicing mutation, three of which were novel; c.1543-1544delGA (p.D515QfsX2), c.733A>G (p.K245E) and c.1634-1G>C (splicing).

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Case Report

Patient 1, a 30-year-old man developed gradual but progressive weakness in all four limbs over a time span of four years since year 2006. Initially, he noticed bilateral lower-extremity weakness that did not impact his life significantly. Three years ago, he noticed atrophy in both the thenar muscles of the hands and the distal legs. Meanwhile, his muscle strength declined, especially, in the lower limbs and he faced difficulty climbing the stairs. However, no walker was needed and he could complete ten sets of leg squats. He visited our department (Neurology Department, Chinese PLA General Hospital) in year 2009 and motor power of both sides rated by the Medical Research Council Scale revealed proximal upper extremities to be 5/5 and distal upper extremities to be 3-4/5. He was unable to walk with his heels or toes showing obvious muscle atrophy in all four distal extremities, especially, in the lower legs. Gower sign was positive, deep tendon and ankle reflexes were diminished. From then on, patient suffered gradual increase in the weakness as he witnessed problem in walking and lifting his feet. He fell many times, had to hold onto the handrails when climbing the stairs and felt exhausted after completing ten sets of leg squats. Patient returned to our hospital in year 2010 with bilateral motor power scores in the sternocleidomastoid and trapezius muscles to be 5/5, deltoid, biceps, and triceps muscles revealed power of 3/5, hand and wrist muscles were 2/5, illio-psoas muscles was 2-3/5, quadriceps femoris muscles examination showed power of 5/5, gastrocnemius muscles were 3/5 and anterior tibial

muscles were 2/5. Patient reported no pain, sensory disturbances or muscle cramps. None of his family members have symptomatic muscle weakness. His serum creatine kinase (CK) levels were elevated (528.8 U/L; normal range 24-194 U/L and lactic dehydrogenase (LDH) levels were normal (187.9 U/L; normal range 100-240 U/L). Electromyography (EMG) showed myogenic changes. A muscle biopsy of the right gastrocnemius muscle showed several rimmed vacuoles with internal nuclei seen in many degenerated and atrophic myofibers (Figure).

A sequence analysis of the polymerase chain reaction (PCR) products amplification was conducted from patient's coding exons 2-12 of the GNE gene (GenBank accession number: GNE, NM 005476). We identified a glycine to cysteine transition at nucleotide position 38 in exon 2 (c.38G>C) that converts cysteine to serine at codon 13 (p.C13S) in the epimerase domain and a G-A deletion at nucleotide position 1543-1544 in exon 9 (c.1543-1544delGA) converting aspartic acid to glutamine at codon 151 (p.D515QfsX2) in the kinase domain. All mutations were further confirmed by single nucleotide polymorphism analysis using the SnaPshot™ Multiplex System Kit (Applied Biosystems, Foster City, CA, USA).

Patient 2, a 31-year-old man had developed progressive muscle weakness over eight years since year 2009. Initially, he presented with fatigue while walking that gradually progressed to difficulty in climbing the stairs, performing leg squats and gripping heavy materials but he could still walk on a leveled road. He had no history of dysphagia or choking while drinking. His parents are cousins but none of his family members were affected. He went to a a local hospital in year 2013 and a muscle biopsy taken at that time from the left calf revealed highly possible sporadic inclusion body myositis (sIBM, which is a feature of inflammatory cell infiltration and muscle fiber necrosis accompanied by regeneration and vacuoles on H&E and Gomori staining). He was treated with coenzyme Q, mecobalamin and vitamin B1 which proved ineffective. He visited our hospital in year 2015 and on admission his general physical examination was normal, neurological examination showed intact cranial nerves and a motor examination according to the Medical Research Council Scale[3] revealed power of 5/5 in the proximal upper extremities, 4/5 in the distal upper extremities, 4/5 in the proximal lower extremities and 2-3/5 in the distal lower extremities with muscle atrophy in the distal parts of all four limbs. Tendon reflexes in all four limbs were diminished and the bilateral Babinski reflexes were absent. Serum CK was mildly elevated at 487.2 U/L (normal range 24-194 U/L) and LDH level was normal. EMG of the upper and lower extremities demonstrated myogenic conversion. A repeat biopsy of the right calf muscles revealed atrophic muscle fibers of different sizes that appeared as small, circular and irregular in shape. Rimmed vacuoles were seen in many atrophic myofibers which contained basophilic substances. Materials in the rimmed vacuoles were stained red with gomori trichrome stain. No inflammatory cell infiltration was noted.

Genetic analysis was performed by targeted NGS to identify the causative genes. We recognized a novel homozygous mutation of the GNE gene showing an A-to-G transi-tion at the nucleotide position (c.733A>G) in exon 4 which changed the amino acid at codon 245 from lysine to glutamic acid (p.K245E) in epimerase domain.

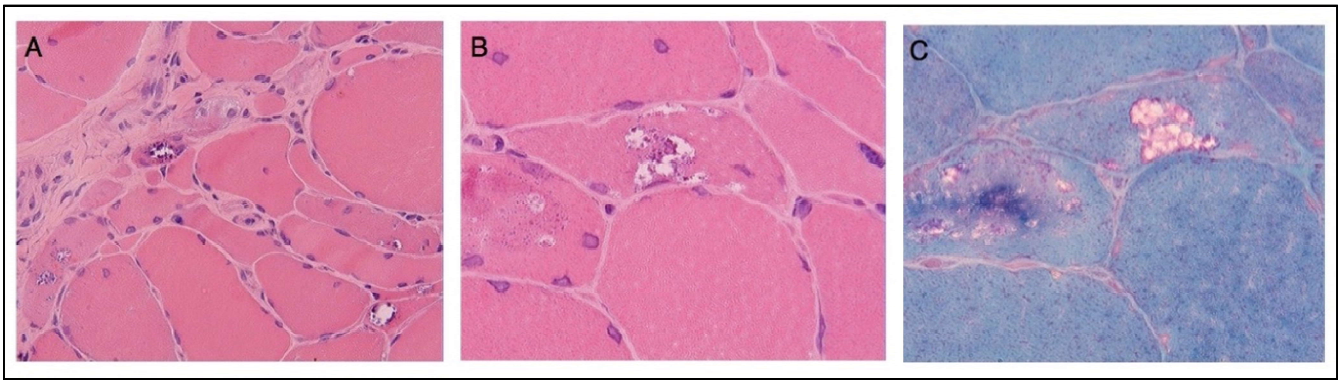

Figure: Muscle biopsy from right gastrocnemious muscle showed: A) Several rimmed vacuoles with internal nuclei found in many degenerated and atrophic myofibers. (HE, X100) B) Atrophic fibers appeared as small angular and rimmed vacuoles in some of the fibers, which contain basophilic materials. (HE, X200). C) Gomori trichrome stain showed red stained basophilic materials in rimmed vacuoles (Gomori, X200).

Table: Clinical presentation and GNE mutations in three unrelated Chinese patients.

No.	Sex	Age (in yrs)	Age of onset	family member	Limbs muscle strength (MRC grade)				atrophy	CK (U/L)	EMG	Biopsy	mutation (*is marked as novel)		domain	
					proximal Upper	distal upper	proximal lower	distal lower	quadriceps sparing				Allele1	Allele2	site1	site2
1	M	30	26	Neg	3	2	3	2	Y	both H+sL	528.8	M	RVs	c.38G>C/ p.C13S	c.1543-1544delGA/ p.D515QfsX2	E	K
2	M	31	23	Neg,CM	5	4	4	2	Y	both H+sL	1108	M	Rvs	c.733A>G/ p.K245E*	c.733A>G/ p.K245E	E	E
3	F	41	37	Neg	5	5	4	1	Y	Neg	513	M	RVs	c.527A>T/ p.D176V	c.1634-1G>C/ (splicing)*	E	K

Neg: negative; CM: consanguineous marriage; H+sL: hands and distal legs; M: myogenic; RVs: rimmed vacuoles: E: epimerase, K: Kinase, CK normal range: 24-194 U/L.

This mutation was further confirmed by Sanger sequencing.

A follow-up visit was done in year 2017 and the patient reported the use of a cane while walking for nearly a year now.

Patient 3, a 41-year-old female initially presented at the age of 37 years(year 2010) with progressive weakness of bilateral foot dorsiflexion. She walked more slowly than others and developed a bilateral foot drop with a high stepping gait. Patient went to see a doctor in year 2011 and simultaneously underwent EMG which demonstrated myopathic changes. She was diagnosed with myopathy and took mecobalamin and vitamin E for one whole year but no improvement was noted. When she visited our hospital in year 2014 she was able to do some leg squats but could not run and had to hold onto the handrails when climbing stairs with an obvious gait abnormality with difficulty in walking on heels or toes. None of her family members have muscle weaknesses. Her bilateral motor power rated by the Medical Research Council Scale was 5/5 in the upper extremities, 4/5 in the proximal lower extremities and 1/5 in the distal lower extremities. Her muscle tension and tendon reflexes of the upper extremities were normal while the tendon reflexes of the lower extremities were diminished. There was no muscle atrophy, sensory disturbance or discordant movement. A blood test showed moderately elevated serum CK levels (513 U/L; normal range 24-194 U/L) and slightly elevated LDH levels (287.3 U/L; normal range 100-240 U/L). Results of a repeated EMG was consistent with the first test indicating myopathy. We performed a biopsy of the right calf muscle which showed rimmed vacuoles in many atrophic myofibers with basophilic materials inside the vacuoles.

Genetic analysis performed by targeted NGS found a compound heterozygous mutation in the GNE gene of c.527A>T (p.D176V)/c.1634-1G>C (splicing). The former is a reported GNE mutation showing an A-to-T transition at nucleotide position 527 in exon 3 that changes an amino acid at codon 176 from aspartic acid to valine in epimerise domain. The latter is a splicing mutation which resulted from c.1634-1G>C in exon 11 kinase domain and this has never been reported before. All the clinical information of these three patients are listed in Table.

Discussion

Here, we present three cases of unrelated patients with GNE myopathy based on the clinical presentation, muscle biopsy and gene testing results. Homozygous or compound heterozygous mutations in the GNE gene are the cause of autosomal recessive GNE myopathy. GNE codes for a bifunctional enzyme (UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase) with both epimerase and kinase activities that has a central role in the sialic acid biosynthetic pathway 2.We identified three novel GNE mutations adding to the growing. Patient 1 harboured compound heterozygous GNE mutations of the epimerase domain at c.38G>C (p.C13S) on exon 2 and of the kinase domain at c. 1543-1544delGA (p.D515QfsX2) on exon 9. To our knowledge, the c.1543-1544delGA (p.D515QfsX2) has not been reported elsewhere. Patient 2 had a novel homozygous mutation at c.733A>G (p.K245E) on exon 4, while Patient 3 had a novel compound heterozygous mutation at c.1634-1G>C (splicing) on exon 11 which

lies next to a previously reported mutation of c.527A>T (p.D176V).

Hundred alleles from normal Chinese individuals were screened to determine the significance of these three novel mutations, result showed that these were neither detected in the 100 ethnically matched control chromosomes nor in the single nucleotide polymorphism database. Polyphen-2 software was used to do the functional prediction of c.1543-1544delGA (p.D515QfsX2) and c.733A>G (p.K245E). They were found to be damaging with scores of 0.956 (sensitivity 0.63, specificity 0.92) and 0.973 (sensitivity 0.60, specificity 0.93), respectively. The third novel mutation of c.1634-1G>C, indicated splicing mutations that are obviously malignant. As described above, these findings indicate three novel GNE mutations to be pathogenic.

The combination of clinical features, ancillary studies, muscle biopsy findings and mo-lecular genetic studies helped confirm the diagnosis of GNE myopathy. Three patients had their initial signs of disease at ages 26, 23 and 37 years, respectively, presenting with slowly progressive, distally predominant muscle weakness and atrophy. However, their quadriceps muscles all retained normal or close-to-normal strength. Patient 1 was the most highly affected individual presenting with severely decreased muscle strength in both upper and lower extremities as well as atrophy in both the hands and distal legs. Patient 2 showed atrophies in both hands and distal legs as well but his upper limbs were slightly affected compared to patient 1. Patient 3 had less severe symptoms amongst the three and she presented with typical distal weakness phenotype without atrophy. While most cases presented similar to patient 3, some patients have atypical phenotypes such as limb-girdle weakness, asymmetric hand weakness or other hip muscles.[4-6] Nishino et al[1] also reported that the disease does not remain limited to the distal musculature but slowly progresses to involve more proximal leg muscles and the upper limbs, which can provide an explanation to our patients 1 and 2 developing upper-limb weakness. The severity of upper-limb involvements in our patients was not in proportion to disease durations, on which larger samples studies are needed. All three of these patients showed mild to moderately elevated CK levels, myogenic changes in the EMG and pathological features including rimmed vacuoles in the muscle fibers. All of the above patients meet the clinical and pathological aspects of diagnostic criteria on typical GNE myopathy; in addition, none of them suffered from cardiac or respiratory problems.

Cho et al.[7] report homozygous mutations in the kinase domain which causes typical clinical features of GNE myopathy but compound heterozygous GNE mutations show various clinical features and varied disease course. What's more, the presence of at least one allele mutation in the epimerase domain may develop unusual clinical features[8] but as in our case, shown in Table, patient 3 manifested typical GNE myopathy phenotype carried compound heterozygous in EK (both epimerase and kinase) domains, patient 1 and 2 manifested atypical clinical features carried mutations in EK (compound heterozygous mutations in both epimerase and kinase) and EE (homozygous mutation in the epimerase domain) separately, suggesting that correlations between disease severity and mutation location might not fit with the previously reported findings. Thus, future studies are required to explore the genotype-phenotype correlation.

Lu et al.[9] reported a first, large-scale report on Chinese GNE myopathy patients and the incidence of distal myopathy with rimmed vacuoles among their sample group was 0.88% (37/4,223), Celeste et al.[10] estimated the worldwide prevalence of GNE myopathy to be 4-21/1,000,000 which indicates that GNE myopathy is a rare disease in China and across the world.

Conclusion

Here, we report three cases of GNE myopathy with three novel GNE mutations which has broadened the mutational spectrum of the GNE gene both in China and across the globe.

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