Journal of Human Genetics (2011) 56, 335–338

www.nature.com/jhg

ORIGINAL ARTICLE

Clinical and molecular genetic analysis in Chinese patients with distal myopathy with rimmed vacuoles

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Distal myopathy with rimmed vacuoles (DMRVs) is an autosomal recessive vacuolar myopathy that has been reported in different ethnic populations with the common mutations of UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (*GNE*) gene. We presented the clinical, pathological and genetic characteristics of eight Chinese DMRV patients from six unrelated families. Six previously reported Chinese DMRV patients from four unrelated families were also reviewed for comparison in *GNE* mutations. In the present eight patients with DMRV, direct sequencing analysis revealed one homozygous mutation of c.1760T>C (p.I587T) and seven compound heterozygous mutations in the *GNE* gene. The latter included two known mutations, c.1892C>T (p.A631V) and c.527A>T (p.D176V), and three novel mutations, c.1523T>C (p.L508S), c.103G>A (p.E35K) and c.153A>G (p.I51M). The allelic frequency of c.1523T>C (p.L508S) was 25% in the Chinese patients with DMRV. Our findings expand the genetic spectrum of DMRV and indicate that the common mutations of *GNE* gene in DMRV may be variable among different ethnic populations.
Journal of Human Genetics (2011) **56,** 335–338; doi:10.1038/jhg.2011.15; published online 10 February 2011

Keywords: Chinese; distal myopathy with rimmed vacuoles; *GNE*; hereditary inclusion body myopathy

INTRODUCTION

Distal myopathy with rimmed vacuoles (DMRVs; OMIM605820), also known as hereditary inclusion body myopathy or inclusion body myopathy 2 (OMIM600737), is an autosomal recessive muscular disorder with early adult onset and quadriceps-sparing muscular involvement. Its muscle pathology is characterized by numerous rimmed vacuoles and filamentous inclusions in the sarcoplasm and nuclei of affected myofibers.[1,2] This disease is caused by mutations of UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase *(GNE)* gene.[3,4] Over the last 9 years, more than 70 *GNE* mutations have been described to be associated with DMRV/hereditary inclusion body myopathy in patients of different ethnic origin. Among the *GNE* missense mutations, three seem to be the founder mutations. The predominant mutation is c.2135T>C (p.M712T) that was initially identified in patients of Persian–Jewish descent,[4–6] but subsequently found worldwide in non-Jewish populations.[7,8] The c.1714C>G (p.V572L) mutation is the most frequent in Japan[9] and as well in Korea with an allelic frequency of 68.8%.[10] The c.527A>T (p.D176V) mutation is one exclusively found in Japanese population.[9,11] To date, only six cases of DMRV with *GNE* mutations have been reported from four Chinese unrelated families (Table 1).[12–14] In this study, we present eight new cases of DMRV with *GNE* mutations from six unrelated Chinese families.

SUBJECTS AND METHODS

Subjects

Eight patients (Table 1, 1.1–6) from six unrelated families were included in this study. The first 7 patients (1.1–5) were previously reported as suspected DMRV without genetic confirmation in Chinese literature.[15] These eight patients have met the following criteria for DMRV: (1) sporadic or possibly autosomal recessive inheritance, (2) onset in the second or third decade of life, (3) weakness beginning in the distal leg muscles with or without distal to proximal progression, (4) normal to moderate increase in serum creatine kinase (CK) level, (5) myopathic or mixed changes on electromyogram and (6) rimmed vacuoles formation and rare muscle fiber necrosis on muscle biopsy. All patients had muscle biopsies in Qilu Hospital of Shandong University, and given informed consent for this study.

Muscle pathology

The muscle specimens were snap frozen in cooled isopentane, and then stored at −80°C until analysis. Cryostat sections were prepared and stained with hematoxylin–eosin, modified Gomori trichrome and various histochemical methods. Small portions of these specimens were fixed in 4% glutaraldehyde and subsequently sectioned for electron microscopy.

Molecular studies

Genomic DNA was extracted from frozen muscle specimens or peripheral blood leukocytes by using a genomic DNA extract kit (Tiangen, Peking, China).

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Received 30 September 2010; revised 11 January 2011; accepted 13 January 2011; published online 10 February 2011

Table 1 Clinical and pathological features, and *GNE* gene mutations in Chinese patients with distal myopathy with rimmed vacuoles

					Lower limbs				Upper limbs				Muscle Pathology					
																EM		
Patient	*Sex*	*Age (years)*	*Age onset (years)*	*FMH*	*TA*	*GC*	*QF*	*IP*	*Dis*	*Pro*	*CK (UI^{-1})*	*EMG*	*RVs (%)*	*Necrotic fibers*	*Biopsy site*	*MB*	*IB*	*GNE mutation*
1.1	Female	25	22	+	3	3	5	5	4	4	213	M	12.5	A few	(BB)	+	+	p.L508S/p.A631V
1.2	Female	28	24	+	4	4	5	3	4	4	260	M/N	0.21	—	(BB)	+	+	p.L508S/p.A631V
2	Female	23	21	–	4	4	5	5	4	4	<200	M/N	13.8	—	(BB)	–	–	p.L508S/p.A631V
3.1	Female	27	20	+	0	3	5	3	2	4	206	M/N	64.4	—	(BB)	+	–	p.I51M/p.L508S
3.2	Female	30	19	+	0	3	5	3	3	3	307	N	34.8	—	(BB)	+	+	p.I51M/p.L508S
4	Female	23	22	–	3	5	5	3	5	4	ND	N	0.24	—	(BB)	ND	ND	p.E35K/p.L508S
5	Female	27	20	–	2	3	5	3	5	4	<200	M	16.8	—	(BB)	ND	ND	p.D176V/p.L508S
6	Female	32	25	–	3	3	5	2	5	4	622	M	37.2	—	(BB)	ND	ND	p.I587T/p. I587T
7[a]	Male	36	31	Cons	1	5	5	4	4	5	1085	M	NA	—	NA	NA	+	p.H509Y/p.H509Y
8[a]	Female	43	33	–	3	2	5	5	5	5	ND	M	NA	—	NA	NA	NA	p.D176V/p.V572L
9.1[b]	Female	38	21	+	0	0	1	NA	2	3	294	M	NA	—	(GC)	NA	NA	p.I241S/p.R246W
9.2[b]	Male	28	18	+	1	3	5	NA	4	4	384	M	NA	—	(VL)	NA	NA	p.I241S/p.R246W
10.1[c]	Male	50	30	+	1	4	5	NA	NA	NA	322	M/N	NA	—	(QF)	NA	NA	p.W513X/p.I241S
10.2[c]	Female	41	26	+	0	1	2	NA	NA	NA	177	M	NA	—	NA	NA	NA	p.W513X/p.I241S

Abbreviations: BB, biceps brachii; CK, creatine kinase; Cons, consanguinious family; Dis, distal; EM, electron microscopy; EMG, electromyogram; FMH, family history; GC, gastrocnemius; GNE, UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase; IB, inclusion body; IP, iliopsoas; M, myogenic patterns (reduced amplitude and duration of motor unit action potential, increased proportion of polyphasic wave and pathologic interference pattern); MB, myeloid bodies; MCV, motor nerve conduction velocity; N, neurogenic pattern (reduced MCV or SCV; autonomous potential); NA, not available; ND, not done; Pro, proximal; QF, quadriceps femoris; RVs, rimmed vacuoles; SCV, sensory nerve conduction velocity; TA, tibial anterior; VL, vastus lateralis.
[a]Reported by Wang *et al*.[14]
[b]Reported in Taiwan[12]
[c]Reported in Taiwan[13]; muscle strength was assessed in accordance with the manual muscle test, which is based on the Medical Research Council's recommendations.

Control DNA was extracted from peripheral blood leukocytes (provided by the Institute of Medical Genetics, Shandong University) of 200 unaffected healthy Chinese individuals. A total of 11 coding regions (exons 2–12) and their intron–exon boundaries of *GNE* gene were amplified by PCR using primers and conditions as previously described.[4] The PCR products were subjected to sequencing by Biosune Biotechnology of Shanghai (Shanghai, China). Confirmation of p.I51M mutation was performed by *Bts*CI restriction enzyme analysis using forward primer 5′-CAATCACGCGAGCTCTCTC-3′ and reverse primer 5′-CAAAGAGGTGCC CTATGGTG-3′, and p.E35K mutation was confirmed by mismatch PCR/Re using forward primer 5′-TAACCGTGCA GATTATTCTAAACTTGCCCCGATCATGTTTGGCATTAAAATC-3′ and reverse primer 5′-GTGACTACTCTAAGGCCAC-3′, and *Taq*-enzyme analysis. The confirmation of p.L508S mutation was performed by PCR/amplification refractory mutation system. Amplification of normal *GNE* gene was performed by using forward primer (5′-CCCCCTTTCTGACACTTT-3′) and reverse primer (5′-ACATTCTAGCTCCTGAACCA-3′), and mutated *GNE* gene by forward primer (5′-CCCCCTTTCTGACACTTC-3′) and reverse primer (5′-ACATTCTAGCTCCTGAACCA-3′).

RESULTS

Table 1 summarized the clinical and pathological data, as well as *GNE* mutations in the Chinese patients with DMRV, which included those (patients 7–10.2) previously reported for comparison. The age of onset was 23.7 ± 4.7 years (mean ± s.d.). All the patients presented distal muscle weakness in the lower extremities. Electromyogram studies showed myopathic or mixed pattern in all the patients, except patient 3.2 and 4. Their serum creatine kinase levels were slightly elevated, ranging from normal to 1085 $IU\,l^{-1}$. In our patients with DMRV, the most prominent pathological feature was the presence of rimmed vacuoles in 0.21–64.4% of the myofibers. Electron microscopy exhibited numerous myeloid bodies, autophagic vacuoles and amorphous structures in rimmed vacuoles. Sarcoplasmic or intranuclear filamentous inclusion bodies were identified in four of six cases.

Direct nucleotide sequencing disclosed five families with compound heterozygous *GNE* mutations and one family with homozygous *GNE* mutation in our patients with DMRV (Figure 1). Of these six different mutations, p.L508S, p.I51M and p.E35K were novel, and the others, p.A631V, p.D176V and p.I587T had been reported. All the three novel mutations were not detected in 200 normal, unrelated, ethnically matched controls(Figure 2c). Mutated amino acids for three novel mutations are conserved across all 10 examined species (Figure 2b), suggesting that they are not polymorphisms. *GNE* consists of two functional domains, an UDP-GlcNAc 2-epimerase domain and a ManNAc kinase domain. Three of the six mutations are located in the UDP-GlcNAc 2-epimerase domain of GNE and the other three are within the ManNAc kinase domain[16] (Figure 2a). The most common mutation was p.L508S, which was found in five families with compound heterozygous mutations.

DISCUSSION

It has become clear that DMRV may occur in different ethnic populations. This study is the largest series of Chinese DMRV patients, in which six unrelated families with clinically and pathologically suspected DMRV are found to have either compound heterozygous or homozygous mutations of the *GNE* gene. The clinical manifestations of our patients with *GNE* mutations are consistent, as described in Table 1, and resemble those of the previously reported classic DMRV/hereditary inclusion body myopathy.[17,18]

The homozygous M712T mutation on exon 12 was shown to be a founder mutation in Middle Eastern Jewish patients with DMRV, whereas the mutations in Japanese patients with DMRV were more complex and involved almost all exons, no matter whether they are homozygous or compound heterozygous.[9,19,20] Of 55 unrelated Japanese DMRV patients, the p.V572L mutation is the most common and accounts for 57% of the mutant alleles.[9] This mutation is also the most frequent in eight Korean patients with DMRV. The common

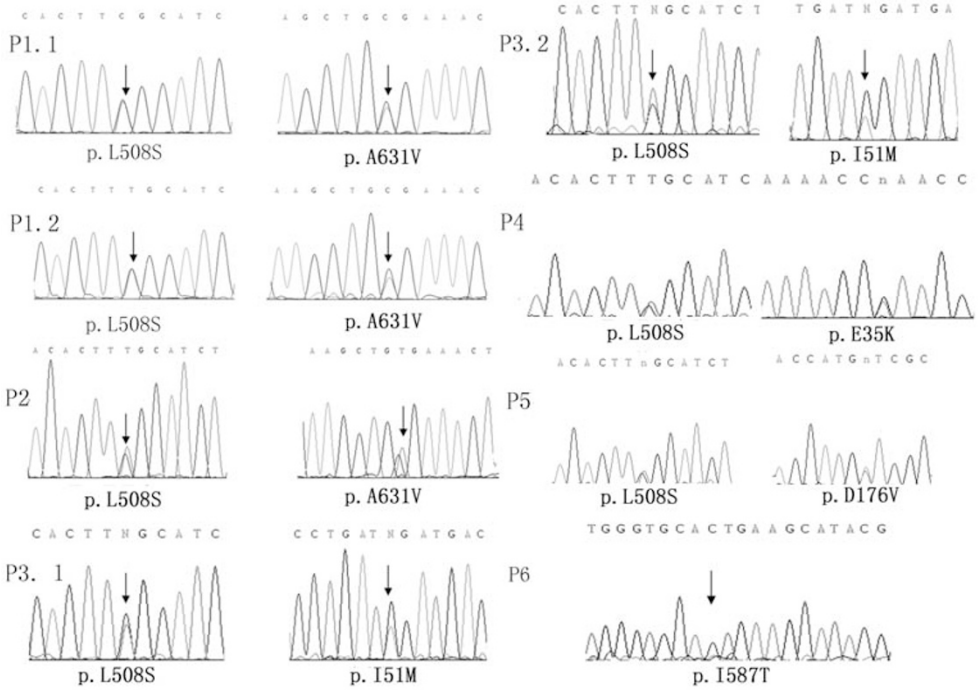

Figure 1 Sequencing of *GNE* gene mutations identified in eight Chinese patients with distal myopathy with rimmed vacuoles.

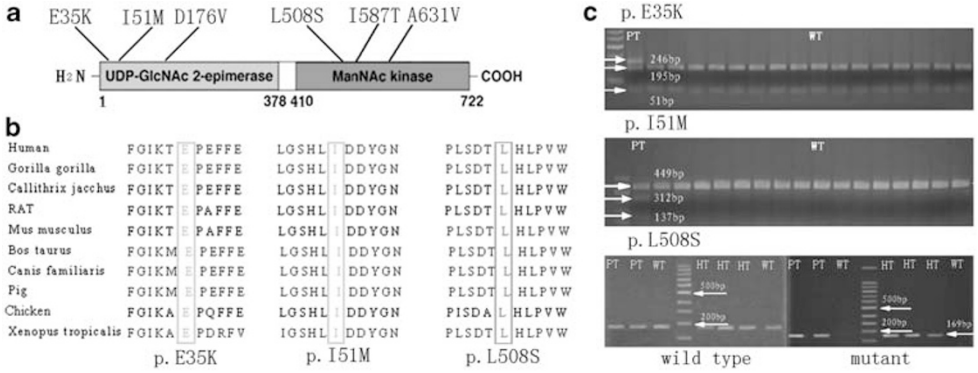

Figure 2 (**a**) Overview of GNE domain structure and localization of the GNE mutants that we studied. (**b**) Amino-acid sequence alignment of human GNE and orthologs from other species, showing phylogenetic conservation of three mutations that we found. The sequences were retrieved from the Entrez protein database and aligned to each other with the use of Clustal W (http://www.ebi.ac.uk/clustalw). (**c**) Mutation screening of *GNE* p.E35K, p.I51M and p.L508S. PCR amplification of normal allele of *GNE* (left) and p.L508S mutated allele of *GNE* (right). These mutations were not found in 200 healthy controls. HT, heterozygous control; PT, patient; WT, wild-type control.

founder effect might exist in Japanese and Korean populations given their neighborliness. In contrast, in the present group of Chinese DMRV patients, there is no p.M712T mutation and only one p.V572L mutation (in patient 8). Although similarity of *GNE* mutations might exist between Japanese and Chinese populations (because there are two Chinese patients carrying p.D176V mutation that was reported only in Japanese patients),[11,20] the common *GNE* mutation of DMRV in China is clearly different from that in Japan. Because five of six families in this study showed the p.L508S mutation in at least one allele of *GNE* gene, this mutation seems to be common in Chinese population. As this mutation has not yet been described in DMRV patients from other ethnic populations, the common mutation of *GNE* gene may vary in different ethnic populations, even among the Northeast Asian countries. The mutations in this study are not confined to any single specific region of the enzyme outside its negative feedback regulatory domain located at codes 249–275.

In conclusion, our findings expand the genetic spectrum of DMRV and suggest that p.L508S is likely the common mutation of *GNE* gene in Chinese population. Furthermore, the common mutations of *GNE* gene might be different among ethnic populations.

ACKNOWLEDGEMENTS

We thank Dr Jian-Qiang Lu, MD, FRCPC (University of Alberta, Canada) for his critical review of the manuscript, and the Institute of Medical Genetics, Shandong University for providing the DNA of the normal controls. This work was supported by grants from the NSFC General Projects of China (Grant No. 30670744/C090301).

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