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ORIGINAL ARTICLE

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Mutation analysis of the *GNE* gene in Korean patients with distal myopathy with rimmed vacuoles

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Abstract Distal myopathy with rimmed vacuoles (DMRV; MIM 605820) is an autosomal recessive neuromuscular disorder characterized by weakness of the anterior compartment of the lower limbs, sparing the quadriceps muscles. Recently, mutations in the UDP-*N*-acetylglucosamine-2-epimerase/*N*-acetylmannosamine kinase (*GNE*) gene have been identified as the genetic basis of DMRV. To investigate the mutation spectrum of the *GNE* gene in Korean patients with DMRV, we performed clinical and genetic analysis of nine unrelated patients suspected to have DMRV. Direct sequencing analysis revealed that eight out of nine patients (88.9%) were either homozygous or compound heterozygous for *GNE* gene mutations, including three known (C13S, R129Q, and V572L) and two novel mutations (M29T and A591P). The allelic frequencies of the V572L and C13S mutations were 68.8% (11/16) and 12.5% (2/16), respectively. These results suggest that screening for *GNE* gene mutations in patients suspected to have DMRV would be helpful for molecular diagnosis of DMRV in the Korean population.

Keywords Distal myopathy with rimmed vacuoles · DMRV · *GNE* gene · Mutation

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Introduction

Adult-onset distal myopathy is a primary muscle disorder characterized clinically by progressive muscular weakness and atrophy beginning in the hands or feet, and pathologically by myopathic changes in the skeletal muscles. Distal myopathy with rimmed vacuoles (DMRV; MIM 605820) is a major entity of distal myopathy developing in early adulthood with weakness of the anterior compartment of the lower limbs, and is characterized by the presence of prominent rimmed vacuoles in muscle fibers with modest elevation of serum creatine kinase (CK) (Nonaka et al. 1981, 1998).

DMRV is caused by mutations in the gene encoding the bifunctional enzyme UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (*GNE*) (Eisenberg et al. 2001; Nishino et al. 2002). Interestingly, previous studies have shown that predominant mutations exist in certain populations, such as the M712T mutation in Middle Eastern Jews and in non-Jewish Iranians, and V572L mutation in Japanese patients (Arai et al. 2002; Eisenberg et al. 2001; Nishino et al. 2002; Noguchi et al. 2004; Tomimitsu et al. 2002, 2004; Vasconcelos et al. 2002), while there are no such mutations in Italian patients with DMRV (Broccolini et al. 2002, 2004; Del Bo et al. 2003; Eisenberg et al. 2003). Unraveling the spectrum of mutations, and the existence of predominant mutations, in each population may be helpful in the determination of diagnostic steps; i.e., genetic testing for predominant mutations in a patient with distal myopathy who is suspected to have DMRV could avoid invasive muscle biopsy for exact diagnosis.

Table 1 Summary of clinical features and the results of mutation analysis of the UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (*GNE*) gene in Korean patients with distal myopathy with rimmed vacuoles (DMRV). *DM* Distal muscle, *LE* lower extremity, *PM* proximal muscle, *UE* upper extremity

Patient	Sex	Age at examination (years)	Age at onset (years)	Family history	Affected muscles	Serum CK (IU/l)	EMG	Muscle biopsy findings (biopsy site)	GNE mutations
1	F	35	29	–	LE/DM	275	Myopathic	Not done	C13S/V572L
2	F	29	26	–	LE/DM	413	Myopathic	Not done	C13S/M29T
3	M	25	16	+	LE/DM	3,858	Myopathic	Not done	No mutation
4	F	22	21	–	LE/DM → PM	352	Myopathic	Myopathy with rimmed vacuoles (vastus lateralis)	V572L/V572L
5	F	24	20	–	LE/DM	222	Myopathic	Myopathy with rimmed vacuoles (biceps bracii)	V572L/A591P
6	F	28	23	–	LE and UE/DM	231	Myopathic	Myopathy with rimmed vacuoles (vastus lateralis)	R129Q/V572L
7	M	48	20	–	LE and UE/DM → PM	271	Not done	Not done	V572L/V572L
8	M	15	13	–	LE/DM	1,422	Myopathic	Not done	V572L/V572L
9	F	28	27	–	LE and UE/DM	211	Myopathic	Myopathy with rimmed vacuoles (vastus lateralis)	V572L/V572L

To date, there have been only two reports of DMRV with *GNE* mutations in the Korean population (Han et al. 2005; Lee et al. 2004). In the present study, we present the results of an analysis of mutations in the *GNE* gene and the implications for molecular diagnosis of Korean patients with DMRV.

Materials and methods

Patients

Blood samples were obtained from nine unrelated patients suspected of having distal myopathy during the 2-year study period. All patients were evaluated neurologically and were referred for genetic testing with informed consent. Based on the medical records, an extensive clinical review, including the family history, serum CK level, and electromyelogram (EMG) findings was performed. These patients fulfilled the following criteria of distal myopathy: (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) slight to moderate increase in serum CK level, and (5) mainly myopathic changes on EMG. Of the nine patients, four underwent muscle biopsy without immunohistochemical studies.

Methods

Genomic DNA was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification Kit following the manufacturer's instructions (Promega, Madison, WI). All coding exons of the *GNE* gene and their flanking introns were amplified by polymerase chain reaction (PCR) using primers designed by the authors (available on request). Direct sequencing was performed with a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA) on an ABI Prism 3100 genetic analyzer (Applied Biosystems). Identified mutations were analyzed with reference to the Human Gene Mutation Database (http://www.archive.uwcm.ac.uk/uwcm/mg/hgmd0.html), and any novel mutations not reported in the database were confirmed on 1,000 control chromosomes by sequencing.

Results

As summarized in Table 1, only patient 3 had a family history of distal myopathy, i.e., an elder sister of the patient had weakness of the distal muscles in the lower extremities but other family members, including both parents and two elder brothers, were clinically unaffected. The mean age of onset was 21.7 ± 5.2 years (mean ± SD). All patients showed weakness of the distal muscles in the lower extremities as the initial presenting symptom, and three patients also complained of involvement of upper extremities. EMG study revealed myopathic changes in all patients except patient 7, and rimmed vacuoles were observed in all patients who underwent muscle biopsy. The level of serum CK in patients with *GNE* gene mutations was slightly elevated, ranging from 207 to 1,422 IU/l.

Mutation analysis revealed that eight out of nine patients had *GNE* mutations: four patients were homozygous for the V572L mutation, and the remaining four patients were compound heterozygous for C13S/M29T, C13S/V572L, V572L/A591P, and R129Q/V572L mutations (Table 1). Therefore, the allelic frequencies of the

V572L and C13S mutations were 68.8 and 12.5%, respectively. The M29T and A591P mutations were novel and were not observed in 1,000 control chromosomes (Fig. 1).

Discussion

DMRV, also known as Nonaka myopathy, is a distinct clinical disease entity in young adults, characterized by the preferential involvement of anterior tibial muscle from the early stage of disease (Nonaka et al. 1981, 1998). Recently, *GNE* gene mutations have been identified in DMRV (Eisenberg et al. 2001; Nishino et al. 2002) and over 40 mutations have been found across the whole coding region of the *GNE* gene thus far. However, a few mutations, such as M712T and V572L, are known to be predominant in Middle Eastern Jews and non-Jewish Iranians, and in Japanese, respectively (Arai et al. 2002; Eisenberg et al. 2001; Nishino et al. 2002; Noguchi et al. 2004; Tomimitsu et al. 2002, 2004; Vasconcelos et al. 2002). According to the first report on *GNE* gene mutations by Eisenberg et al. (2001), all 47 Middle Eastern Jews in the study were homozygous for the M712T mutation, and haplotype analysis around the mutation indicated one unique ancestral founder chromosome in this community. Similarly, Arai et al. (2002) reported that the V572L mutation in the kinase domain was shared by all the DMRV families examined, suggesting a strong founder effect in Japanese DMRV patients. However, although V572L is the predominant

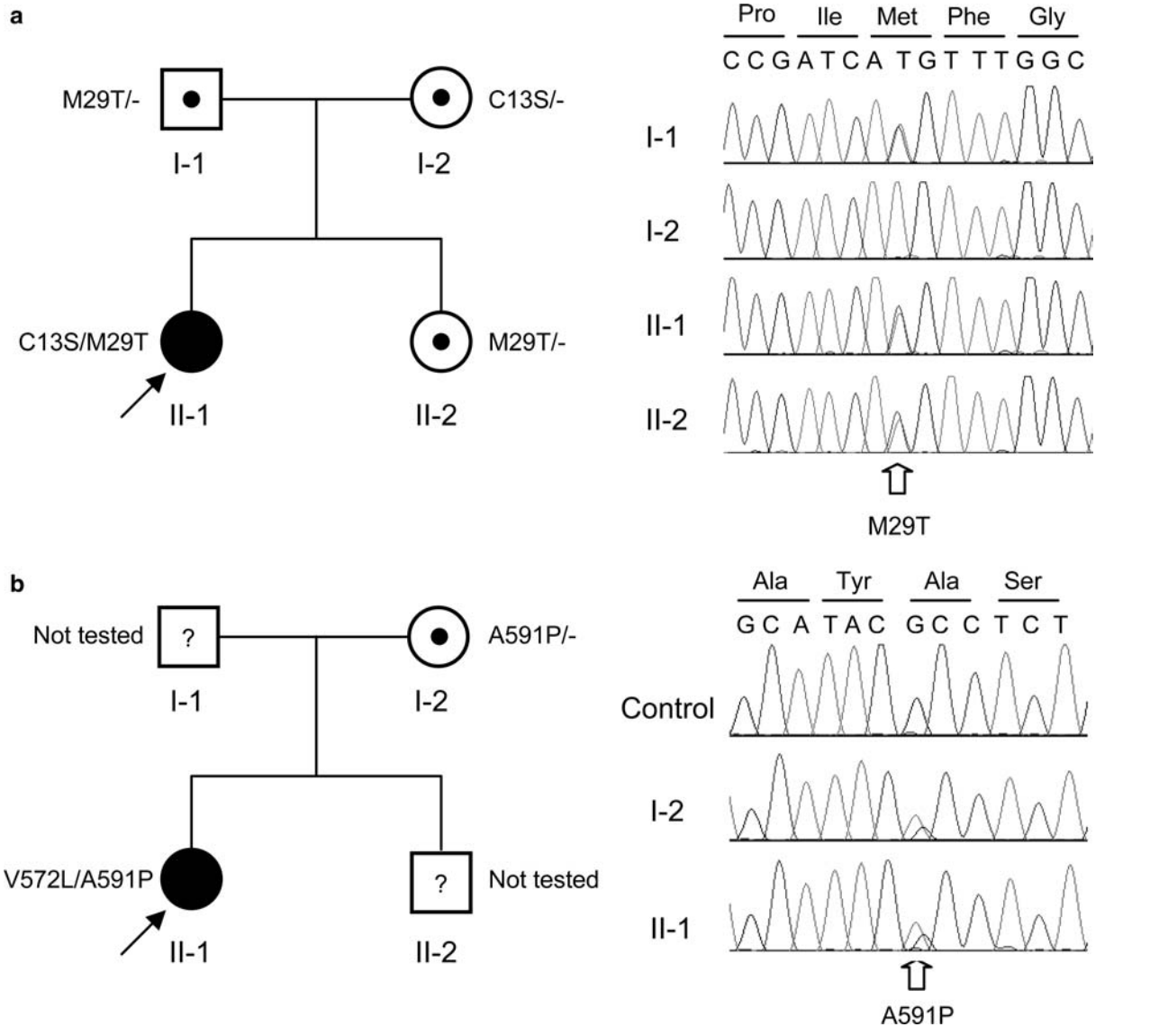

Fig. 1a,b Pedigrees and sequencing results of novel UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (*GNE*) gene mutations identified in the present study. **a** The proband (patient 2), her father, and a younger sister had the Met29Thr (c.86T > C) mutation. **b** The proband (patient 5) and her father had the Ala591Pro (c.1771G > A) mutation. *Circles* Females, *squares* males, *shaded symbols* affected, *open symbol with dot* carriers, *open symbol with question mark* possible carrier

mutation in the Japanese population, subsequent studies reported variable frequencies of this mutation: 63.5% (Nishino et al. 2002) and 57.1% (Tomimitsu et al. 2004).

In the present study, we identified five mutations in the *GNE* gene from eight Korean patients with DMRV. Among them, three mutations, C13S, R129Q, and V572L, have been previously reported in the Japanese population (Arai et al. 2002; Kayashima et al. 2002; Nishino et al. 2002; Noguchi et al. 2004; Saito et al. 2004; Tomimitsu et al. 2002, 2004; Yabe et al. 2003). The V572L mutation was the most frequent mutation observed, with an estimated frequency of 68.8%, which is comparable to that in the Japanese population. The C13S mutation, which has recently been reported in the Japanese population (Noguchi et al. 2004; Saito et al. 2004; Tomimitsu et al. 2004), was the second most frequent mutation in our subjects, with an estimated frequency of 12.5%. The R129Q mutation has also been observed in Japanese patients with DMRV (Tomimitsu et al. 2004). The remaining two mutations, M29T and A591P, were novel: they have not been reported previously and these mutations were not observed in 1,000 control chromosomes. Since the V572L mutation is the most frequent mutation in Korean as well as in Japanese patients with DMRV, and Korea is geographically very close to Japan, a common founder effect might exist in these populations.

One patient without a *GNE* gene mutation (patient 3) presented with progressive distal leg weakness and gait difficulty since the age of 16 and had a older sister with similar clinical features. The CK level was moderately elevated in this patient and the EMG study suggested myopathic changes. Because the patient refused muscle biopsy, it was not possible to confirm (or not) a diagnosis of DMRV in this patient.

In conclusion, the identification of the mutation spectrum of the *GNE* gene in Korean patients with DMRV, together with the high prevalence of the V572L mutation, suggests that DNA-based genetic testing could be a helpful diagnostic tool in Korean patients who are clinically suspected to have distal myopathy.

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