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ARTICLE

Genetics of GNE myopathy in the non-Jewish Persian population

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GNE myopathy is an autosomal recessive adult-onset disorder characterized by progressive muscle atrophy and weakness, initially involving the distal muscles, while often sparing the quadriceps. It is caused by variants in the *GNE* gene that encodes a key bifunctional enzyme in the sialic acid biosynthetic pathway. We investigated the clinical and molecular characteristics of 18 non-Jewish Persian patients from 11 unrelated GNE myopathy families. In addition, we reviewed the previously reported cases and suggest genotype–phenotype correlations for the identified variants. Comprehensive clinical and laboratory evaluations were carried out. Sequencing of the *GNE* gene was performed using genomic DNA from the patients. Screening of the identified variants was performed in all relevant family members. Molecular analyses identified three causative homozygous *GNE* variants in 11 families: c.2228T>C (p. M743T) in 7, c.830G>A (p.R277Q) in 2, and one novel variation (c.804G>A) in 2 families that results in a synonymous codon change (p.L268=) and likely creates a novel splice site affecting the protein function. This study confirms that c.2228T>C (p.M743T) is the most prevalent disease-causing variant in the non-Jewish Persian population, but other *GNE* variants can cause GNE myopathy in this population. The patients with all three different variants had similar ages of onset. The youngest patient was an 18-year-old girl in whom the c.830G>A (p.R277Q) variant was identified, whereas the oldest onset age (31 years) was seen in a male patient with c.804G>A (p.L268=). The results of this investigation expand our knowledge about the genotype–phenotype correlations in GNE myopathy and aid in clinical management and therapeutic interventions.

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INTRODUCTION

GNE myopathy, also known as hereditary inclusion body myopathy (HIBM or IBM2) and distal myopathy with rimmed vacuoles (DMRV), is a rare young adult-onset non-inflammatory progressive disabling myopathy that is inherited as an autosomal recessive trait. The disease, first described in four Jewish families of Persian descent,[1] is characterized by onset of distal muscle weakness of the lower limbs, especially the tibialis anterior muscles, which progresses to the proximal muscles. Notably, the quadriceps, for unknown reasons, is selectively spared, despite marked weakness of all other hip muscles.[2] Upper limbs, especially the scapular and proximal muscles, are usually involved in the advanced stages.[1] Typically, the initial disease manifestation is foot drop and the consequent altered gait resulting from the involvement of the foot dorsiflexion muscles. As the disease progresses patients become wheelchair bound ~10–12 years after onset.[3]

The typical histopathological findings in GNE myopathy include cytoplasmic rimmed vacuoles and congophilic inclusions mainly in angular atrophic fibers, some arranged in groups using Gomori trichrome and Congo red stains. Presence of necrotic or basophilic degenerative fibers and endomysial fibrosis are not among usual findings but can be seen in typical cases. Chronic inflammatory cell infiltration is less frequent and is a distinguishing histopathologic feature from sporadic inclusion body myositis.[4–6] The presence of diagnostic findings in biopsy depends on the site of muscle biopsy. Sometimes choosing the site of biopsy is a challenge because distal muscles could be completely wasted and proximal muscles are spared.

GNE myopathy results from variants in the *GNE* gene,[7,8] which encodes the bifunctional rate-limiting enzyme, UDP-*N*-acetylglucosamine (UDP-Glc*N*Ac) 2-epimerase/*N*-acetylmannosamine (Man*N*Ac) kinase. The *GNE* encoded enzyme consists of 722 amino acids and possesses two functional domains: an N-terminal epimerase domain (encoded by exons 2–6) and a C-terminal kinase domain (encoded by exons 7–12).[9,10] This enzyme plays a key role in the biosynthesis of *N*-acetylneuraminic acid, a member of the sialic acid family, catalyzing the rate-limiting initial two steps of the cytosolic synthesis of *N*-acetylneuraminic acid from UDP-Glc*N*Ac.[9,10] Sialic acids are the most ubiquitous terminal monosaccharides on complex glycoproteins

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and glycolipids, which are located on the surface of eukaryotic cells, with important roles in biological recognition processes, including cell–cell interactions and binding of cellular receptors to their target bacteria, viruses, parasites, and toxins.[11] In addition, sialic acids play important roles in determining the survival time and biologic activity of various glycoproteins, hormones, and enzymes, *in vivo*.[12]

The *GNE* gene, located on chromosome 9p12-13 is ~62.6 kb and consists of 13 exons encoding multiple GNE protein isoforms, its longest consisting of 753 amino acids. GNE is expressed in almost all body tissues but the highest expression has been observed in the liver.[9,12] Interestingly, the tissues with the highest GNE expression, including liver, lung, and kidney, remain intact in GNE myopathy and the disease selectively affects skeletal muscles, which have relatively low levels of the enzyme.[13]

In the mouse, GNE is expressed very early and during all stages of development.[13] Studies showed that the targeted inactivation of GNE in early mice embryos is lethal.[14] These observations suggest an important role for this enzyme in development.

GNE also plays an important role in cell regulation through interaction with two important regulatory proteins, collapsin response mediator protein 1 and the promyelocytic leukemia zinc-finger protein.[15]

The exact pathogenesis of GNE myopathy remains unknown. It has been shown that normal sialylation is crucial for the stabilization and function of skeletal muscle glycoproteins and that modifications in the sialylation of cell surface glycoproteins can influence cell adhesion and signal transduction and cause myofibrillar degeneration, resulting in loss of normal muscle function.[16–18] Studies have demonstrated that some skeletal muscle proteins, such as neprilysin,[19] alpha-dystroglycan,[16] *O*-linked glycans,[20] and neural cell adhesion molecule (NCAM)[17] are hyposialylated in GNE myopathy, a distinctive feature in comparison with other myopathies with similar clinical manifestations. The suggested impact of *GNE* variants on sialylation remains controversial and under study.

Here, we investigated the clinical features and molecular basis of GNE myopathy in 18 patients from 11 affected families from the non-Jewish Persian population.

PATIENTS AND METHODS

Patients

Eighteen HBIM patients from 11 families from the Iranian non-Jewish population were identified. The families were mainly from central and southern regions of Iran (Figure 1). The diagnosis of GNE myopathy was based on the presence of slowly progressive myopathy with a characteristic distribution of weakness (with prominent involvement of distal muscles and relative quadriceps sparing) and positive biopsy findings (presence of rimmed vacuoles).

Written consents for participation in clinical and molecular studies were obtained from all family members. The study was conducted in accordance with the Declaration of Helsinki.

Methods

Genomic DNA was extracted from peripheral blood of all patients, their parents and healthy siblings, using QIAamp DNA blood Midi kits (Qiagen, Hilden, Germany).

Muscle biopsies were taken from 11 patients, at least one affected member from each family, for histology and immunohistochemistry studies.

Affected members of each family were first tested for the Persian Jewish *GNE* founder variant, c.2228T>C (p.M743T) by targeted allele-specific primer extension analysis. For exon sequencing of the *GNE* gene, genomic DNA was PCR amplified and the resultant amplicons were then sequenced using ABI PRISM Big Dye Terminator Cycle Sequencing (Applied Biosystems, Grand Island, NY, USA) on an ABI 3730xl automated sequencer (Applied Biosystems; for details of methods see Supplementary Methods). NP_001121699 was used to denote amino acid changes in hGNE2 and NM_001128227 for nucleotide changes in mRNA variant 1.

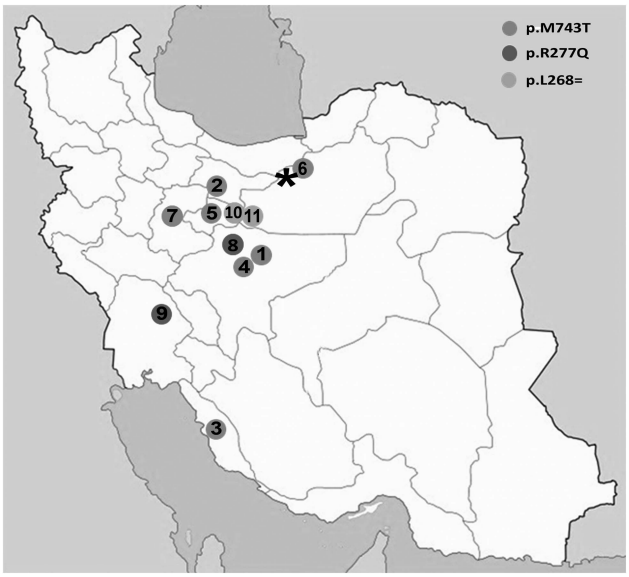

Figure 1 Geographical distribution of *GNE* variants in Iran. Numbers in the map represent the family numbers in Table 1. The black asterix shows the location of Sangsar.

The variants and associated phenotypes were submitted to ClinVar (www.clinvar.com).

RESULTS

Clinical findings

Eighteen patients from 11 Muslim Persian families: 9 (50%) female and 9 (50%) male, were examined at hospitals of Tehran University of Medical Sciences. Details of the clinical findings are shown in Table 1. The mean age at examination and mean onset age of our patients were 34 (24–47) and 25.7 (19–31) years, respectively. Nine (50%) patients from seven families had consanguineous parents. The parents of the patients were healthy. Most (67%) of our patients presented with symptoms indicative of distal lower extremity weakness such as steppage gait or tiptoe walking, whereas the main chief symptom in the others was a gait disturbance due to hip-girdle weakness. One family (10a, b, and c) had hand weakness from the beginning. Pain or muscle cramping was not a major symptom in any of the patients and sensory symptoms were generally absent. In the majority of our patients (94.5%), the quadricep muscles were relatively spared. Magnetic resonance imaging (MRI) of thigh and leg muscles of patient 7b is shown in Figure 2. In the lower extremities, hip adductors, flexors, foot dorsiflexors, and knee flexors were frequently involved, and hip abductors and plantar flexors were involved in lesser degrees. Most patients (94.5%) had some level of upper limb involvement, except for one patient (7a, aged 47 years). Weakness usually started from intrinsic hand muscles with involvement of deep finger flexors in some patients. Elbow flexors and extensors became weak later in the disease course. Three (17%) of our patients were wheelchair bound and four others (22%) used wheelchairs most of the time. Facial, ocular, and bulbar muscles were generally spared and none of our patients had scapular winging or spinal deformities. Neck flexor muscles were only involved in three (17%) wheelchair bound patients (10a, 10c, and 9a, all over 40 years of age), a finding that

Table 1 Clinical features of the patients

							Pattern of muscle involvement							
Family No.—patient	*Variant (NP_001121699)*	*Sex*	*Age (year)*	*OA (year)*	*Consanguinity*	*Initial clinical presentation*	*Prominent weak muscle(s) in lower limbs*	*Relative quadriceps sparing*[a]	*Prominent weak muscle(s) in upper limbs*	*Prominent deep finger flexor involvement*[b]	*Cardiac/ respiratory/ non-muscular involvement*	*CK levels (IU/l)*	*WCB (age)*	*EDX/muscle biopsy*
1	p.M743T	F	26	20	Yes	HGW	HA, FDF	++	IO	Yes	No/no/no	274	No	MP/RVM
2	p.M743T	M	31	26	Yes	FD and HGW	FDF, HA, and HF	++	IO	No	No/no/no	654	No	MP/RVM
3	p.M743T	M	29	23	No	HGW	HA, HF, and KF	++	IO	No	No/no/no	1975	+/−	MP/RVM
4	p.M743T	M	35	30	Yes	FD	FDF, HA, and HF	++	IO	No	No/no/no	360	No	MP/RVM
5	p.M743T	M	29	25	Yes	HGW	HA and HF	++	IO and TM	No	No/no/no	2260	No	MP/RVM
6	p.M743T	F	27	19	Yes	FD and HGW	FDF, HA, HF, and plantar flexors	++	IO mild	No	No/no/no	160	+/−	MP/RVM
7a	p.M743T	M	47	30	No	HGW	FDF and HA	++	Not involved	No	No/no/no	—	+/−	−/−
7b	p.M743T	F	35	27	No	HGW	FDF, HA, HF, and KF	++	IO and TM, elbow extensors	Yes	No/no/no	428	No	MP/RVM
Sangsari cohort[44]	p.M743T	F(4), M(1)	30–64	27–37	?	FD	?	++	?	?	?	?	1 Patient (<13 years after onset)	
ME cohort[7,22]	p.M743T	?	?	17–48	?	FD	?	5% Quadriceps involved	?	?	?	×2–4	>15 Years after onset	
8	p.R277Q	F	24	18	Yes	FD and HGW	FDF, HF, and HA	++	Distal and proximal	No	No/no/no	284	No	MP/RVM
9a	p.R277Q	F	45	27	No	FD and HGW	Proximal and distal	+	IO and proximal	No	No/no/no	—	Yes (40)	−/−
9b	p.R277Q	M	42	27	No	FD and HGW	FDF, HF, and HA	++	IO and TM	No	No/no/no	—	+/−	−/−
9c	p.R277Q	F	32	28	No	FD	FDF, HA, and HF	++	IO	No	No/no/no	172	No	MP/RVM
Taiwanese-1[42]	p.R277Q, p.I272S	F	38	21	No	FD	FDF, hamstrings, and gastrocnemius	—	Deltoid, biceps, triceps, and intrinsic hand muscles	?	No/no/no	294	Yes (30)	Fibrillations and positive sharp waves in the TA and gastrocnemius muscles, but normal motor unit potentials/RVM/atrophy with variation of fiber sizes from the gastrocnemius
Taiwanese-2[42]	p.R277Q, p.I272S	M	28	18	No	FD	FDF and gastrocnemius	+	Triceps	?	IRBBB/no/no	384	No	Normal (vastus lateralis)
Bahamas (4 patients)[7]	p.R277Q, p.D256N	?	?	18–24	No	FD	?	Mildly affected in 2 patients	?	?	?	?	?	?
Italy[7]	p.R277Q p.Q386_C387del/ p.G383fs*15	F	49	21	No	FD	Distal and proximal	+	Distal and proximal	?	No/no/no	Slight increase	?	Neurogenic/RVM
Japanese (3 patients)[33]	p.R277Q with p.D207V (2) and p.V603L (1)	F	22–36	19–34	?	?	?	?	?	?	?	?	?	?

Table 1 (Continued)

Family No.—patient	Variant (NP_001121699)	Sex	Age (year)	OA (year)	Consanguinity	Pattern of muscle involvement: Initial clinical presentation	Prominent weak muscle(s) in lower limbs	Relative quadriceps sparing[a]	Prominent weak muscle(s) in upper limbs	Prominent deep finger flexor involvement[b]	Cardiac/ respiratory/ non-muscular involvement	CK levels (IU/l)	WCB (age)	EDX/muscle biopsy
10a	p.L268=	F	42	28	Yes	HD and HGW	HF, HA, KF, and FDF	++	HD	Yes	No/no/no	200	Yes (39)	MP/RVM
10b	p.L268=	M	34	30	Yes	HD and FD	FDF	++	HD	No	No/no/no	—	No	−/−
10c	p.L268=	F	46	26	Yes	HD and HGW	HF, HA, KF, and FDF	++	Distal and proximal	Yes	No/no/no	220	Yes (40)	MP/−
11a	p.L268=	M	33	31	No	FD and HGW	HA and FDF	++	IO	No	No/no/no	603	No	−/−
11b	p.L268=	M	31	24	No	FD and HGW	HA, KF, and FDF	++	IO	Yes	No/no/no	422	No	MP/RVM
11c	p.L268=	F	24	24	No	HGW	HA and HF	++	IO	No	No/no/no	382	No	MP/−

Abbreviations: CK, creatine kinase; EDX, electrodiagnostic; FD, foot drop; FDF, foot dorsiflexors; HA, hip adductors; HD, hand weakness; HF, hip flexors; HGW, hip-girdle weakness; IO, interossei muscles; IRBBB, incomplete right bundle branch block; KF, knee flexors; MP, myopathic, OA, onset age; RVM, rimmed vacuolar myopathy; TM, thenar muscles; WCB, wheelchair bound; +/−, barely able to walk; −/−, no biopsy was performed.
[a]++: mild, ++: prominent.
[b]Compared to other hand muscles.

probably emerges in later stages. Our patients did not have any cardiac, respiratory, or other non-muscular involvement.

Electromyography (EMG) revealed myopathic changes in the examined muscles of our patients. Active denervation in the form of fibrillation potentials and positive sharp waves were all prominent electrophysiologic findings in more involved muscles and nine patients who had EMG, had active denervation in their tibialis anterior muscles. Serum creatine kinase (CK) levels were either normal or mildly elevated in some patients, but usually below 1000 IU/l. Only five patients had serum CK levels from 1338–5300 IU/l, especially in the early stages of the disease. Electrocardiography (ECG), echocardiography, and pulmonary function tests were performed in all patients and did not reveal any abnormality.

Pathological findings

Histological and histochemical studies of muscle biopsies taken from selected muscles in patients (one sample in each patient) support the diagnosis of rimmed vacuolar myopathy. Histological findings were remarkably variable and were composed of mixed myopathic and neurogenic features. The number of rimmed vacuoles ranged from very few to many and the appearance of the vacuoles varied from small cleft like to large round or irregular structure. Typical necrosis/regeneration was not seen, but endomysial fibrosis was noted in prominently affected fascicles (Figures 3a and b). Interestingly, neurogenic features were variable from small clusters of angulated fibers resembling an adult case of motor neuron disease to a prominent fascicular atrophy resembling Werdnig-Hoffmann disease on haematoxylin and eosin stain (Figures 3c and d). The details of pathologic findings are elaborated in Table 2.

Molecular analysis

Molecular analyses of *GNE* in the 11 families diagnosed clinically with GNE myopathy identified all families carrying variants that affect or are likely to affect the protein fuction. Targeted variant analysis for the known GNE myopathy founder variant, c.2228T>C (p.M743T), revealed that eight patients from seven families were homozygous for the lesion. This variant alters the methionine at position 743 to threonine in exon 13 (NCBI Reference Sequence: NG_008246.1) and is within a kinase domain. We also identified four patients from two families carrying the homozygous missense variant, c.830G>A. This variant in exon 5 replaces an arginine with a glutamine at position 277 in the epimerase domain, c.830G>A (p.R277Q). In addition, a synonymous variant c.804G>A (p.L268=) in exon 5 (NCBI Reference Sequence: NG_008246.1) was identified in six patients from two families. Although this variant was deep into the exon and did not alter a codon, there is evidence that the variant may be causative as it segregated with the disease as the parents were heterozygous and healthy siblings were either heterozygous or wild type for the identified variant whereas the affecteds were homozygous for the change. The sequence surrounding the variant is shown in Supplementary Figure 1a. This synonymous exonic variant most likely causes a splice site aberration, as revealed by the Human Splicing Finder (HSF) program (http://www.umd.be/HSF/) (Supplementary Figure 1b). The HSF calculated consensus values (CV) of potential splice sites and branch points, and predicted this variant to be 'probably damaging' creating a potential splice site.[21] This finding was confirmed by MutationTaster (http://www.mutationtaster.org/), another software application, which predicted this variant to be probably damaging and placed the variant in the 'affect protein function' class.

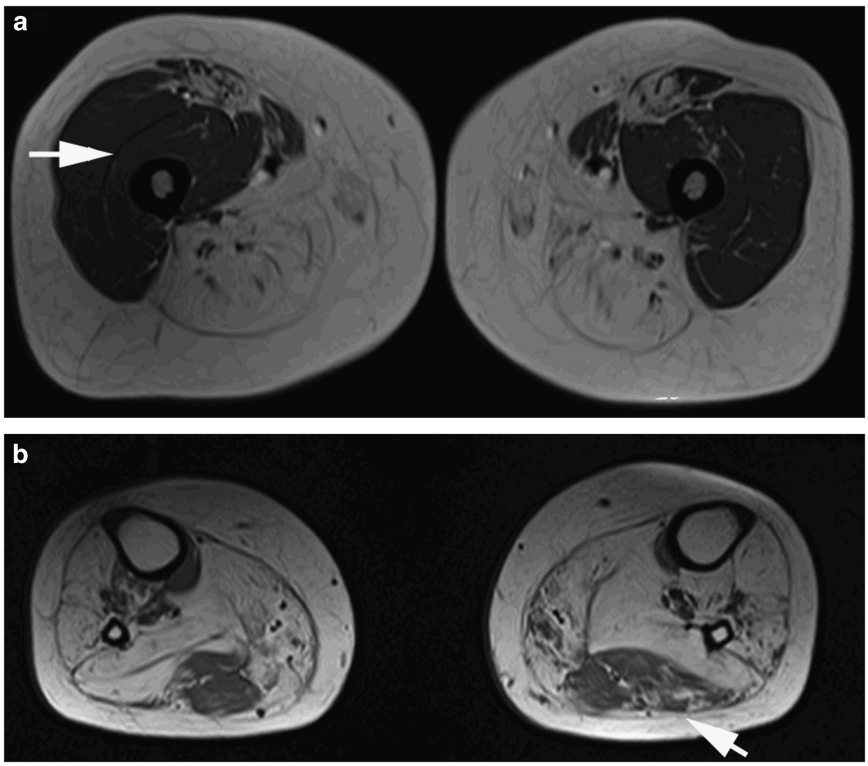

Figure 2 Thighs (**a**) and legs (**b**) T1-weighted MR images (patient 7b). (**a**) Atrophy and fat replacement of posterior thigh muscles with relative sparing of quadriceps (white arrow) and sartorius muscles. (**b**) Fatty replacement in anterior and posterior leg muscles with relatively less involvement of the lateral head of gastrocnemius muscle (white arrow).

Phenotype spectrum of variants

To date, no genotype–phenotype correlation has been described in GNE myopathy. Patients with the same *GNE* genotype may have very different clinical manifestations, not fully explained. The results of this study have expanded the phenotypic spectrum associated with variants in the *GNE* gene.

In our study, the mean 25 years of age at onset in patients with the homozygous c.830G>A (p.R277Q) variant was similar to those for patients homozygous for the c.2228T>C (p.M743T; 26.75 years) or c.804G>A (p.L268=; 27.1 years) variants. The average age of onset of Middle-Eastern patients homozygous for the c.2228T>C (p.M743T) variant (based on data from 140 patients) was 30 years.[7,22] The earliest and the latest recorded age of onset in patients from the Middle East were 17 and 48 years, respectively.[5,17,23,24] The mean onset age of Japanese and Indian patients was 26 years.[5,20] The average onset age in our 18 patients was similar, 25.7 years.

Three of our c.2228T>C (p.M743T) patients who were >6 years after the onset used wheelchairs, but none of them was wheelchair bound, even 17 years after the GNE myopathy onset (7a, 47 years; Table 1). The Middle Eastern patients harboring c.2228T>C (p.M743T) became wheelchair bound on average 15 years after the disease onset,[5] whereas ambulatory loss occurred earlier in Indian (3–9 years after the disease onset)[25] and Japanese (12 years)[3] patients. Of our patients homozygous for c.830G>A (p.R277Q; Table 1), one (9b, 42 years) used a wheelchair intermittently (15 years after onset) and another (9a, 45) became wheelchair bound 13 years after onset.

Very recently, Khademian *et al.* investigated the prevalence of c.2228T>C (p.M743T) variant in a relatively isolated community of Northern Iran, from Sangsar (also known as Mahdishahr; 35° 42′ 39″ N and 53° 21′ 14″ E). The frequencies of heterozygous and homozygous c.2228T>C (p.M743T) variant in Sangsar were 3.91 and 0.63%, respectively;[26] which are similar to those of Persian Jewish population.[27] Sangsar is only 6 km away from Shahmirzad, a city with many Jewish families. It is estimated that a subset of the present day Sangsari population is of relatively recent Jewish descent, because marriages of individuals from these two cities is very common, however, no haplotype analysis has been performed to confirm this. The average age of onset in the Sangsari patients was 32 years, similar to that of the Middle-Easterners homozygous for the c.2228T>C (p.M743T) variant. Three out of five Sangsari patients (female, 32, 40, and 64 years) had only difficulty in walking and only one patient (40 years) was wheelchair bound. The fifth individual (male) homozygous for c.2228T>C (p.M743T) did not have any obvious myopathy symptoms. None of our families were of Jewish ethnicity or from cities with a significant Jewish population, and only one of our patients (6, Table 1) was from an area close to Sangsar.

In most GNE myopathy cases, serum CK was elevated.[28] The serum CK level in our patients ranged from 160 to 2260 IU/l in cases homozygous for c.2228T>C (p.M743T), a kinase domain variant, whereas 172–274 IU/l in patients with the epimerase domain variant, c.830G>A (p.R277Q). Patients harboring the c.804G>A (p.L268=) variant had CK levels ranging from 200 to 608 IU/l. Very high CK levels (>1300 IU/l) were observed in younger patients who had a mean age of onset of 18.5 years.

Several atypical features have been identified in GNE myopathy patients, including sparse reports of major quadriceps involvement,[5] lacking distal weakness,[5] or limb-girdle myopathy.[29] Incomplete penetrance of the disease has also been described; three individuals

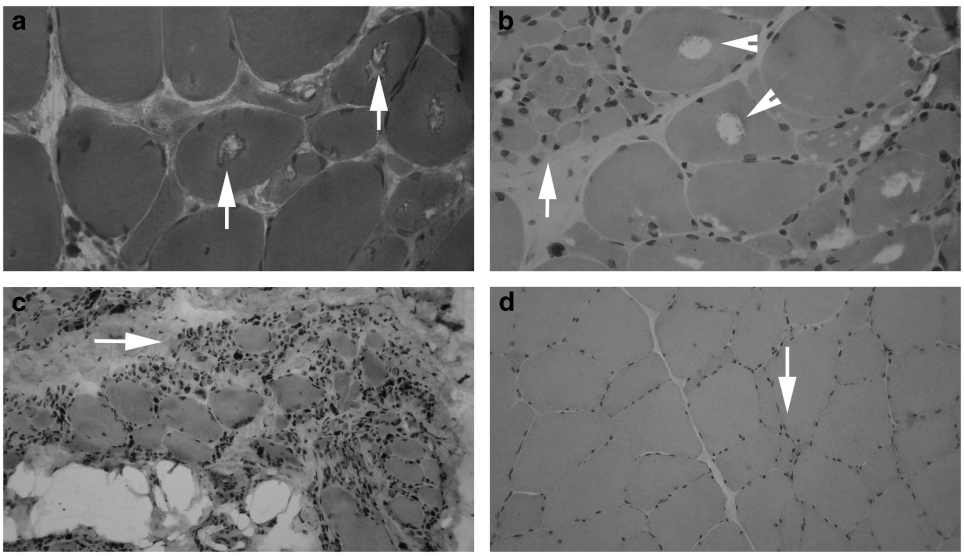

Figure 3 Pathological characteristics of GNE myopathy. (**a**) Red-rimmed cytoplasmic vacuoles showed by arrows (Gomori trichrome, high power, patient 9c muscle biopsy from right anterior tibialis). (**b**) Group atrophy (arrow) associated with prominent cytoplasmic vacuoles (arrowheads; H&E, high power, patient 9c muscle biopsy from right anterior tibialis). (**c**) Large group atrophy (arrow) resembling 'spinal muscular atrophy' (H&E, medium power, patient 11b muscle biopsy from left biceps). (**d**) Dispersed or small group of angulated atrophic fibers (arrow) resembling 'neurogenic atrophy' (H&E, high power, patient 8 muscle biopsy from left biceps). The full colour version of this figure is available at *European Journal of Human Genetics* online.

(two Muslim Arabs and one Persian homozygous for c.2228T>C (p.M743T), and one Japanese homozygous for p.D176V variant), who still remained asymptomatic in their sixth to seventh decades of life.[5,24,26] Interestingly, these three individuals were all female. Unusual biopsy findings of perivascular, perimysial, or endomysial inflammation have also been reported.[4,5]

Full involvement of quadriceps has been observed in 5% of Middle Eastern patients,[5,20] with onset age of 19–35 years.[5] These patients became wheelchair bound earlier (5–8 years after onset).[5] The involvement of quadriceps muscles was seen only in patients with at least one variant in the epimerase domain,[12,30–32] whereas patients with a homozygous variant in the kinase domain did not have quadriceps involvement; suggestive of a variant-dependent pattern for muscle involvement. Quadriceps involvement was observed only in one patient (5.5%) in this study, patient 9a who was homozygous for epimerase domain variant, c.830G>A (p.R277Q; however, not as severely involved as other pelvic girdle muscles), but not in patients with c.2228T>C (p.M743T), supporting this hypothesis. Studies in GNE myopathy patients have shown that the epimerase activity in lymphocytes is decreased, indicative of the partial functional loss of enzyme activity.[24]

Lack of distal weakness (even 5 years after disease onset) has been reported in two GNE myopathy patients from a Persian Jewish family homozygous for the c.2228T>C (p.M743T) variant. CT and MRI investigations revealed significant atrophy of the posterior hip compartment, and relative sparing of the quadriceps muscles. Distal muscles were normal on CT but had increased MRI signals on T2 in the tibialis anterior region. All patients in this study showed distal weakness.

Limb-girdle myopathy, instead of distal myopathy was very recently described in six Korean patients,[29] three being wheelchair bound. The age of onset in these patients was 16–21 years (mean 19.3 ± 1.8). Their disease started with weakness of the proximal part of the lower limbs and a waddling gait. CT scan showed involvement of hamstring and biceps brachii muscles, whereas the anterior tibial muscles were relatively spared. The *GNE* variants identified in these patients included p.V603L, p.C44S, p.G576Efs9*, p.M292V, and p.A662V. A recent study on Korean patients has reported that both homozygous p.V603L and p.C44S can cause distal myopathy as well. The mean onset age for these patients were 23.2 (p.V603L) and 30 (p.C44S) years.[33]

Cardiac involvement has been reported in a number of GNE myopathy patients, with a higher prevalence (18%) in Japanese patients.[28] Moreover, a recent animal study revealed the important role of GNE in the early development of cardiac muscles.[34] Interestingly, none of our patients nor other reported Iranian non-Jewish patients[4,22] had any heart problems.

Facial weakness is another atypical feature in GNE myopathy that was reported[5] in three patients in a Jewish and a Karaite family from the Middle East who were homozygous for c.2228T>C (p.M743T). This abnormality was not present in the patients of this study.

Prominent involvement of deep finger flexors is typically seen in sporadic inclusion body myopathy.[35] Interestingly, none of our patients with c.830G>A (p.R277Q; in the epimerase domain) exhibited out-of-proportion involvement of deep finger flexors, whereas two patients with c.2228T>C (p.M743T) and two with the c.804G>A (p.L268=) variant had prominent involvement.

DISCUSSION

GNE myopathy has been identified worldwide and in populations of diverse ethnicities, but the highest prevalence has been reported in the Persian Jewish population (1:1500) with a disease gene carrier frequency of 1–9%.[27,36] The prevalence of the disease in Japan (where the phenotype is often referred to as Nonaka myopathy) has been estimated at 1 in 1 million individuals.[8] To date, around 150 GNE myopathy cases of Persian Jewish descent have been reported.[7,22]

Here, we studied 11 non-Jewish families from Iran affected with GNE myopathy and performed comprehensive clinical and molecular investigations of the 18 affected patients. Two disease-causing

Table 2 Histological findings of biopsy samples

Family No.—patient	*Site of biopsy*	*Atrophic change*	*Group atrophy*	*Rimmed vacuoles*	*Fibrosis/adipose tissue replacement*	*Necrosis/phagocytosis*	*Degeneration/regeneration*	*Inclusion by light mic*	*Nuclear clumps*	*Angular atrophic fibers*	*Other*
1	Lt biceps	Moderate	Yes	Many	No/no	No/no	No	No	No	No	
2	Lt gastrocnemius	Mild	No	Few scattered	No/no	No/no	No	No	Few	Yes	Fiber-type grouping
3	Lt gluteus maximus	Moderate	Yes	Yes	Slight/no	No/few	Yes	Few	Yes	Yes	
4	Rt biceps	Moderate	Yes	Many	Yes/no	Rare/rare	Rare	Few	Yes	Yes	
5	Lt biceps	Mild	No	Rare (only one)	No/no	No/rare	Rare	No	No	Yes, many	
6	Lt ant tibialis	Moderate	Yes	Many	Yes/no	No/few	Few	Yes	Yes	Yes	Cytoplasmic discoloration on TG and CR
7b	Lt ant tibialis	Severe	Yes	Many	Yes/no	No/no	Yes	Yes	Yes	Yes	
8	Lt deltoid	Mild	No	Few scattered	No/no	No/no	No	No	No	Rare	Uniform type 1 fibers
9c	Rt ant tibialis	Severe	Yes	Many	Yes/slight	No/no	Yes	Yes	Yes	Yes	Round vacuoles
10a	Lt biceps	Mild	Yes (small group)	Some scattered	Slight/no	No/no	Few	No	Yes	Yes	
11b	Lt biceps	Severe	Yes	Yes	Yes/no	No/few	Yes	Yes	Yes	Few	Resembling SMA in H&E

Abbreviations: ant, anterior; H&E, hematoxylin and eosin stain; light mic, light microscope; Lt, left; Rt, right; SMA, spinal muscular atrophy.

homozygous *GNE* missense variants, c.830G>A (p.R277Q) and c.2228T>C (p. M743T) were identified to be responsible for the disease in nine families. We also identified one novel variant (c.804G>A) among six patients from two families, which leads to a synonymous codon change (p. L268L) and likely disrupts normal splicing. We were unable to obtain fresh blood or other samples for RT-PCR studies to clearly demonstrate the RNA defect.

It is likely that the c.2228T>C (p.M743T) variant was of non-Jewish origin, particularly as it was present in the non-Jewish population. It likely entered the small Jewish population and became prevalent. A similar transfer of the common Jewish type 1 Gaucher disease variant, p.N409S, has been shown to have entered the Ashkenazi Jewish population from non-Jewish Europeans where it is also frequent.[37] Since the c.2228T>C (p.M743T) variant is found in the general non-Jewish population, it is likely that it and other rare variants occurred in the non-Jewish population and that the homozygous patients' parents are distant relatives, but they are unsure of prior multigenerational relationships, particularly because the parents are from the same town.

All three variants were homozygous in the patients of this study, whereas only seven families were consanguineous. Information about the allele frequency and distribution of these variants may explain the identification of homozygous patients in non-consanguineous families, however, such data are not available in Iran. In addition, the high rate of consanguinity in the general population in Iran might also contribute to homozygosity in patients 'not clearly known' to be consanguineous.

The homozygous variant (c.2228T>C, p.M743T) is the founder variant in Persian Jewish GNE myopathy cases.[7] In patients from other ethnicities, several variants have been identified, most of which are compound heterozygotes for missense variants.[30,31] The c.2228T>C (p.M743T) founder variant has been estimated to have arisen about 2500 years ago.[23] This variant has been found in the homozygous state in Iranian Jewish[7,22] and non-Jewish,[22] Tunisian, [32] Middle Eastern Muslim,[38] Egyptian Muslim,[39] and Japanese[40,41] patients and in heterozygous state in a few patients from Italy,[36] Tunisia,[32] and Japan.[42] The most prevalent variant in the Japanese[41] and Korean[33] patients is p.V603L, followed by p.D207V in Japanese and p.C44S in Koreans. Importantly, 13%[40] of all reported cases (and 50% of Japanese cases)[41] with GNE myopathy carry at least one copy of p. V603L. Patients homozygous for this variant have a severe phenotype with earlier onset and faster progression of the disease.[40]

Most of the reported GNE myopathy variants in the *GNE* gene are missense. Only a few nonsense, frameshift, splice site variants, and indel variants[31,40] have been described in *GNE*, of which none was found in the homozygous state,[28] suggesting that this protein may have a critical role in embryonic development and viability, and that the total functional loss of GNE might be lethal in humans, as observed in mice.[14]

At amino acid position 277 of GNE, two other variants have been reported: p.R277W and p.R277Q. The p.R277W variant was identified in families of European ancestry,[22,43,44] a family from China,[43] a family of Italian descent,[45] and a family from Japan).[40] The c.830G>A (p.R277Q) variant has been identified in the heterozygous state in patients from the Bahamas (with p.D256N),[7] Taiwan (with p.I272S),[46] Italy (with p.Q386_C388del/p.G352fs*15),[47] and Japan (with p.D207V and p.V603L).[40] This study is the first to report homozygous c.830G>A (p.R277Q) in GNE myopathy. Clinical variations were noted among our patients who were homozygous for the c.830G>A (p.R277Q) variant and these previously reported cases. The average onset age of the patients in this study was ~5 years later than the

previous cases. Although foot drop was the common initial presentation in all patients, three of our patients (with the c.830G>A (p.R277Q) variant) exhibited hip-girdle weakness as well. The quadriceps muscles were involved in only one (25%) of our patients (9a, Table 1) with this variant; however, still with less severity than other thigh and pelvic girdle muscles, whereas three of nine previously reported patients had affected quadriceps. One of the previous cases[48] (Taiwanese-1, Table 1) had an incomplete right bundle branch block, but none of our or other previously reported patients had any cardiac abnormality. The involvement of upper limbs was more marked in the Taiwanese patients than that of the others. In our patients who were homozygous for the c.830G>A (p.R277Q) variant, mean CK values were lower than those in previously reported patients harboring the same variant, but in heterozygous state.

Currently, no clear genotype–phenotype correlations have been established for GNE myopathy. Varying clinical features in patients with different *GNE* variants suggest that different variants do not have equivalent functional impacts. Various clinical features associated with the same variant suggest the presence of other modifying gene variants, different genetic backgrounds, or epigenetic factors that might influence the clinical manifestations. In our analysis, we identified one novel variation that led to a synonymous codon change c.804G>A (p.L268=) that potentially creates a novel splice site. The G>A transition in the consensus splice site sequence (gene sequence) was predicted to alter splicing, by several splice site software programs thereby resulting in an altered enzyme structure and GNE myopathy.

Currently, there is no effective treatment for GNE myopathy; therefore the main focus of efforts is on prevention by prenatal carrier identification and genetic counseling for carrier couples. Recent preclinical studies with oral monosaccharides reversed the muscle hyposialylation in the GNE myopathy mouse model.[49,50] A phase II clinical trial with oral sialic acid extended release tablets and two phase I trials with Man*N*Ac are underway (www.clinicaltrials.gov).

In summary, clinical features of GNE myopathy in non-Jewish Persian patients from Iran were investigated and molecular analysis identified three disease-causing variants in the *GNE*: c.2228T>C (p.M743T), c.830G>A (p.R277Q), and c.804G>A (p.L268=). The latter is a novel variant that is predicted to cause abnormal splicing. The location of the most prevalent Middle Eastern variant, c.2228T>C (p.M743T), in the kinase domain and occurrence of the other two variants, c.804G>A (p.L268=) and c.830G>A (p.R277Q), in the epimerase domain suggest that GNE myopathy families, even from the same ethnic group, can have different *GNE* variants. This is the first report of patients homozygous for c.830G>A (p.R277Q) in the Persian population. Our findings, along with the other studies, emphasize the clinical heterogeneity of this disease. The results of this study expand the knowledge on the phenotype and molecular genetic heterogeneity of GNE myopathy, even in the non-Jewish Persian population.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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