SHORT COMMUNICATION

Tomohiko Kayashima · Hidenori Matsuo · Akira Satoh
Tohru Ohta · Koh-ichiro Yoshiura · Naomichi Matsumoto
Yoshibumi Nakane · Norio Niikawa · Tatsuya Kishino

Nonaka myopathy is caused by mutations in the UDP-*N*-acetylglucosamine-2-epimerase/*N*-acetylmannosamine kinase gene (*GNE*)

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Abstract This is the first report on mutations of the UDP-*N*-acetylglucosamine-2-epimerase/*N*-acetylmannosamine kinase gene (*GNE*) in Nonaka myopathy or distal myopathy with rimmed vacuoles (OMIM 605820), an autosomal recessive neuromuscular disorder. Sequence and haplotype analyses of *GNE* in two siblings with Nonaka myopathy from a Japanese family revealed that both patients were compound heterozygotes for a C→T transition (A460V) in exon 8 and a G→C transition (V572L) in exon 10. Their parents and a normal elder brother were all carriers for one or the other of the mutations. *GNE* mutations are known to cause two other disorders: sialuria (OMIM #269921) and autosomal recessive inclusion body myopathy (IBM2, OMIM #600737). Mutations associated with sialuria are located in the epimerase domain, and those associated with IBM2 are in the epimerase or the kinase domain or both, whereas the mutations we observed in the Nonaka myopathy patients were located in the sugar kinase domain of the gene. Thus, Nonaka myopathy is the third disease known to be caused by *GNE* mutations.

Key words Nonaka myopathy · Distal myopathy with rimmed vacuoles · Autosomal recessive inclusion body myopathy (IBM2) · UDP-*N*-acetylglucosamine-2-epimerase/*N*-acetylmannosamine kinase gene (*GNE*) · Sialuria · Compound heterozygote

Introduction

Nonaka myopathy or distal myopathy with rimmed vacuoles (OMIM 605820) is an autosomal recessive neuromuscular disorder characterized by early adult onset, weakness of distal muscles in the lower limbs, and a mildly elevated serum creatine kinase (CK) level. Muscle biopsies have shown that numerous rimmed vacuoles are distinguishable in affected muscle fibers without inflammation (Nonaka et al. 1981). Although the disease appears to be common in Japan, with an estimated prevalence of one in 1 million persons, a similar distal myopathy has also been described as an autosomal recessive inclusion body myopathy (IBM2, OMIM #600737) in several families of Persian-Jewish and Kurdish-Iranian-Jewish origin (Argov and Yarom 1984; Massa et al. 1991). Linkage analysis revealed the IBM2 locus at chromosome 9p13-p12 (Eisenberg et al. 1999), and mutation analysis of the UDP-*N*-acetylglucosamine-2-epimerase/*N*-acetylmannosamine kinase gene (*GNE*) identified compound heterozygous missense and nonsense mutations in IBM2 families (Eisenberg et al. 2001). *GNE* is an enzyme involved in the biosynthesis of sialic acid, and it has 98% identity to the rat or mouse orthologue. Each of the two enzymatic domains of the *GNE* protein also has homology to the respective bacterial protein. Mutations in human *GNE* also cause a metabolic disorder, sialuria (OMIM #269921). Clinical and pathological similarities as well as linkage data at 9p1-q1 in Japanese families with Nonaka myopathy (Ikeuchi et al. 1997) suggest that Nonaka myopathy may be allelic with IBM2. We report here the result of a mutation analysis of *GNE* in a Japanese family with Nonaka myopathy.

T. Kayashima (✉) · K. Yoshiura · N. Matsumoto · N. Niikawa
Department of Human Genetics, Nagasaki University School of
Medicine, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan
Tel. +81-95-849-7120; Fax +81-95-849-7121
e-mail: d399022y@stcc.nagasaki-u.ac.jp

H. Matsuo
Department of Neurology, Kawatana National Hospital, Nagasaki,
Japan

A. Satoh
Section of Neurology, Nagasaki Kita Hospital, Nagasaki, Japan

T. Ohta · N. Niikawa · T. Kishino
Nagasaki University Gene Research Center, Nagasaki, Japan

Y. Nakane
Department of Neuropsychiatry, Nagasaki University School of
Medicine, Nagasaki, Japan

T. Kayashima · K. Yoshiura · N. Matsumoto · N. Niikawa ·
T. Kishino
CREST, JST, Kawaguchi, Japan

Fig. 1. *GNE* mutations in a Japanese family with Nonaka myopathy. **a** Sequence chromatograms of two heterozygous missense mutations. **b** Family tree showing haplotypes and *GNE* mutations. *GNE* is located between D9S1788 and D9S1791. *GNE* 1430 and *GNE* 1765 indicate base substitutions in exon 8 and 10, respectively. W and M are wild-type and mutant alleles, respectively, in *GNE* 1430 or *GNE* 1765

Subjects and methods

The affected family had two patients. Patient 1, a 33-year-old Japanese man (II-2, Fig. 1b), was first noticed to have walking difficulty at age 21. The walking disturbance and muscle atrophy in his lower limbs, especially in the anterior tibial muscles, gradually progressed. Neurological examinations at age 26 revealed marked weakness and atrophy of the anterior tibial muscles and hand muscles. Tendon reflexes were diminished without any other neurological abnormalities. The serum CK level was mildly elevated. An electromyogram demonstrated a mixed pattern of neurogenic and myogenic changes with positive sharp wave, fibrillation, and short duration polyphasic potential.

Patient 2 (II-3), a 32-year-old younger brother of Patient 1, was first noticed by neighbors to have a walking abnor-

Fig. 2. Muscle biopsy of the anterior tibial muscle of patient 2 (II-3). Numerous rimmed vacuoles (arrowheads) and variations in fiber size without inflammation were observed in hematoxylin-eosin (HE)-stained (a) and Gomori trichrome-stained (b) preparations

mality at the age of 24 years. He himself gradually became aware of weakness in the anterior tibial muscles and atrophy in the hand muscles. Neurological examinations confirmed the muscle atrophy and weakness. Biceps and triceps reflexes were normal, but ankle and knee reflexes were diminished. The serum CK level was mildly elevated. An electromyogram showed a mixed pattern of neurogenic and myogenic changes, and muscle biopsy revealed rimmed vacuoles in the anterior tibial muscle fibers without inflammation (Fig. 2). The parents and an elder brother (II-1) were all phenotypically normal, and consanguinity of the parents was denied.

DNA was extracted from peripheral blood lymphocytes of the five family members, including the patients, after informed consent was obtained. DNA was amplified by polymerase chain reaction (PCR) using primer sets for 13 exons of *GNE* as described previously (Eisenberg et al. 2001), and PCR products were sequenced on an autosequencer (ABI PRISM 3100, PE Applied Biosystems, Foster City, CA, USA). PCR was also performed using primers for 13 microsatellite markers spanning chromosome 9 (Dib et al. 1996), and PCR products were subjected to haplotype analysis.

Results and discussion

Two different missense mutations were found in the family: a C→T transition (A460V) in exon 8 and a G→C transition (V572L) in exon 10 of *GNE* (Fig. 1a). The parents and unaffected brother all had one or the other of these mutations. Amino acid positions of these novel mutations, A460V and V572L, are well conserved in both rat and mouse, and the V572L mutation is located at a position highly conserved even in bacteria. The same mutations were not found in 80 normal Japanese individuals. The haplotype analysis revealed that both patients were compound heterozygotes for the mutations (Fig. 1b).

UDP-*N*-acetylglucosamine-2-epimerase/*N*-acetylmannosamine kinase (*GNE*) is an enzyme involved in the biosynthesis of sialic acid, with which modification of cell surface molecules is crucial for their function in many biological processes, including cell adhesion and signal transduction (Hinderlich et al. 1997; Stäsche et al. 1997; Keppler et al. 1999). *GNE* is bifunctional, having epimerase activity in its N-terminal region and, independently, sugar kinase activity in the C-terminal region (Effertz et al. 1999). As mentioned above, mutations in its gene (*GNE*) are known to cause two different disorders, sialuria and IBM2. Sialuria is a seemingly autosomal dominant metabolic disorder characterized by cytoplasmic accumulation and increased urinary excretion of free *N*-acetylneuraminic acid, and three heterozygous mutations at codons 263 to 266 in the epimerase domain of *GNE* were identified in patients with the disorder (Seppala et al. 1999). In contrast, the *GNE* mutations in the families with IBM2 were located in the epimerase domain, the kinase domain, or both (Eisenberg et al. 2001), while those identified in our patients with Nonaka myopathy were confined to the sugar kinase domain. Thus, Nonaka myopathy is the third clinical entity associated with *GNE* mutations. Although the biochemical mechanisms of mutant *GNE* in myopathy, e.g., epimerase and/or kinase activity, remain unknown, it is plausible that domain-specific mutations, either dominant or recessive, result in phenotypic differences among these disorders. Nonaka myopathy is similar in its clinical features to IBM2, and the main difference between them is the distribution of muscle weakness. Nonaka myopathy is less sparing of the quadriceps within 10 years of onset. Further mutation analysis of Nonaka myopathy and IBM2 will clarify the genotype–phenotype correlation between the two, and help us understand the role of *GNE* in neuromuscular disorders.

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