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Single nucleotide polymorphisms in the dystroglycan gene do not correlate with disease severity in hereditary inclusion body myopathy

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Abstract

Aberrant glycosylation of dystroglycan occurs in certain muscular dystrophies, including hereditary inclusion body myopathy (HIBM). HIBM harbors a widely varying clinical severity and age of onset, which raised the suspicion of the presence of disease modifier genes. We considered the highly polymorphic dystroglycan gene (*DAG1*) as a feasible candidate modifier gene. *DAG1* genomic DNA was sequenced for 32 HIBM patients, mainly of Persian-Jewish descent. Five novel *DAG1* single nucleotide polymorphisms (SNPs) were identified, bringing the total number of SNPs to 19. However, no direct correlation between *DAG1* SNPs and clinical severity of HIBM could be detected. Several identified SNPs substitute an amino acid and might modulate dystroglycan function or glycosylation status, and deserve further research. These data are valuable for future studies on the role of *DAG1* in HIBM and other muscular dystrophies, especially those dystrophies that involve abnormal glycosylation of dystroglycan.
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Introduction

Hereditary inclusion-body myopathy (HIBM; MIM 600737) is an autosomal recessive neuromuscular disorder characterized by adult onset of weakness and atrophy of the distal lower limb muscles, with relative sparing of the quadriceps [1–4]. Age of onset and disease progression are highly variable [1–7]. The disorder results from predominantly missense mutations in the UDP-*N*-acetylglucosamine (GlcNAc) 2-epimerase/*N*-acetylmannosamine (ManNAc) kinase gene (*GNE*; MIM 603824) on chromosome 9p13.2. A *GNE* founder mutation (M712T) was originally described in Persian-Jewish HIBM families [8], followed by reports of numerous other *GNE* mutations in patients worldwide [5,7,9–15]. UDP-GlcNAc 2-epimerase/ManNAc kinase is a ubiquitously expressed, bifunctional, rate-limiting enzyme that is crucial in sialic acid biosynthesis [16–18]. HIBM-associated *GNE* mutations result in a reduced activity of both the GlcNAc 2-epimerase and the ManNAc kinase enzymes [19–22], most likely leading to a disturbance of sialic acid metabolism and ultimately muscle fiber degeneration. The exact pathologic mechanism remains unknown [18,20–25].

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GNE mutations result in hyposialylation of skeletal muscle proteins, but the extent of hyposialylation appears to be variable and related to the specific *GNE* mutation [20–23]. Recently, we and others demonstrated decreased glycosylation of α-dystroglycan [23,24], an integral component of the dystrophin–glycoprotein complex [26], in HIBM patients' muscles. These findings, similar to those apparent in other congenital muscular dystrophies such as Muscle–Eye–Brain disease (MEB), Fukuyama congenital muscular dystrophy (FCMD), and Walker–Warburg syndrome (WWS) [27,28], provided a possible explanation for the cause of muscle weakness in patients with *GNE* mutations. Specifically, decreased availability of sialic acid could lead to reduced sialylation of the *O*-mannosyl linked glycans present in the mucin-like domain of α-dystroglycan [24]. These glycans appear to be critical for interactions of muscle α-dystroglycan with laminin and other extracellular matrix ligands [26,29].

However, it remains uncertain why individuals with the same *GNE* mutations manifest vastly different degrees of severity with respect to their myopathy. One possibility relates to dystroglycan itself. The gene coding for both α- and β-dystroglycan, *DAG1* (dystrophin-associated glycoprotein 1; MIM 128239) on chromosome 3p21 [30,31], is highly polymorphic, with several amino acid substituting single nucleotide polymorphisms (SNPs). In the current study, we investigated if SNPs in the *DAG1* gene were correlated with any particular age of onset or severity of the HIBM phenotype.

Materials and methods

Patients and DNA

DNA of 28 patients with HIBM (patient #1–28), of Persian-Jewish descent, was collected by the HIBM Research Group, Encino, CA (Dr. D. Darvish). Four additional HIBM patients (Patient #29–32) were enrolled in a clinical protocol, approved by the institutional review board of either the National Human Genome Research Institute or the National Institute of Neurological Disorders and Stroke. The diagnosis of HIBM was based on clinical features, muscle pathology, and the presence of *GNE* gene mutations. Written informed consent was obtained from each patient. DNA was isolated from patients' peripheral blood using standard procedures.

PCR and sequencing

The three *DAG1* (GenBank NM_004393) exons and their intron/exon boundaries were PCR amplified from genomic DNA of all 32 patients and 8 control individuals, employing the primers and conditions listed in Table 1. PCR amplification was followed by direct sequencing using a Beckman CEQ 2000 automated sequencer, using the manufacturer's CEQ Dye Terminator Cycle Sequencing kit and protocols (Beckman Coulter, Fullerton, CA). BLAST analyses were performed for sequence homology searches (http://www.ncbi.nlm.nih.gov/BLAST/). Detected polymorphisms were compared with SNP databases (Entrez SNP; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp).

Results

Genetic and clinical information regarding the 32 HIBM patients are presented in Table 2; 29 patients were homozygous for the Persian-Jewish M712T founder mutation in *GNE*, and 3 patients carried other *GNE* mutations. The age of onset of symptoms varied between 20 and 59 years and the current ambulation status varied from walking unassisted to wheelchair bound.

Table 1
Primers and intron/exon boundaries for *DAG1*[a]

Exon No.[b]	Size (bp)	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Annealing temperature (°C)	5′splice site exon/intron	Intron Size (bp)	3′splice site intron/exon
1	326	TGCAACACTCGCGTG TCAGGCG	CAGCAGGTGTTCCGC GGAAGGC	61	GCGCTGG/gtgagtg	39986[c]	ttttcag/GCTCTGT
2	720	GTTTCTGTATAAGAG ACCATG	GATGCTATTCTGAGT ATATAG	58	CATCAAG/gtgagac	19978[d]	cttctag/GTATCAG
3-A	776	GCTTCCCCAGCAGGC ATTCTGA	GCCTCCGGACGCGTT TGGGAAG	61	—	N/A	—
3-B	880	GTCTGCTCAGCTTGG CTACCCT	TGGCATGCATGAAAT ACTCGTG	61	—	N/A	—
3-C	863	GACAAGCTGAAGCTG ACCCTGA	CAGAATGAGTGGCAT GCTGGAG	61	—	N/A	—
3-D	470	GCATCATTGCCATGAT CTGCTA	GTCTGCAGGCCACGG TCTCCA	61	CTTTATT	N/A	—

[a] Primer sequences and intron/exon sizes are derived from *DAG1* GenBank Accession No. NM_004393.
[b] *DAG1* consists of three exons. Exon 3 was amplified in 4 overlapping fragments (3A-D).
[c] Intron 1.
[d] Intron 2.