CASE REPORT

A novel missense mutation in the GNE gene in an Iranian patient with hereditary inclusion body myopathy

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Hereditary inclusion body myopathy (hIBM) is an adult-onset hereditary myopathy, usually with distal onset and quadriceps sparing. This myopathy is autosomal recessive and associated to UPD-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (GNE) gene mutations. In this study, we report a novel GNE homozygous point mutation c.1834T>G that results in amino acid substitution of cysteine 612 to glutamine in an Iranian patient. This mutation is located in exon 10 within the kinase domain of the protein.

Key words: GNE, hIBM, neuromuscular, sialic acid

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INTRODUCTION

Hereditary inclusion body myopathy (hIBM) is a neuromuscular disorder that is characterized by early adulthood onset, ethnic predisposition, distal and proximal weakness, and quadriceps sparing.[1] Typical muscle pathology of hIBM includes rimmed vacuoles and filaments inclusion without inflammatory cells.[2] The first patient affected with hIBM was described in 1984 among Iranian Jews.[3] In the Iranian Jewish population, the frequency is greater than 1 in 1 500 individuals.[4]

Autosomal recessive hIBM2 is related to GNE gene mutations.[5] In a pathological point of view, it is widely accepted that the presence of autophagic or rimmed vacuoles are the most characteristic feature of myopathy associated with GNE mutations.[6]

The GNE gene encodes a bifunctional enzyme UPD-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase which has a key role in sialic acid biosynthesis. GNE is ubiquitously expressed with the highest level in liver and relatively low levels in skeletal muscle.[4,7] GNE gene is located on short arm of chromosome 9 and its major transcript consists of 13 exons that 11 of them[2-12] are coding.[8,9]

In this study, we report clinical data and genetic analyses of the GNE gene in a patient from an Iranian family affected by autosomal recessive hIBM.

MATERIALS AND METHODS

Clinical data

A 48-year-old woman who was born in Iran from non-Jewish, non-consanguineous marriage, referred to our lab. Although our case had no family history of neurodegenerative disorders, she had a history of immune thrombocytopenic purpura (ITP) and hypothyroidism, and also her elder brother had ITP. The disease onset age was at 28 years with weakness in lower extremities that progressed gradually to upper limbs. Neurological examination demonstrates bilateral foot drop, positive Gower's sign, and significant weakness of lower extremities. Manual muscle testing revealed 5/5 strength in quadriceps muscles, but other proximal and distal muscles of lower extremities were 3/5. In upper extremities, strength of proximal muscles was 4/5 and flexor pollicise lunges and flexor digit rum profoundus were 5/5 and 2/5, respectively. Sensation and cranial nerves were intact.

Electromyographic study of left biceps and deltoid muscles revealed normal motor unit interference pattern with low amplitude and short duration motor unit

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action potential without abnormal spontaneous activity. Left tibialis anterior revealed a few polyphasic motor unit action potentials. Nerve conduction studies were within normal limits.

Serum creatine kinase was increased to 347 IU/L (CK normal: 32 to 267 IU/L).

Muscle biopsy was performed on her left biceps and fresh muscle sample was frozen on isopentane cooled in liquid nitrogen and frozen muscle sections were stained by routine histochemistry panel including hematoxylin and eosin, Gomori trichrome, PAS, oil red O, NADH-TR, SDH, COX, and ATPase reactions. The biopsy was interpreted as a rimmed vacuolar myopathy [Figure 1]. No inflammatory infiltrate was found. Based on her phenotype and the myopathology findings, the diagnosis of hereditary inclusion body myopathy was proposed.

Genetic analysis

Genomic DNA was extracted from peripheral blood of the patient by salting out method.[10] PCR was performed using a set of primers to amplify all coding exons and flanking intron region of GNE gene.[9] The PCR product was directly sequenced with ABI 3730xl (Life Technologies, USA) automated sequencer and analyzed with sequence analysis software (Finch TV, Perkin Elmer, USA).

RESULTS

Sequence analysis of the GNE gene exons and flanking region in patient revealed a homozygous mutation thymine to guanine transversion at position 1834 in exon 10 of the gene (RefSec: NM_01128227.2). Further analysis on her daughters showed that both of them were carrier for the mutation [Figure 2]. The mutation c.1834T>G results in amino acid substitution of cysteine 612 to glycin (p.C612G) and change in splice site. The mutation is of the residue conserved in mammals, zebrafish, and mouse, and is predicted to be pathogenic using the mutation taster program (http://www.muatationtaster.org/).

Confirmation of the pathogenicity of the mutation was done by PON-P (pathogenic or not pipeline) program (http://bioinf.uta.fi/PON-P).

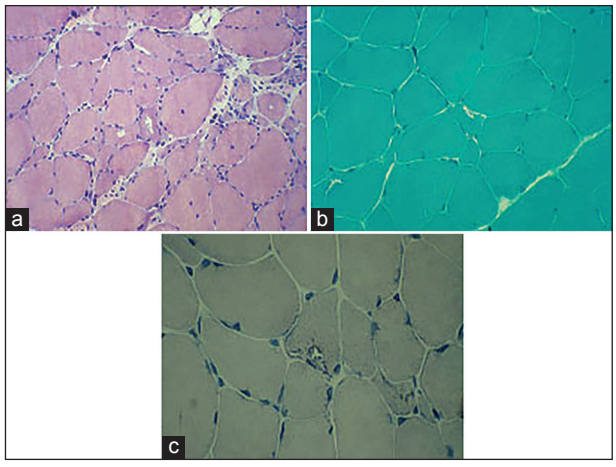

Figure 1: (a) Prominent muscle fiber size variation with small group atrophy and some nuclear clumps, two of the central fibers contain granular-rimmed cytoplasmic vacuoles (H&E). (b) Slightly red-rimmed cytoplasmic vacuole (Gomori trichrome stain). (c) Basophilic-rimmed cytoplasmic vacuole with congophilic inclusions

DISCUSSION

The coding exons of UPD-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (GNE) gene encode a bifunctional enzyme that initiates and regulates the biosynthesis of N-acetylneuraminic acid (NeuAc), a precursor of sialic acids. Sialic acid is a crucial factor in many biological processes, although its specifications associated with hIBM remain to be clarified. It is a rate-limiting enzyme in the sialic acid biosynthetic pathway. The N-terminal portion of GNE (amino acids 1-378) has UDP-GlcNAc 2-epimerase catalytic activity,[11] which catalyzes the epimerization of UDP-GlcNAc to ManNAc with release of UDP. The C-terminal portion (amino acids 410-722) has ManNAc kinase catalytic activity, which phosphorylates ManNAc to ManNAc-6-P and phosphoenolpyruvate. ManNAc-6-P is then further condensed to sialic acid. So GNE mutations have been shown to reduce sialic acid production.[12]

There are over 80 GNE gene mutations described that are revealed to hIBM in patients of different ethnic background till now. The majority of mutations in GNE are missense.[9,13-17] The M712T missense mutation located in exon 12 of GNE gene is the most common mutation in the Middle-Eastern families. However, this mutation was also found in Italian[18] and Japanese[19] patients.

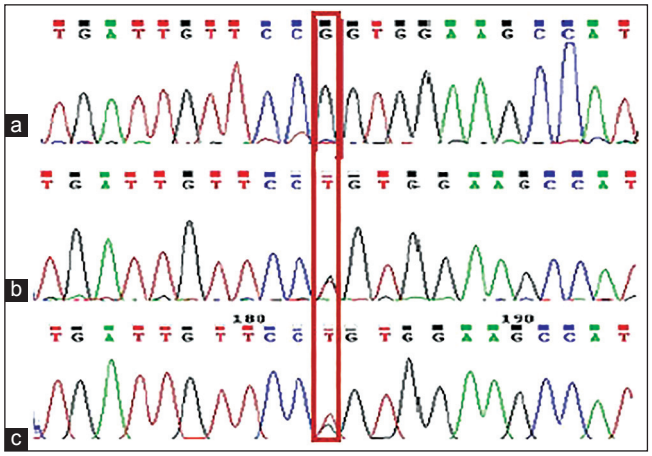

Figure 2: Sequencing results of GNE gene. Nonsynonymous homozygous mutation c.1834 T>G is found in exon 10 of the gene (a) and same mutation at the location is found in the patient's daughters in heterozygous form (b and c)

In this study, we report a novel GNE homozygous point mutation c.1834T>G in an Iranian patient. The c.1834T>G mutation is located in exon 10 within the kinase domain of the protein. In Leiden database, another substitution at this location (c.1834T>C) was also reported and considered as a pathogenic mutation (http://www.dmd.nl).

This mutation is probably pathogenic because of its absence in dbSNP database, affect of the mutation in a phylogenetically conserved residue within the GNE protein, and presence of a different nucleotide change (c.1834T>C that exchange Cys 612 to Arg) at the same spot that is marked as pathogenic. However, no experimental evidence for the mutation pathogenicity was done.

AUTHORS' CONTRIBUTION

MS contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. SH contributed in the acquisition of data, analysis and interpretation of data, approval of the final version of the manuscript, and agreed for all aspects of the work. DK contributed in the acquisition of data, analysis and interpretation of data, approval of the final version of the manuscript, and agreed for all aspects of the work. KB contributed in the conception and design of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. YN contributed in the conception and design of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. MB contributed in the writing a manuscript, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

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