A Family with Distal Hereditary Motor Neuropathy and a K141Q Mutation of Small Heat Shock Protein \(HSPB1\)

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Abstract

We herein describe a Japanese family with distal hereditary motor neuropathy carrying a K141Q mutation of small heat shock protein \(HSPB1\). Two patients among them had late onset disease (older than 50 years). The muscles of the distal legs were weak and atrophic. Sensory and autonomic dysfunction were not seen. Even eight years after onset, one patient could still walk without support. A nerve conduction study revealed axonal degeneration of the motor nerves of the legs. A heterozygous K141Q mutation was detected in the affected patients. The late onset and mild clinical phenotype might reflect the mild biochemical alteration of HSP27 induced by the K141Q mutation.

Key words: Charcot-Marie-Tooth disease, distal hereditary motor neuropathy, heat shock protein

(Intern Med 53: 1655-1658, 2014)
(DOI: 10.2169/internalmedicine.53.2843)

Introduction

Mutations of several genes are known to cause distal hereditary motor neuropathy (dHMN). There is often an overlap with Charcot-Marie-Tooth disease (CMT2) and the juvenile form of amyotrophic lateral sclerosis. Heat shock protein (HSP) 27 is one of the causative proteins resulting in dHMN or CMT2F. Its clinical phenotypes differ based on the \(HSPB1\) mutations (1). In Japan, only three families with dHMN and \(HSPB1\) mutations have been reported so far (2-4). We herein report another family with dHMN with a mutation in this gene.

Case Reports

Case 1 (the proband)

A 69-year-old man presented with gait disturbance which had appeared one year earlier. He did not have numbness in his feet. Before the onset, he had been exercising at a gym near his house. He had not been exposed to any toxic organic solvent or heavy metals. There were some individuals showing similar symptoms in his family.

During the initial examination, the patient was alert, and there were no abnormal finding for his cranial nerves. Muscular atrophy was obvious in his legs, and pes cavus was observed (Fig. 1A). The muscle strength was decreased to 4/4 in the anterior tibial muscles. The strength of the other muscles was normal. Sensations of light touch, pain, temperature, vibration, and position were normal. The tendon reflexes were symmetrical and decreased in all four limbs. No pathological reflex was evoked. The patient’s coordination was normal. He could not stand on his heels. He did not have any autonomic symptoms, such as orthostatic hypotension or urinary incontinence. The complete blood cell count was normal. The erythrocyte sedimentation ratio was 13/33 mm (one hour/two hours). The parameters of liver function and renal function were within the normal limits. The serum level of creatine kinase was mildly elevated, to 488 IU/L. The levels of fasting blood glucose, electrolytes, and lipids were normal. Anti-nuclear antibody, proteinase 3-anti-neutrophil cytoplasmic antibody (PR3-ANCA), and myeloperoxidase (MPO)-ANCA, and the levels of vitamin B1, B12 and folic acid were normal.

The cell count of the cerebrospinal fluid (CSF) was 2/3 mm\(^3\). The levels of protein and glucose in the CSF were 46 mg/dL and 64 mg/dL, respectively. A nerve conduction...
study (Table) revealed a decreased amplitude of the compound muscle action potentials in the tibial nerves. The amplitudes of the other motor nerves were relatively spared. The amplitude of the sensory nerve action potential was not decreased, except for the right sural nerve. The conduction velocities were not decreased in either the motor or sensory nerves. He refused to undergo a sural nerve biopsy.

**Case 2 (the son of the proband’s cousin)**

The second patient was sixty years old at the time of our examination. He had experienced difficulty walking for eight years. His grandmother had also experienced difficulty walking. The patient’s calf was atrophic and pes cavus was also found (Fig. 1B). The muscle strength was 1/1 at the anterior tibial muscles and 2/2 at the gastrocnemius muscles. The strength of the other muscles was normal. He did not have any sensory symptoms, such as numbness. The sensations of light touch and temperature were normal, but the vibration sensation was slightly decreased. All tendon reflexes were decreased. No pathological reflex was evoked. He could walk by himself without any support.

**Other family members** (Fig. 2A)

Three cousins of the proband had similar symptoms. One of them had previously been examined at another hospital. He had undergone sural nerve biopsy and was diagnosed with axonal type CMT, but had not undergone a genetic diagnosis. Although the surviving patients had some level of walking disability, their activity of daily life was relatively preserved. The disease onset was after age fifty in all of the patients. Although we asked the family members to undergo re-examination of their neurological condition, they rejected our proposal.

**Gene analysis of the proband and his family** (Fig. 2B)

Genomic DNA was extracted from the peripheral blood leukocytes of the patients using the Gentra Puregene Blood
Kit (Qiagen, Duesseldorf, Germany). A panel of sixty genes, including 40 known CMT disease-causing genes and 20 candidate genes were screened (AARS, ANKG, APTX, ARHGEF10, CARS, CNTF, CNTN2/TAG1, DARS, DHI, DNM2, EGR2, EPRS, FARSA, FARSB, FGD4, FIG4, GAN, GARS, GDAP1, GJB1, HARS, HK1, HOXD10, HSPB1, HSPB8, IARS, KARS, KCC3, LARS, LITAF, LMNA, MARS, MED25, MFN2, MPZ, MTMR2, NARS, NDRG1, NEFL, PEPD, PMP22, PRPS1, PRX, QARS, RAB7, RARS, SBF2, SCN8A, SETX, SH3TC2, SOX10, TARS, TDP1, TRPV4, TTR, VARS, WARS and, YARS). Using the Primer 3 program, we designed 861 oligonucleotide primers covering the entire coding exons and exon-intron junctions, with an amplicon length of 350-500 base pairs. Briefly, all fragments were amplified by multiplex polymerase chain reaction (PCR) (Qiagen Multiplex PCR Kit; Qiagen) and then were mixed to build the amplicon DNA library. As an initial input, 50 ng of the DNA library was fragmented and tagged simultaneously with the Nextera transposome, then multiple index 1 (i7) and index 2 (i5), as well as common adapters (P5 and P7, respectively) were ligated. After small DNA fragments (shorter than 300 bp) were removed using the AMPure PCR purification system (Agencourt Bioscience, Beverly, USA), the library was adjusted to a working concentration of 2 nM. The target re-sequence analysis was performed using a next-generation sequencer (MiSeq®, Illumina, San Diego, USA). After cluster generation through a bridge PCR, paired-end sequencing (150×2) was performed on a flow cells; clusters were imaged using light emitting diode (LED) and filter combinations specific to each of the four fluorescently-labeled deoxyribonucleotides. After base-calling, filtering, and quality scoring, fastq files were generated. Using the CLC Genomics Workbench 6 software program (CLC bio, Aarhus, Denmark), the output reads were aligned with the reference sequence, and thereafter the variants were called and annotated for the analysis.

To confirm the mutation revealed by next-generation sequencer, the proband and two members of the family underwent a genetic analysis by the Sanger method for direct sequencing. In the two affected individuals, we detected a heterozygous c.421A>C (p.K141Q) missense mutation in the HSPB1 gene. The proband’s younger brother, who was neurologically normal, did not have this mutation.

**Discussion**

This family is the fourth reported Japanese family with autosomal dominant dHMN with a HSPB1 mutation. The K141Q mutation was first reported by Ikeda et al. (4). The ages of onset of their two cases were 47 years and in the fifties. Compared with these patients, the onset in our Case 1 occurred at an older age. In the first reported family with this mutation, severe dysfunction of the autonomic nervous system was reported. Unlike that family, our patients did not complain of orthostatic hypotension or neurogenic bladder. However, the dysautonomia in the first reported family could have been due to complicated diabetes mellitus. The sensory involvement was minimal or subclinical in our cases, as well as in the previously reported cases.
Since the first report of HSPB1 mutation (5), 16 different autosomal dominant mutations and one autosomal recessive mutation have been reported in families with CMT2 and dHMN (6-12). HSP27 is one of a stress-induced chaperone protein and forms oligomers to maintain a misfolded protein in a refolding-competent state. The upregulation of HSP27 has been reported to be required for the survival of motor and sensory neurons injured by apoptotic stress (13). In fact, higher levels of serum HSP27 have been reported in diabetic patients with better nerve function (14). Individuals with mutations in the C-terminal domain of HSP27 show a more severe phenotype, with ages at onset as young as four and seven years (2).

The K141Q substitution is located in the α-crystallin domain of HSP27. The K141Q mutation does not dramatically affect the quaternary structure of HSP27. The chaperone-like activity associated with the K141Q mutation is only a little less than that of the wild-type protein. However, oligomers formed by proteins with the K141Q mutation are slightly larger and less stable than those formed of the wild type (15). The effects of the K141Q mutation on the aggregation of neurofilament light polypeptide or incorporation of neurofilament medium polypeptide into the cytoskeletal network remain to be clarified. Unlike patients with mutations in the C-terminal domain, patients with the K141Q mutation show late onset and a mild clinical phenotype, reflecting the minimal biochemical changes associated with this mutation (15).

The authors state that they have no Conflict of Interest (COI).

References