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A novel Lys141Thr mutation in small *heat shock protein 22* (*HSPB8*) gene in Charcot–Marie–Tooth disease type 2L

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Abstract

Charcot–Marie–Tooth disease (CMT) is a group of clinically and genetically heterogeneous peripheral neuropathies. *HSPB8* gene encodes *heat shock protein 22* (HSP22) which belongs to the superfamily of small stress induced proteins. Mutations in *HSPB8* are implicated to CMT2L and distal hereditary motor neuropathy 2A (dHMN2A). All three reported *HSPB8* mutations are interestingly located in the Lys141 residue. In the present study, we examined a Korean axonal CMT patient who presented distal limb atrophy, sensory loss, areflexia, and axonal loss of large myelinated fibers. Whole exome sequencing identified a novel missense mutation c.422A>C (p.Lys141Thr) in *HSPB8* as the underlying cause of the CMT2 patient. The mutation was regarded as a *de novo* case because both unaffected parents have no such mutation. The patient with *HSPB8* mutation is the first case in Koreans. Clinical heterogeneities have been revealed in patients with Lys141 mutation; the present patient revealed similar phenotype of CMT2L. In addition, the lower limb MRI revealed a similarity between our *HSPB8* and *HSPB1* patients. It seems that the Lys141 site in the alpha-crystallin domain of *HSPB8* is regarded as a mutational hot spot for peripheral neuropathy development, and mutations even in the same codon can exhibit different CMT phenotypes.

Keywords: Charcot–Marie–Tooth disease; CMT2L; Exome; *HSPB8*; HSP22; Korean

1. Introduction

Charcot–Marie–Tooth disease (CMT), which is also called hereditary motor and sensory neuropathy is the most common inherited peripheral nerve disorders. CMT pertains to a group of genetically and clinically heterogeneous disorders with weak genotype–phenotype correlation. So far, mutations in more than 60 genes have been reported to be implicated to development of CMT or CMT-related diseases. CMT is conventionally divided into the demyelinating form (CMT1) and the axonal defective form (CMT2). CMT1 exhibits markedly reduced nerve conduction velocities (NCVs), whereas CMT2 shows slightly reduced or normal NCVs [1].

CMT2 has been categorized into many subtypes with respect to their distinctive clinical symptoms cum genetic causes: CMT2A to CMT2Q. On a phenotypic basis, sensory signs are often lacking in CMT2 patients, making it occasionally difficult to distinguish CMT2 from distal hereditary motor neuropathy (dHMN) which closely resembles axonal CMT but does not involve sensory

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abnormality. The dHMN is also a heterogeneous disorder group that shares the common feature of a motor neuron predominant involvement with length dependent manner [2].

Mutations in the small *heat shock protein 22* (*HSPB8*, also called *HSP22*) gene located on 12q24.23, have been reported to be implicated to CMT2L (OMIM 608673) [3] and dHMN2A (OMIM 158590) [4]. So far, three *HSPB8* mutations have been reported as the genetic causes of CMT2 or dHMN [3,4]. Interestingly, all three mutations are nonsynonymous substitution mutations of the same Lys141 residue: c.421A>G (Lys141Glu), c.423G>C (Lys141Asn) and c.423G>T (Lys141Asn). Therefore, the Lys141 residue which is located in the α-crystallin domain seems to be very important for protein function in the peripheral nerves. The α-crystallin domain is a highly conserved region within the small heat shock protein (HSP) superfamily and mutations in this motif decrease its chaperone activity [5]. Mutations in the *HSPB1* (*HSP27*) are also associated to CMT2F (OMIM 606595) and dHMN2B (OMIM 608634) [6–9], while mutations in the *HSPB3* (*HSPL27*) is associated to dHMN2C (OMIM 604624) [10]. Most disease causing mutations in the *HSPB1* and *HSPB3* have also been identified in the α-crystallin domain.

The expression of *HSPB1* and *HSPB8* is induced in response to various environmental stresses [11,12]. *HSPB8* inhibits the accumulation of insoluble Htt protein containing 43 glutamine residues (Htt43Q). The C-terminal domain of *HSPB8* contains the specific sequence necessary for chaperone activity. Carra et al. suggested that decreased *HSPB8* chaperone activity may influence the development of some neuropathies [11].

Exome sequencing has recently been introduced to identify the genetic causes of rare genetic diseases. Particularly, exome sequencing is an efficient tool for genetic diagnosis of CMT, because it is genetically and clinically heterogeneous, and exhibits a weak genotype–phenotype correlation [13–17]. In this study, we identified a novel causative mutation in *HSPB8* from a Korean CMT2 sporadic case by exome sequencing. This is the first report for *HSPB8* mutation in Korean CMT patients.

2. Materials and methods

2.1. Patient and clinical assessment

This study included an axonal CMT case (Sample ID: FC31) of Korean origin. In addition, 200 healthy controls were recruited from the Department of Neurology. All participants included in this study provided written informed consent according to the protocol approved by the Ethics Committee of Ewha Womans University Hospital (Seoul, South Korea).

Patient was evaluated by taking a detailed history and gait abnormalities including walking on heels and on toes. Muscle strength of flexor and extensor muscles was measured manually using the medical research council (MRC) scale. In order to determine physical disability, CMT neuropathy score (CMTNS) was used. Motor nerve conduction velocities (MNCVs) were measured for the median, ulnar, peroneal, and tibial nerves. Sensory nerve conduction velocities (SNCVs) were obtained over the finger-wrist segment from the median and ulnar nerves by orthodromic scoring. Density of myelinated fibers (MFs), axonal diameter, and myelin thickness were determined directly from semi-thin transverse sections using a computer-assisted image analyzer (AnalySIS, Soft Imaging System, Germany). Ultrastructural analysis was performed using the H-7650 electron microscope (Hitachi, Japan). MRIs were obtained from thigh, and lower leg using the 1.5-T system (Siemens Vision, Siemens, Germany).

2.2. Exome sequencing and identification of causative mutations

Total DNA was isolated from peripheral blood by using a QIAamp blood DNA purification kit (Qiagen, Hilden, Germany). The exome was captured using a SeqCap EZ ver 2.0 (Roche-NimbleGen, Madison, WI, USA), and sequencing was performed using the HiSeq 2000 Genome analyzer (Illumina, San Diego, CA, USA). The UCSC assembly hg19 (NCBI build 37.1) was used as the reference sequence. The variants were annotated by the ANNOVAR (ver 2011Feb20) program, and were compared with common variants registered in dbSNP137 (http://www.ncbi.nlm.nih.gov/snp/) and 1000 Genomes Project (http://www.1000genomes.org/) databases.

Functionally significant variants were first selected from about 60 CMT-relevant genes. The candidate variants considered as causative were confirmed by Sanger's sequencing method. Mutations were considered to be an underlying cause when they were detected in only the affected member of a family, and were not detected in more than 200 control samples. Conservation analysis of protein sequences was conducted using MEGA5 ver 5.05 program. *In silico* analyses were performed using SIFT (http://sift.jcvi.org/), PolyPhen, and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) programs. The cDNA numbering was achieved with +1 corresponding to the A of the ATG initiation codon.

3. Results

3.1. Clinical and electrophysiological features

The patient (Supplementary Fig. 1, male/27-year-old) first noticed gait disturbances at 13 years. He visited our neurologic clinic at 15 years due to walking difficulty and gait ataxia. When we examined him again at 27 years, he had difficulty in running but was still able to walk unaided. Follow up neurologic examination during a lapse of 12 years revealed the inception of muscle