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A novel missense variant (Gln220Arg) of *GNB4* encoding guanine nucleotide-binding protein, subunit beta-4 in a Japanese family with autosomal dominant motor and sensory neuropathy

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ABSTRACT

Dominant intermediate Charcot–Marie–Tooth disease F (CMTDIF) is an autosomal dominant hereditary form of Charcot–Marie–Tooth disease (CMT) caused by variations in the guanine nucleotide-binding protein, subunit beta-4 gene (*GNB4*). We examined two Japanese familial cases with CMT. Case 1 was a 49-year-old male whose chief complaint was slowly progressive gait disturbance and limb dysesthesia that appeared at the age of 47. On neurological examination, he showed hyporeflexia or areflexia, distal limb muscle weakness, and distal sensory impairment with lower dominancy. Nerve conduction studies demonstrated demyelinating sensorimotor neuropathy with reduced action potentials in the lower limbs. Case 2 was an 80-year-old man, Case 1's father, who reported difficulty in riding a bicycle at the age of 76. On neurological examination, he showed areflexia in the upper and lower limbs. Distal sensory impairment in the lower limbs was also observed. Nerve conduction studies revealed mainly axonal involvement. Exome sequencing identified a novel heterozygous nonsynonymous variant (NM_021629.3:c.659T > C [p.Gln220Arg]) in *GNB4* exon 8, which is known to be responsible for CMT. Sanger sequencing confirmed that both patients are heterozygous for the variation, which causes an amino acid substitution, Gln220Arg, in the highly conserved region of the WD40 domain of GNB4. The frequency of this variant in the Exome Aggregation Consortium Database was 0.000008247, and we confirmed its absence in 502 Japanese control subjects. We conclude that this novel *GNB4* variant is causative for CMTDIF in these patients, who represent the first record of the disease in the Japanese population.

1. Introduction

Inherited neuropathy is a group of clinically and genetically heterogeneous disorders characterized by progressive motor, sensory, or autonomic peripheral neuropathy. Almost 90 genes are associated with neuropathies (Suppl. Table 1). Inherited neuropathies that do not form part of another syndrome are known as hereditary motor and sensory neuropathy (HMSN) or Charcot–Marie–Tooth disease (CMT) [Jerath and Shy, 2015] .

CMT has a prevalence of 1/2500 to 1/250 [Braathen et al., 2011; Patzkó and Shy, 2011], and is classified into axonal, demyelinating, or intermediate types according to the nerve conduction velocity [Jerath and Shy, 2015]. Dominant intermediate Charcot–Marie–Tooth disease F (CMTDIF, MIM: 61585) is an autosomal dominant hereditary CMT caused by a heterozygous mutation in the guanine nucleotide-binding protein, subunit beta-4 gene (*GNB4*; MIM: 610863) [Soong et al., 2013; Liu and Zhang, 2014]. GNB4 is widely expressed and is a component of heterotrimeric G proteins that play a key role in transducing ligands, such as hormones, neurotransmitters, chemokines, local mediators, and sensory stimuli binding to G protein-coupled receptors, into

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intracellular responses [Hamm, 1998; Rosskopf et al., 2003].

To date, one autosomal dominant family and two sporadic cases of CMTDIF have been reported [Lee et al., 2010; Soong et al., 2013; Laššuthová et al., 2017]. The family has a *GNB4* missense point mutation (NM_021629.3:c.659T > C [p.Gln220Arg]), and the two sporadic cases have *de novo* missense point mutations (NM_021629.3:c.265A > G [p. Lys89Glu] and NM_021629.3:c.169A > G [p.Lys57Glu]). These CMTDIF patients originate from Taiwan and the Czech Republic [Lee et al., 2010; Soong et al., 2013; Laššuthová et al., 2017]. Here, we report the first known Japanese familial cases of CMTDIF caused by a heterozygous variant (c.659T > C [p.Gln220Arg]) in *GNB4*.

2. Materials and methods

2.1. Human subjects

The patients in this study are members of a Japanese family originating from Fukuoka Prefecture, a western province of Japan (Fig. 1A). Written informed consent was obtained from all participating individuals. This study was approved by the Ethics Committees of Kurume University School of Medicine and Kyushu University, Faculty of Medicine.

2.2. Whole-exome sequencing

We carried out whole-exome sequencing of both patients. Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). Exonic regions were enriched using SureSelectXT Human All Exon v5 (Agilent Technologies, Santa Clara, CA, USA). Paired-end sequencing (of 75 bp reads) was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA). Raw sequencing reads were aligned using the Burrows–Wheeler Aligner-MEM algorithm and analyzed by SureCall 2.1 software (Agilent Technologies). Allele frequencies were annotated using data from the 1000 Genomes Project [http://www.1000genomes.org] and the Human Genetic Validation Database [http://www.genome.med.kyoto-u.ac.jp/SnpDB/index.html].

Because our cases were suffering from neuropathy, we prioritized variants in 89 genes known to be responsible for hereditary neuropathy (Suppl. Table 1). We examined the frequency in control populations of candidate variants using the Exome Aggregation Consortium (ExAC) database [http://exac.broadinstitute.org]. The functional consequence of the variants was evaluated *in silico* using the PolyPhen-2 prediction tool [http://genetics.bwh.harvard.edu/pph2/].

2.3. Sanger sequencing

The region spanning the variation site in exon 8 of *GNB4* was PCR-amplified using 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s with the following primers: 5′-GACTGGGCATTCTGGAGAT-3′ (forward) and 5′-CCTTGAAGGCAATTTGTTGTT-3′ (reverse). PCR products were sequenced using the ABI PRISM Big Dye Terminator (v 3.1) Cycle Sequencing Kit (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) and ABI PRISM 3130-Avant Genetic Analyzer (Applied Biosystems).

2.4. Copy number analysis of MPZ and PMP22 by real-time PCR

Copy number variation in genes encoding myelin protein zero (*MPZ*) and peripheral myelin protein 22 (*PMP22*) causes hereditary neuropathy [Høyer et al., 2011; Van Paassen et al., 2014], so we performed real-time PCR assays to measure *MPZ* and *PMP22* copy numbers. Genomic DNA was extracted from peripheral blood of the patient and an unrelated control using the QIAamp DNA Blood Kit (Qiagen). Real-time PCR was performed using the power SYBR Green PCR Master Mix (Applied Biosystems) and TaqMan Copy Number Reference Assay RNase P (Applied Biosystems) with the TaqMan Genotyping Master Mix (Applied Biosystems) as an endogenous control on the ABI PRISM 7000 Sequence Detection System (Applied Biosystems). Primers were designed for all six exons of *MPZ* and all four exons of *PMP22*, and primer sequences are listed in Suppl. Table 2. Each reaction was performed in triplicate. Data were analyzed using relative quantitation. The calibration curve was prepared using unrelated control DNA.

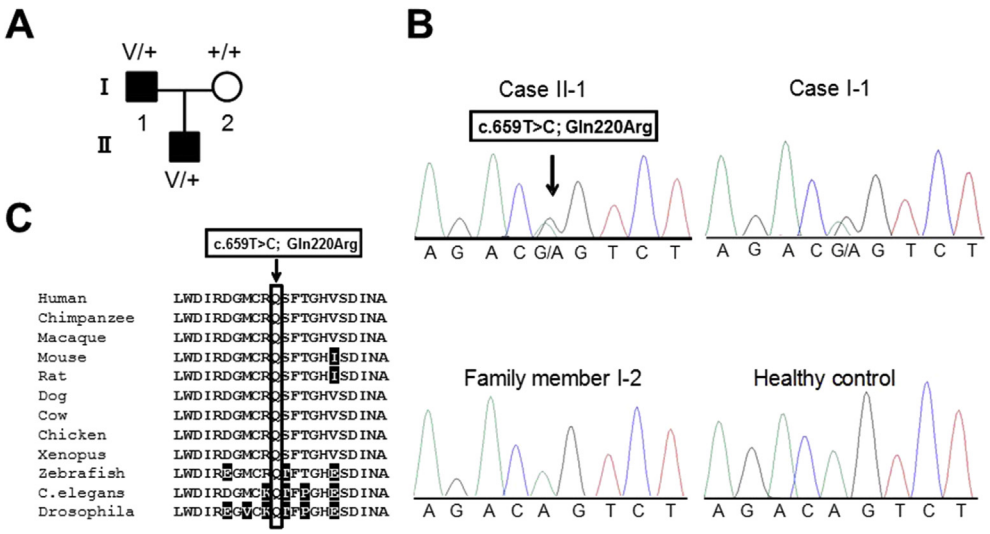

Fig. 1. (a) Family pedigree. Squares, males; circles, females; solid symbols, affected individuals; open symbols, unaffected individuals. V, variant allele; +, wild-type allele. (b) Sequencing of *GNB4* exon 8 in control and three family members. A heterozygous T to C substitution was detected at position 659, causing a glutamine-to-arginine substitution in codon 220 of *GNB4* in patients I-1 and II-1. (c) Alignment of GNB4 amino acid sequences and counterparts in the region containing the p.Gln220Arg variation among species. The 220th glutamine is highly conserved (data from NP_067642.1, XP_001168001.1, XP_002802962.1 (NP_001248094.1), NP_038559.2, NP_001013932.1, XP_545211.1, NP_001092503.1, XP_003641822.2, NP_001016952.1, NP_998646.1, AAK55963.1, and NP_001303567.1 in NCBI). Outlined characters show non-conserved amino acids.

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