Short Communication

A novel RAB7 mutation in a Chinese family with Charcot–Marie–Tooth type 2B disease

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Charcot–Marie–Tooth type 2B (CMT2B) disease is a hereditary motor and sensory neuropathy subtype characterized by prominent loss of sensation, distal muscle weakness and wasting skin ulcers. Recurrent ulcers often require amputation of lower limbs. To date, only four mutations of the RAB7 gene, which encodes the small GTPase, have been associated with CMT2B. A Chinese family with CMT2B was identified. Direct DNA sequencing performed on the affected individuals in this family revealed a novel mutation (p.Asn161Ile) in RAB7. The mutation is located in a potential mutational hotspot region, implicating the importance of this region for RAB7 protein. This is the first report of RAB7 mutation in Asian population.

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1. Introduction

Charcot–Marie–Tooth disease (CMT) is a progressive peripheral nerve disorder causing muscle weakness, wasting, and sensory loss affecting the hands and legs. Foot deformations such as pes cavus are also features of CMT (Dyck and Lambert, 1968). CMT neuropathies fall into two major classifications: CMT1, which is typified by reduced nerve conduction velocity (NCV) and CMT2, CMT2 is characterized by normal NCV but decreased nerve conduction amplitudes (Gemignani and Marbini, 2001).

The CMT2B (OMIM: 60882) disease is an autosomal dominant axonal neuropathy with sensory loss, distal muscle weakness and wasting, and frequent foot ulcers and subsequent infections (Cogli et al., 2005). Disease onset is usually early adulthood or younger, but the disease can occur in later life, as well (Cogli et al., 2005). HSN1 (Hereditary sensory neuropathy 1, OMIM: 162400) which is caused by mutations in SPILC1 (OMIM: 605712) has a similar phenotype to CMT2B (Reilly and Shy, 2009); thus, CMT2B and HSN1 are difficult to distinguish by phenotype alone.

Four RAB7 mutations (p.L129F, p.K157N, p.N161T, and p.V162M; OMIM: 602298) (http://www.hgmd.org/) have been reported in CMT2B patients (Houlden et al., 2004; Meggouh et al., 2006; Verhoeven et al., 2003). Here, we identified a three generation Chinese family with CMT2B. Linkage analysis revealed that the family's disease was mapped to RAB7. Direct DNA sequencing identified a novel mutation (p.N161I) in the RAB7 gene, this is the first report that RAB7 gene mutation cause CMT2B in Asian population.

2. Materials and methods

2.1. Ethics statement

This study was approved by the ethics committee of Huazhong University of Science and Technology. All participants in the study agreed with informed consent to participate in the investigation.

2.2. Linkage analysis

Genomic DNA was extracted from blood samples using standard methods. Three polymorphic microsatellite markers linked to RAB7 – D3S1551, D3S1290, and D3S3264 – were selected for linkage analysis. PCR amplification of microsatellite markers was performed using labeled universal primer and tailed primer methods. Markers were genotyped using an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Genotypes were analyzed using the GeneMapper 2 Software program (Applied Biosystems, Foster City, CA). Pairwise logarithms of odds (LOD) scores were obtained via Linkage Package 5.2. Assumptions for linkage analysis included an autosomal dominant model with a 95% penetrance rate, a gene frequency of 0.0001, and an allele frequency of...
1/n (n = number of alleles observed). A haplotype was constructed using the Cyrillic program.

2.3. Mutation screening

Mutation screening was carried out by direct DNA sequencing. All RAB7 gene exons were PCR amplified and sequenced. PCR primers were as follows:

- exon2/F5: ggcctgcctgctgcttgcc
- exon3/F5: gctgcaaggctacgctggc
- exon4/F5: cacgctctggcgctctggc
- exon5/F5: gcggcactgctggcaacc

PCR was performed in standard PCR buffer (50 µl) containing 1.5 mM MgCl₂, 0.2 mM dNTP, 0.5 µM of each primer, 1 unit of Taq DNA polymerase, and 25 ng of human genomic DNA. The amplification program was one cycle for 3 min for denaturation at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 58 °C, 30–45 s at 72 °C, and one 7-min extension step at 72 °C. PCR products were purified using the Tiangen Gel Extraction Kit (Tiangen, Beijing, China). DNA sequencing analysis was performed using the BigDye Terminator Cycle Sequencing v3.1 kit and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

2.4. RFLP analysis

The c.482A > T point mutation in the RAB7 gene eliminated an HpyCH4IV restriction site, which was used to confirm the mutation and whether the mutation co-segregated with respect to the family’s disease. The RAB7 Exon 4 which contained the mutation was PCR amplified from all family members and from 100 unrelated, healthy Chinese individuals who served as controls. PCR products were digested by 1 unit HpyCH4IV restriction enzyme at 37 °C for 12 h, and then separated on a 1% agarose gel.

3. Results

A three generation Chinese family suffering from CMT2B were identified and characterized. The proband was a 45 year-old female who reported diminished sensation to pain in her lower limbs, occurring at age of 10. Her sensory loss was accompanied by shooting pain, whereas touch and thermal sensations were normal. These clinical features are similar to those reported for CMT2B. When the proband received surgical wound debridement, she felt no pain except tension and a sense of traction. The proband had no muscle atrophy but her mother and brothers had clawed toes, hammerhead thumbs, and they developed ulcerations in their teen years or later. The proband’s mother, at the age of 30, developed a severe ulcer on her right foot, which was eventually amputated. All members of the family studied lacked a steppage gait, pes cavus, and atrophy of the lower leg or other abnormal phenotypes of the upper limbs.

Needle electromyography (EMG) was conducted on the first female study subject. Her right tibial nerve motor NCV (MNCV) was 13.2 m/s and the left was 22.0 m/s. The right peroneal nerve MNCV was 36.9 m/s and the left was 38.9 m/s. The superficial peroneal sensory NCV (SNCV) was reduced (right, 23.5 m/s; left, 11.5 m/s), and sural SNCVs could not be measured. Thus, both sensory and motor nerve conduction velocity was decreased.

To confirm that the CMT2B-like phenotype of the study family was due to mutations in RAB7, linkage analysis was performed using three markers that span the RAB7: D3S1551, D3S1290, and D3S3264. These three markers co-segregated with the disease in the family (Fig. 1). A maximum LOD score of 1.43 was obtained by applying a two-point analysis method, and these data suggest that the disease gene in the family is RAB7.

Direct DNA sequencing of all exons and exon-intron boundaries of the RAB7 gene (Genbank No: NM_004637) in an affected family member revealed an A-to-T transition in nucleotide 482, causing an asparagine to be replaced with an isoleucine residue (p.Asn161Ile). All members of the study family carried this mutation, and unaffected members did not. Furthermore, 100 unrelated normal controls did not carry the mutation. Alignment of amino acid residues across different species suggested that Asn161 is highly conserved (Fig. 2).

To confirm that this mutation was associated with the disease in the family, RFLP analysis was conducted. All five affected family members had both the wild type (WT) allele (278 bp and 222 bp)
and the mutant allele (500 bp) (Fig. 3). However, unaffected family members and the 100 unrelated controls had only the WT allele. Thus, the Asn161Ile mutation is the disease-causing mutation in the CMT2B family.

4. Discussion

In this study, we identified a novel mutation (p.N161I) in the RAB7 gene in a three generation Chinese family with CMT2B. All affected
members carried the mutation, which did not exist in unaffected family members and 100 unrelated healthy controls.

Interestingly, our study is the second mutation report on Asn161 amino acid in the RAB7 gene associated with CMT2B. Previously, Houlden and co-workers identified an Asn161Thr mutation in the RAB7 gene which cause CMT2B. Both N161 and V162 in the RAB7 gene are evolutionarily conserved amino acids in all exocytic and endocytic Rab GTPases (Merithew et al., 2001).

More than 60 different RAB proteins expressed in human cells are known to be important in vesicle transportation (Cogli et al., 2010). Rab7 is a small GTPase in RAB family, controlling vesicular transport to late endosomes and lysosomes in the endocytic pathway (Cogli et al., 2009). To date, only four RAB7 gene mutations have been reported to be associated with CMT2B (p.L129F, p.K157N, p.N161T and p.V162M). The four CMT2B-causing mutations of RAB7 have similar biochemical effects, which increase $K_{\text{off}}$ both for GTP and GDP. All reported CMT2B disease-causing mutants were predominantly in GTP-bound form, in contrast with Rab7 WT protein (De Luca et al., 2008; Spinosa et al., 2008).

CMT2B-associated Rab7 mutants impair growth factor receptor trafficking and, in turn, alter p38 and ERK1/2 signaling from perinuclear, clustered signaling endosomes (BasuRay et al., 2013). Therefore, the impaired neurite growth may be due, in part, to the impeded shuttling of phosphorylated Erk1/2 to the nucleus (BasuRay et al., 2013).

Thus, we identified a novel mutation c.482A>T in the RAB7 gene associated with CMT2B in a Chinese family, this is the first report that RAB7 mutation cause CMT2B in Asian CMT2B patients. Our findings directly contribute to better understanding the role of RAB7 in the development of hereditary axonal neuropathy.

Competing interests

The author(s) declare that they have no competing interests.

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References