Mutations in the Small GTP-ase Late Endosomal Protein RAB7 Cause Charcot-Marie-Tooth Type 2B Neuropathy

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Charcot-Marie-Tooth type 2B (CMT2B) is clinically characterized by marked distal muscle weakness and wasting and a high frequency of foot ulcers, infections, and amputations of the toes because of recurrent infections. CMT2B maps to chromosome 3q13-q22. We refined the CMT2B locus to a 2.5-cM region and report two missense mutations (Leu129Phe and Val162Met) in the small GTP-ase late endosomal protein RAB7 which causes the CMT2B phenotype in three extended families and in three patients with a positive family history. The alignment of RAB7 orthologs shows that both missense mutations target highly conserved amino acid residues. RAB7 is ubiquitously expressed, and we found expression in sensory and motor neurons.

The inherited neuropathies of the peripheral nervous system show considerable clinical and genetic heterogeneity (De Jonghe et al. 2000). Some forms, the ulcer-mutilating neuropathies, are characterized by prominent sensory loss, often complicated by severe infections, arthropathy, and amputations (Auer-Grumbach et al., in press). So far, two loci and one gene have been reported for autosomal-dominant ulcer-mutilating neuropathies. However, molecular genetic studies have demonstrated that a third locus must exist (Auer-Grumbach et al. 2000b; Bellone et al. 2002). Hereditary sensory neuropathy type I (HSN I [MIM 162400]) maps to chromosome 9q22.1–q22.3 and is caused by mutations in the serine palmitoyltransferase, long chain base subunit-1 (SPTLC1) gene (Bejaoui et al. 2001; Dawkins et al. 2001). Charcot-Marie-Tooth type 2B (CMT2B [MIM 600882]) or hereditary motor and sensory neuropathy type IIB (HMSN IIB) was assigned to chromosome 3q13–q22 in an American family (Kwon et al. 1995). CMT2B is clinically characterized by marked distal muscle weakness and wasting and a high frequency of foot ulcers, infections, and amputations of the toes because of recurrent infections. CMT2B is mild to severe, with sensory loss and all modalities equally affected. Spontaneous pain is absent. Motor deficit is often the first and most prominent sign of the disease. The distal leg muscles are more affected than the hand muscles. Nerve-conduction-velocity studies indicate a primarily axonal neuropathy that allows clinical diagnosis in asymptomatic individuals (reviewed in Auer-Grumbach et al., in press).

We performed a molecular genetic study of three families with an ulcer-mutilating phenotype who were previously linked to the CMT2B locus: an American family (CMT-195) (Kwon et al. 1995), a Scottish family (CMT-90) (De Jonghe et al. 1997), and an Austrian family (CMT-140) (Auer-Grumbach et al. 2000a). To determine whether these families share a common disease haplotype, we analyzed 15 STR markers in the CMT2B region. These markers included six new polymorphic STR markers (D3SCMT126A, D3SCMT126B,
D3SCMT126C, D3SCMT126D, D3SCMT126F, and D3SCMT126G), which we isolated using sequence information from public databases. For each marker, alleles associated with the ulcer-mutilating phenotype were identified, and a disease haplotype was reconstructed in each family (fig. 1). No common disease haplotype was observed among families CMT-195, CMT-90, and CMT-140, suggesting the absence of a genetic relationship among these families with CMT2B. However, a common disease haplotype spanning seven STR markers, D3S3584–D3SCMT126C, was shared between the Austrian family CMT-140 and a small branch of another Austrian multigenerational pedigree, CMT-126 (patients III-5, IV-2, IV-3, and V-1), originally excluded from the CMT2B locus (Auer-Grumbach et al. 2000b; fig. 1). The five remaining patients (III-1, III-2, III-3, III-7, and IV-4) with a CMT2B phenotype in all pedigrees and were belonging to the small linked branch of family CMT-126, which was also present in the patients served a c.385C→T (Leu129Phe) mutation as the patients in family CMT-140 (fig. 2A), indicated a close familial relationship between CMT-140 and part of CMT-126, who originated from the same Austrian province. In CMT-126, we found no obvious differences in neurological and electrophysiological findings between patients with the Leu129Phe mutation and those without the RAB7 mutation, except that the phenotype was more severe in the branch with the Leu129Phe mutation, including the occurrence of ulcers and amputations. Also, the ulcer-mutilating CMT phenotype of the remaining patients in family CMT-126 is likely to be caused by a mutation in another gene (since SPTLC1 was excluded; data not shown) and further supports the presence of a third locus for ulcer-mutilating neuropathies.

In addition to this study, we selected a set of 24 unrelated patients diagnosed with an HSN or ulcer-mutilating CMT phenotype for mutation analysis of RAB7. In three patients, two Austrian and one Belgian, we observed Leu129Phe (HSN-15.1) once and Val162Met twice (HSN-8.1 and PN-626.1). These three patients with RAB7 mutations have a family history of CMT and ulcer-mutilations, but the additional patients were not cooperative for genetic studies. Haplotype analysis confirmed that the Austrian patient HSN-15.1, with a family history of CMT2B, shared the linked haplotype with the Austrian families CMT-140 and part of CMT-126. These findings indicate a founder effect for the Leu129Phe mutation in families CMT-126, CMT-140, and HSN-15.1. However, the Austrian (HSN-8.1) and Belgian (PN-626.1) patients with the Val162Met mutation do not share a common disease haplotype and are not related to the Scottish CMT-90 and American CMT-195 families (data not shown).

The alignment of RAB7 orthologs shows that both missense mutations target highly conserved amino acid residues (fig. 2C). The Val162Met mutation affects a valine that is conserved among all species. The Leu129Phe mutation is located next to a conserved GTP-binding domain (NKID). The leucine residue at position 129 is not conserved in Arabidopsis and yeast.

Vitelli and coworkers (1996) reported expression of two transcripts of 2.5 kb and 1.8 kb for the human RAB7 gene in different cell types. The expression information of human RAB7 and mouse Rab7 in the Unigene database suggests ubiquitous expression (Unigene Clusters: Hs.356386 and Mm.4268). We found expression in all tested tissues; in human, high expression was found in skeletal muscle (fig. 3A), and, in mouse, high expression was found in liver, heart, and kidney (fig.
Figure 1  Haplotype analysis of chromosome 3q13-22 STR markers in families with CMT2B. Pedigrees of four families with an ulceromutilating phenotype linked to the CMT2B locus: A, Austrian family CMT-140; B, Austrian family CMT-126; C, Scottish family CMT-90; and D, American family CMT-195. Symbols: open diamond = unaffected; filled diamond = affected; half-filled diamond = probably affected (i.e., CMT-126 II-3); slashed diamond = deceased; arrow = recombination; box = disease-associated haplotype. Pedigree structure and sex are disguised to preserve anonymity. The best genetic and physical order of STR markers is according to NCBI (GenBank/LocusLink). The six newly developed STR markers (D3SCMT126A, D3SCMT126B, D3SCMT126C, D3SCMT126D, D3SCMT126F, and D3SCMT126G) are localized between D3S2324 and D3S1587. Genotypes are represented by allele sizes in base pairs, and 0-0 = failed genotype. The RAB7 gene is located on the same contig (NT_005523) as markers D3SCMT126B and D3SCMT126C. For genotyping, fragment analysis was performed on an ABI Prism3700 DNA Analyzer and processed with the ABI GENESCAN 3.5 and GENOTYPER 3.6 software (Perkin Elmer Applied Biosystems).
Figure 2  DNA and protein sequence analysis of RAB7. A, Electropherogram showing the c.385C→T sequence variation in part of exon 3, resulting in the Leu129Phe missense mutation in families CMT-140 and CMT-126 and patient HSN-15.1. B, Electropherogram of the c.484G→A sequence variation in part of exon 4, resulting in the Val162Met missense mutation in families CMT-90 and CMT-195 and patients HSN-8.1 and PN-626.1. The corresponding genomic sequence of a control person is shown below. DNA sequencing was performed using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech), and the sequencing reactions were loaded on the ABI Prism3700 DNA Analyzer (Perkin Elmer Applied Biosystems). The data were collected and analyzed via the ABI DATA COLLECTION version 1.1 and DNA SEQUENCING ANALYSIS version 3.6 software, respectively. C, ClustalW multiple protein alignment of the RAB7 orthologs of the region surrounding the Leu129Phe and Val162Met mutations. RAB7 orthologs: Human (Homo sapiens), mouse (Mus musculus), rat (Rattus norvegicus), fly (Drosophila melanogaster), slime mold (Dictyostelium discoideum), nematode (Caenorhabditis elegans), mouse-ear cress (Arabidopsis thaliana), and baker’s yeast (Saccharomyces cerevisiae). The highly conserved motif involved in guanine nucleotide binding is boxed. Both amino acid mutations are shaded and indicated by an arrow.

3B). PCR analysis of cDNA from E13 mouse dorsal root ganglia and ventral horn showed expression of Rab7 in sensory and motor neurons, respectively (fig. 3C).

RAB7 belongs to the Rab family of Ras-related GTPases. These Rab proteins are essential for the regulation of intracellular membrane trafficking. The Rab proteins regulate vesicle transport through the recruitment of specific effector or motor proteins and may have a role in linking vesicles and target membranes to the cytoskeleton (Echard et al. 1998; Nielsen et al. 1999). RAB7 is involved in transport between late endosomes and lysosomes, and recent studies demonstrate that the Rab7-effector protein RILP (Rab interacting lysosomal protein) induces the recruitment of dynein-dynactin motors and regulates transport toward the minus-end of microtubules (Cantalupo et al. 2001; Jordens et al. 2001). Expression of Rab7 dominant-negative artificial mutants in mammalian cells inhibits lysosomal degradation and
disperses lysosomes. One such mutant, rab7N125I, is localized in the GTP-binding domain of Rab7 (Press et al. 1998). In vitro studies, performed in hamster BHK kidney cells, demonstrated that this mutant rab7N125I protein exists preferentially in a nucleotide-free form and has been shown to have a dominant-negative effect on late endocytic transport (Press et al. 1998). In contrast, in human fibroblasts overexpressing Rab7, late endocytic vesicles accumulated in the perinuclear region, probably because of an increased motility in the minus-end direction of microtubules (Lebrand et al. 2002). Overexpression studies of wild-type Rab7 also demonstrated that the Rab7 protein is involved in the Golgi targeting of glycosphingolipids (Choudhury et al. 2002). It is interesting that the SPTLC1 gene, mutated in patients with ulceromutilating HSN type I, is involved in the biosynthesis of sphingolipids (Bejaoui et al. 2001; Dawkins et al. 2001).

To date, 60 human RAB genes have been identified, and the majority are likely to control highly specialized functions in many cell types. Mutations in RAB genes may, therefore, cause a wide range of inherited diseases. In addition, there is evidence for alteration in RAB function in the pathogenesis of acquired diseases, such as infection due to intracellular micro-organisms (Seabra et al. 2002). It is unclear how dysfunction of Rab7 causes sensory and motor neuropathy in patients with CMT2B. However, other Rab proteins are involved in trafficking mechanisms associated with neurite outgrowth and polarized sorting in neurons (Tang 2001). The Rab3A protein regulates neurotransmitter release and other forms of regulated secretion (Geppert et al. 1997). The Rab23 protein is implicated in neural patterning, as demonstrated in the mouse sonic hedgehog signaling pathway (Eggenschwiler et al. 2001).

In conclusion, we report two missense mutations in the Rab7 gene as the cause for the ulceromutilating inherited peripheral neuropathy CMT2B. It will be a challenge to explain why mutations in such a universally expressed protein as Rab7 lead to an axonal pathology in CMT2B.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:


German Human Genome Project, http://www.rzpd.de/ (clones for partial human Rab7 cDNA sequences)


References


