ed. These observations confirm that REM sleep and dreaming, while linked, may depend on independent generators.

In conclusion, our case demonstrates the existence of total dream loss as a distinct neuropsychological dysfunction after deep bilateral occipital lobe damage, in the absence of REM sleep changes.

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References

A Novel RAB7 Mutation Associated with Ulcero-Mutilating Neuropathy

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There are two known autosomal dominant genes for the hereditary ulcero-mutilating neuropathies: SPTLC1 (hereditary sensory neuropathy type 1) and RAB7 (Charcot–Marie–Tooth disease type 2B). We report a family with autosomal dominant ulcero-mutilating neuropathy, developing in the teens and characterized by ulcers, amputations, sensory involvement in the feet but no motor features. Sequencing the RAB7 gene showed a novel heterozygous A to C mutation, changing asparagine to threonine at codon 161. The mutation is situated adjacent to a previously identified valine to methionine mutation at codon 162, implying a hotspot for mutations in the highly conserved C terminus of RAB7.

The hereditary sensory neuropathies (HSNs) are a heterogeneous group of disorders characterized by prominent sensory loss, often with the development of skin ulcers, arthropathy, osteomyelitis, and amputations. Similar features are seen in Charcot–Marie–Tooth disease type 2B (CMT2B), to the extent that some have argued CMT2B would be better classified as a form of HSN. Both HSN type 1 and CMT2B are autosomal dominant conditions and their phenotypes overlap. Age of onset in both disorders is typically in the teens, sensory nerves are prominently affected, and there is a high incidence of ulcero-mutilating complications. One difference between HSN1 and CMT2B is said to be the presence of positive sensory symptoms (paresthesias.

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and lancinating or burning pain) in the former. More marked distal motor involvement is said to favor a diagnosis of CMT2B. Unlike CMT2B, motor nerve conduction velocities in HSN1 families are occasionally slowed, sometimes into the demyelinating range.1,2,3 Using linkage analysis, the gene for HSN1 was localized to chromosome 9q22.4 Mutations in the serum palmitoyltransferase, long chain base subunit-1 (SPTLC1) gene were then identified as the cause.5,6 In CMT2B, genetic linkage analysis identified a locus on chromosome 3q13–q22.7–9 The small GTP-ase late endosomal protein RAB7 was subsequently identified as the disease gene.10 Two mutations have been described: a transition Val162Met mutation in exon 4 and a Leu129Phe mutation in exon 3.

RAB7 is one of more than 60 Ras-related GTP-ases. These proteins regulate vesicle transport by the recruitment of specific effector proteins with a possible role linking vesicles and target membranes to the cytoskeleton.11,12 RAB7 is involved in transport between late endosomes and lysosomes. The mechanism of action of RAB7 genetic mutations in the development of peripheral neuropathy is as yet unknown, although RAB proteins are known to be involved in neurite outgrowth.13

We report the clinical and pathological findings in a further autosomal dominant ulcero-mutilating neuropathy family, with an Asn161Thr mutation in the RAB7 gene. This mutation is in a highly conserved region, adjacent to the reported Val162Met mutation, suggesting a functionally important hotspot for RAB7 mutations. The mutation leads to a virtual absence of expression of a key RAB7 effector, RILP (Rab interacting lysosomal protein), implying the pathogenetic mechanism for this neuropathy may be through this effector pathway.

Materials and Methods

Nerve Biopsy

A sural nerve fascicular biopsy specimen was obtained from the proband with informed consent and processed according to our standard laboratory procedures.14 The procedure was justified on routine diagnostic grounds, having taken place at a time before molecular genetic testing for HSN1 and CMT2B had become available, and after other affected family members had died. In addition to semithin sections for light microscopy and morphometry, and ultrathin sections for electron microscopy, frozen sections were stained immunocytochemically using a polyclonal antibody against RILP15 (gift from Dr C. Bucci).

Genetic Analysis

With informed consent, DNA was extracted from blood samples obtained from affected and unaffected family members. The five exons and flanking intronic regions of the RAB7 gene were amplified by polymerase chain reaction (PCR) (primers available on request). PCR products were purified (96-well plate; Millipore, Billerica, MA) and sequenced using the BigDye Terminator cycle sequencing kit. Sequencing reactions were loaded on an ABI 3100 Genetic Analyzer (Perkin Elmer Applied Biosystems, Foster City, CA). Exons 5 and 6 of the SPTLC1 gene also were sequenced.5,6

Results

Clinical Details

The family reported here is an autosomal dominant, three-generation, English pedigree with ulcero-mutilating neuropathy. The proband is a 56-year-old man. At the age of 16 years, he developed a painful ulcer on the left sole. This lesion never healed and he had numerous operations on this foot. At the age of 47 years, he developed progressive right foot pain, with swelling and deformity. There was also spontaneous lancinating pain in the left foot. His mother had also been affected, with scoliosis, ulcers, and operations on her feet, including amputations. His maternal grandfather had a history of gangrene and leg amputation. His brother and son were clinically normal.

On examination, the proband had gross deformity of the right foot. The middle toe of his left foot had been amputated. He had mild scoliosis. There was nystagmus to lateral gaze. In the limbs, there was no wasting. Tone, power, and coordination were normal. The ankle jerks were absent. Plantar responses were downgoing. Vibration sensation was reduced to the costal margins. Joint position sense was abnormal at the toes. Pin-prick sensation was impaired in the right foot and later the left.

Blood tests were normal including syphilis serology. Plain radiographs of the right foot showed a Charcot–Lisfranc joint. Brain imaging showed cerebellar degeneration. Autonomic function tests were normal.

Nerve Conduction Studies

Motor nerve conduction studies in the proband were normal in the upper limbs. In the lower limbs, distal motor latency was prolonged in the common peroneal nerve at 9.2 milliseconds, with a compound muscle action potential amplitude of 0.1mV. Sensory conduction velocities were normal throughout, but some sensory nerve action potentials were reduced in amplitude (right median, 7µV; left median, 5µV; right ulnar, 3µV; left ulnar, 3µV; right radial, 23µV; left sural, 7µV). Thermal thresholds were abnormal at the right thenar eminence (warming, 13°C; cooling, 2.5°C; difference, 15.5°C). At the lateral border of the right foot, thresholds were also abnormal (warming, 13.4°C; cooling, 3.5°C; difference, 16.9°C). Large fiber nerve conduction studies were normal in the proband’s brother.

Nerve Biopsy

Sural nerve fascicular biopsy from the proband showed moderately severe depletion of the myelinated fiber population (Fig 1A). This was confirmed by mor-
Fig 1. Sural nerve biopsy from the proband. (A) Semithin resin section (thionin and acridine orange) showing loss of myelinated fibers with prominent regenerative clusters (arrows). Bar = 50μm. (B, C) Immunostaining against RILP in nerve biopsy from proband and control, respectively. (brown) Horseradish peroxidase. Bar = 50μm.
phometry: total myelinated fiber density was 3,301/mm² compared with a value of 7,007/mm² from a normal control of the same age. The myelinated fiber size distribution at first sight appeared relatively normal because the loss of the normal small fiber population was counterbalanced by the presence of many small regenerating fibers. There were very few remaining normal small fibers (see Fig 1A). No abnormal axonal or Schwann cell inclusions were seen by light microscopy, but electron microscopy showed some inclusions in small unmyelinated axons suggesting active axonal loss. This was confirmed by the presence of collections of flattened sheets of Schwann cell processes. Some Remak bundles contained more axons than normal, also suggesting regeneration. Immunostaining of the proband’s nerve biopsy specimen with polyclonal antibody against RILP was markedly reduced compared with control (see Fig 1B, C).[15]

**Genetic Analysis**

Sequencing the entire RAB7 gene in the proband showed an A to C heterozygous mutation in exon 4 (Fig 2), causing an amino acid change from asparagine to threonine at position 161. This change was absent in the unaffected brother and in 200 chromosomes from English controls on restriction enzyme digest with the enzyme TaqI (Fermentas, Hanover, MD). No other family individual was available for genetic analysis. Sequencing exons 5 and 6 of the SPTLC1 gene in the proband was normal.

**Discussion**

The index case in this family showed an early-onset, slowly progressive, ulcero-mutilating neuropathy with amputations and no motor features. There was also evidence of central involvement with nystagmus and cerebellar atrophy on brain imaging. The neuropathy was inherited in an autosomal dominant fashion over three generations. Nerve conduction studies supported the diagnosis of a sensory axonal neuropathy, although the electrical features were mild. Sural nerve biopsy showed appearances of a chronic active neuropathy causing axonal degeneration and prominent regeneration.

This family expands the phenotypic spectrum of CMT2B. The findings in the proband suggest there are few distinguishing features between CMT2B and HSN1. Clinically, the presence of positive sensory features, the development of ulcers, and the need for amputations would indicate HSN1. The lack of motor findings is more suggestive of HSN1 than CMT2B. These anomalies, and the evidence for central nervous system involvement, illustrate how establishing the genetic basis of a disease may expand the range of phenotypic manifestations. This phenomenon has been recognized in the hereditary neuropathies since molecular genetic techniques became readily available.[16] Pathologically, the proband’s sural nerve biopsy did show features typical of CMT2, these being an axonopathy with marked regeneration, although previous histological descriptions have largely been restricted to CMT2A.[16]

The Asn161Thr mutation identified in this family is only the third to be reported in the RAB7 gene. This mutation is adjacent to the previously reported Val162Met mutation, suggesting clustering at a potential hotspot for RAB7 mutations. Codons 161 and 162 are both highly conserved (Fig 3), implying functional importance throughout evolution, and are located in the C terminus region of RAB7. The other RAB7 mutation is located at codon 129, close to a conserved guanine nucleotide binding domain.

RAB7 is a protein involved in vesicle transport between late endosomes and lysosomes and is thought to act by the induction of a number of effectors.[17] One of the key effectors of RAB7 is RILP, which induces the
recruitment of dynein-dynactin motors to the late endosome/lysosome, thereby stimulating the transport of these compartments toward the perinuclear region along microtubules.\textsuperscript{15,18} The interaction of RAB proteins with effectors such as RILP may underlie the involvement of these proteins in neurite outgrowth.\textsuperscript{13}

The significant reduction of RILP expression (Fig 1B, C) in the proband’s sural nerve biopsy suggests that the mutation lies in an essential part of the gene, crucial for RAB7 and RILP interaction. Alternatively, the RAB7 mutation may cause a dominant negative effect with a reduction in RAB7 production and hence RILP interaction. It seems less likely that mutant RAB7 causes the disease phenotype directly. If this were the case, more widespread clinical abnormalities might be expected, given the probable role of RAB7 in cellular housekeeping. These putative mechanisms are probably oversimplifications, however, because RAB7 may have additional functions and intermolecular relationships. Another RAB7 effector, RABring7, is thought to act in additional functions and intermolecular relationships.\textsuperscript{19}

In conclusion, the ulcero-mutilating neuropathies are a diverse group of disorders, both clinically and genet-ically. The family described here expands the range of phenotypic manifestations of CMT2B and may give an initial clue to the pathogenetic mechanism of RAB7 mutations.

References


