

SHORT REPORT

SPTLC1 and RAB7 mutation analysis in dominantly inherited and idiopathic sensory neuropathies

C J Klein, Y Wu, K E Kruckeberg, S J Hebring, S A Anderson, J M Cunningham, P J B Dyck, D M Klein, S N Thibodeau, P J Dyck

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Background: The variable clinical features of hereditary sensory and autonomic neuropathy (HSAN I) suggest heterogeneity. Some cases of idiopathic sensory neuropathy could be caused by missense mutations of *SPTLC1* and *RAB7* and not be recognised as familial.

Objective: To screen persons with dominantly inherited HSAN I and others with idiopathic sensory neuropathies for known mutations of *SPTLC1* and *RAB7*.

Patients: DNA was examined from well characterised individuals of 25 kindreds with adult onset HSAN I for mutations of *SPTLC1* and *RAB7*; 92 patients with idiopathic sensory neuropathy were also screened for known mutations of these genes.

Results: Of the 25 kindreds, only one had a mutation (*SPTLC1* 399T→G). This kindred, and 10 without identified mutations, had prominent mutilating foot injuries with peroneal weakness. Of the remainder, 12 had foot insensitivity with injuries but no weakness, one had restless legs and burning feet, and one had dementia with hearing loss. No mutation of *RAB7* was found in any of these. No known mutations of *SPTLC1* or *RAB7* were found in cases of idiopathic sensory neuropathy.

Conclusions: Adult onset HSAN I is clinically and genetically heterogeneous and further work is required to identify additional genetic causes. Known *SPTLC1* or *RAB7* mutations were not found in idiopathic sensory neuropathy.

Hereditary sensory and autonomic neuropathy type I (HSAN I) is an autosomal dominant disorder, typically presenting in the second or later decade of life with prominent small fibre (drC and A σ) sensory involvement. Variable degrees of motor and autonomic dysfunction may also be present. The sensory alterations are associated with the following: mutilating acropathy and peroneal muscle atrophy with weakness; neurogenic arthropathy without weakness; mutilating acropathy, with or without hearing loss, and with or without dementia; and restless legs with burning feet, and possibly other phenotypes.¹

Mutations in serine palmitoyltransferase long chain base subunit-1 (*SPTLC1*) have been identified as the cause of HSAN I, at least for some kindreds.^{2–4} Affected individuals have increased de novo glucosyl ceramide synthesis in lymphoblast cell lines and resultant abnormal neuronal apoptosis, possibly explaining the neuronal degeneration found. In 14 previously reported HSAN I kindreds with identified mutations, all have occurred in four amino acids (C133W, C133Y, V144D, and G387A).^{2–4}

Initially the disorder studied here was designated a form of Charcot–Marie–Tooth type 2B (hereditary motor and sensory neuropathy type 2B (HMSN 2B)). However, the presence of mutilating injuries of the feet may suggest an altered designation (HSAN I).⁵ Several mutations of the endosomal

protein *RAB7* have been described (V162M and L129P) and are associated with sensory loss and mutilation.⁶

Whether mutations of *SPTLC1* or *RAB7* account for the different clinical varieties of HSAN I is the first question addressed here. The second is whether the known mutations of these genes account for some of the cases of cryptogenic sensory predominant neuropathies. Among chronic cryptogenic length dependent sensorimotor polyneuropathies, it is assumed that diverse mechanisms are involved—immune, postinfectious, infectious, metabolic, nutritional (deficiency), toxic, paraneoplastic, and other.⁷ At the time that patients are intensively evaluated, the putative cause may not be readily apparent. There is a possibility that some of these patients may have unrecognised hereditary sensory and autonomic neuropathies, the second question studied here.

SUBJECTS

HSAN kindreds

With institutional review board approval, we assessed DNA isolated from lymphoblast cell lines of patients diagnosed by us (CJK, PJBD, PJD) with HSAN I or idiopathic sensory predominant neuropathies and evaluated at Mayo Clinic Rochester since 1980. Patients with congenital or childhood onset disease were not included. All patients had clinical and electrophysiological studies consistent with HSAN I¹ along with extensive clinical review and studies to exclude alternative causes of sensorimotor polyneuropathy.⁸ Only families with confirmatory examination or testing of an additional family member in a different generation were included. DNA was available from 25 kindreds. The number of individuals known to be affected within a family ranged from two to 28. In nine families, male to male transmission was documented. Sural nerve biopsies had been obtained from persons in six families, and in these biopsy specimens there was axonal loss with no specific interstitial pathological abnormality to suggest another cause for the neuropathy. Male probands were all examined and found not to have α -galactosidase deficiency, the enzyme deficiency responsible for Fabry's disease.

Among 25 HSAN I families, 11 also had peroneal atrophy and mutilating acropathy with foot fractures and ulcers of the feet including amputations; 12 had similar foot injuries without peroneal atrophy or weakness; one had restless legs and burning feet with neuropathy; and one had dementia, hearing loss, and mutilating sensory polyneuropathy.

Idiopathic sensory predominant neuropathies

We studied 92 patients with idiopathic sensory polyneuropathy. Pain (often burning or lancinating associated with paraesthesiae) occurred in 77 of them. All had electrophysiological evidence of sensory nerve involvement. Sensory loss was much more prominent than weakness. In the 25 patients who had weakness, it was mild, affecting intrinsic

Abbreviations: HSAN, hereditary sensory and autonomic neuropathy; HMSN, hereditary motor and sensory neuropathy

Table 1 Primer sets used to sequence the open reading frame of the two genes

<i>SPTLC1</i>	
Exon 1	F 5'-CTGCGGAGGTGATAACGACAC-3' R 5'-AGACCTGTCTCTGCGTCCC-3'
Exon 2	F 5'-AGGGAGAAATTCCTGCTATT-3' R 5'-TTCCCATATTTGCCTA-3'
Exon 3	F 5'-TAAGAGTGACAGCGAACTGA-3' R 5'-GGTAACGTGAGACAAAT-3'
Exon 4	F 5'-CCACCAAGGTAATATGATAGC-3' R 5'-GGAAAACTTGCATAGCG-3'
Exon 5	F 5'-ATTGGGATTGTGGGCTAACAG-3' R 5'-CTCTGCACTCGTCTCACGC-3'
Exon 6	F 5'-AGATGCGTAGCTGATTAG-3' R 5'-ATGACTATAGACACCGTTAAG-3'
Exon 7	F 5'-TTATGTGAAAAGGACTCATGG-3' R 5'-GCAATGACAAGCAATTAC-3'
Exon 8	F 5'-AAAGTGCTGGGAATATGTAA-3' R 5'-CACTAAAAGCGGTCAATAACA-3'
Exon 9	F 5'-GCCATGAAAAGGGTAGTTGT-3' R 5'-GGAACACTGCTTGTGCTAT-3'
Exons 10-11	F 5'-CTCCTTTCCGGCAGATTGAT-3' R 5'-CCTTGGGGCAGGAATAGCTC-3'
Exon 12	F 5'-CTTGGCTGTGTAGTATGAG-3' R 5'-CTTAGCTCAATCTGGT-3'
Exon 13	F 5'-CGCAAGCTTCTGTACGTT-3' R 5'-AGCATCTTCTTTTACCCTC-3'
Exon 14	F 5'-GCAGGATTCAGCGTTC-3' R 5'-CTTTCAGATGTGGCAGATAG-3'
Exon 15	F 5'-CTTACTGTCTCAGCATTGC-3' R 5'-GAAGCTCCAGTTTTAAACG-3'
<i>RAB7</i>	
Exons 1-2	F 5'-GCCGGAACGTGACGCAAGG-3' R 5'-TGAAGAACCGGATGGGAACGC-3'
Exon 3	F 5'-TTAGTTCAAATTGCCAATCCA-3' R 5'-TCAAATAGCCATCCGTTCC-3'
Exon 4	F 5'-GCTCGGAAGGCTACTG-3' R 5'-TGAGTATCAGCCAATTATCTA-3'
Exon 5	F 5'-GCACCCTGGAGATTGGACTCA-3' R 5'-CCACCAGCACTCTAGAGC-3'
Exon 6	F 5'-CTAGCAGGACAAGTTGGACG-3' R 5'-GGGAGCCTCAGACTTCA-3'
Exon 7	F 5'-GTTTCTATCCCTGAGTAACC-3' R 5'-GGGGTAAAAAGACCAAGAC-3'

foot muscles or plantar dorsiflexors. High arches or hammer toes, usually mild in degree, were noted in 20 patients. Charcot joints with radiological confirmation of bone fractures were found in 15 persons including painless foot ulcers in five. The family history was reviewed in all cases and for 13 families it was reported that there were other family members with foot pain or previous foot surgery. Testing of relatives for neuropathy was not carried out. Sural nerve biopsies were done in 18 cases and showed prominent axonal degeneration or fibre loss but no interstitial pathological abnormalities. Male cases (n = 40) had negative examination for α -galactosidase deficiency (that is, for

Fabry's disease), and all had extensive testing for acquired causes of neuropathy.

DNA SEQUENCING OF *SPTLC1* AND *RAB7*

DNA samples from the probands of each of the studied kindreds were examined by mutation analysis of the entire open reading frame of *SPTLC1* and *RAB7* by conventional fluorescent sequencing, using polymerase chain reaction and the primers shown in table 1. Among our idiopathic sensory neuropathy patients, the known mutations of *SPTLC1* (398G→A C133Y, 399T→G C133W, and 431T→A V144D) and *RAB7* (484G→A, V162M and 385C→T, L129P) were screened for, using the appropriate primer sets in table 1. Pyrosequencing (PSQ 96 SNP reagent kit, Pyrosequencing AB, Uppsala, Sweden) of the known mutations of *SPTLC1* and *RAB7* was also done in all kindred and all patients with idiopathic sensory neuropathy, employing conventional sequencing data.

RESULTS

Of our 25 kindreds, only one kindred (or person) had a mutation in the *SPTLC1* gene 399T→G (C133W) (fig 1). This kindred from Newfoundland, Canada, had prominent mutilating foot injuries with peroneal muscle atrophy and weakness. Two of the family members had amputations of the toes or feet and male to male transmission. The mother of our proband at the time of his initial visit had no reported symptoms, but later developed positive sensory symptoms and reported mild sensory loss in her six decade of life. *RAB7* mutations were not found in our studied kindreds. The known *SPTLC1* and *RAB7* mutations were not found in our group of patients with idiopathic sensorimotor polyneuropathy. Four kindreds had *RAB7* polymorphism of C to G at nucleotide position 207, which is not predicted to alter the amino acid leucine and has been described previously (<http://genome.ucsc.edu/cgi-bin/hgGateway@SNPentry.RS11549749>).

DISCUSSION

On the basis of this study, mutations in *SPTLC1* and *RAB7* appear to be an uncommon cause of HSAN I among our American kindreds with patterns of inheritance and clinical features typical of HSAN I. Also, known mutations of these genes were not found in our patients with idiopathic sensory polyneuropathies. Only one of 25 HSAN I kindreds had an *SPTLC1* mutation 399T→G (C133W) and was from Newfoundland, Canada. This kindred had mutilating acropathy leading to amputations with peroneal atrophy and weakness similar to those reported previously with *SPTLC1* mutation.²⁻⁴ Also of note is the extent of variable expression in that family, wherein the patient's mother was clinically very mild and only presented late in life. Ten other kindreds with a similar phenotype (that is, HSAN with or without mutilating acropathy and peroneal muscle atrophy) did not have mutations of either gene. None of the other phenotypes of HSAN I had mutations. These results strengthen the suggestion that genetic heterogeneity exists for the varieties of adult onset, dominantly inherited sensory neuropathies. Second, known mutations of *SPTLC1* and *RAB7* were not responsible for any of our cases of cryptogenic sensory predominant neuropathy. Because we did not examine the entire open reading frame for *SPTLC1* and *RAB7* mutations in the idiopathic sensory cases, we cannot exclude other mutations.

Among some of the familial cases, our results were predicted on clinical grounds. Specifically, a single molecular genetic mechanism is unlikely to have caused the degree of clinical variability observed¹:

- HSAN with or without mutilating acropathy and peroneal muscle atrophy;

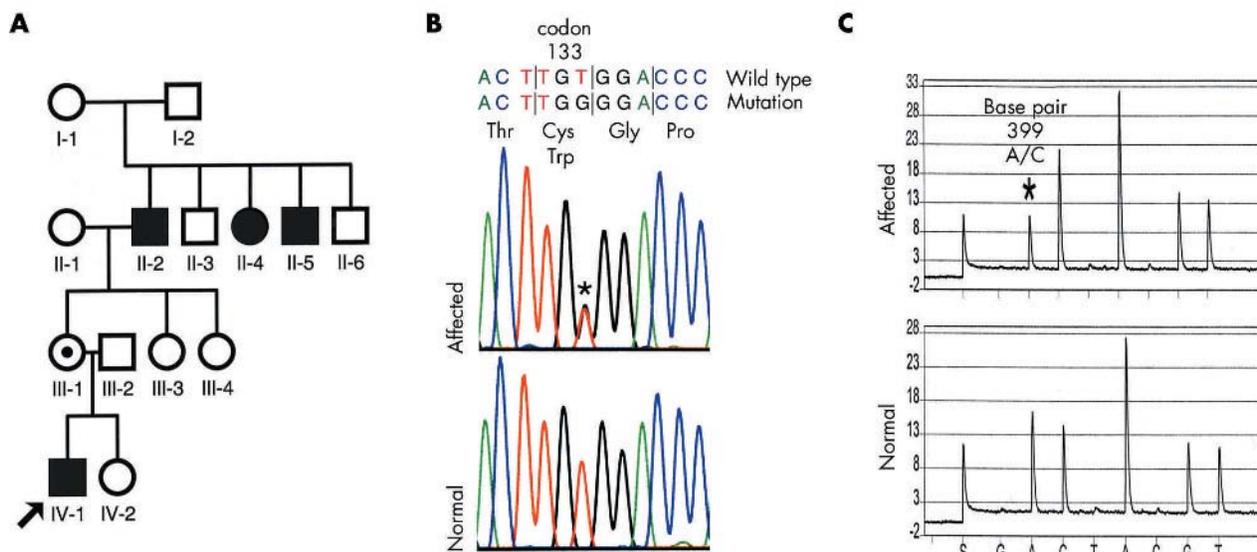


Figure 1 (A) Pedigree of the Newfoundland kindred with the described mutation. II-4 and II-5 had both undergone foot amputations, and the former had those amputations after having burned her feet against an oven without recognising pain. The mother of the proband II-1 was asymptomatic at the time of her son's initial examination. She was later reported to have developed positive prickling tingling of her feet and sensory loss in her sixth decade without mutilating foot injuries. Her son had no foot ulcerations and initially presenting with painful dysaesthetic shooting extremity pain by age 12 years evolving to anaesthesia with peroneal atrophy at age 22 years. (B) The forward complementary missense mutation 399T→G (C133W) within exon 5 of serine palmitoyltransferase, long chain base subunit-1 (*SPTLC1*). (C) Confirmatory reverse strand pyrosequencing analysis of the same mutation (above) and normal (below) showing evidence of the heterozygous A and C base pair population at position 399. Each peak (y axis) represents the luminescent reaction with successful incorporation of the specific purine or pyrimidine base released for incorporation (x axis).

- HSAN with or without plantar ulcers;
- HSAN with or without neurogenic arthropathy;
- HSAN with or without burning feet;
- HSAN with or without mutilating acropathy, with or without hearing loss, with or without dementia;
- HSAN with or without restless legs.

Affected persons within these kindreds tend to have the essential features of their syndrome, providing further support for separate genetic causes and not simply as modifying factors of a similar genetic defect. This is not to say that each of the syndromes listed here will ultimately be found to have a unique and different molecular genetic mechanism. The fact that only one of our 11 HSAN I kindreds with weakness had the gene mutation does suggest that even those with similar phenotypes may be genetically heterogeneous.

Compared with the earlier investigations,²⁻⁶ we found far fewer *SPTLC1* and *RAB7* mutations in similarly affected HSAN I families—that is, with peroneal atrophy and mutilating foot injuries. Our data might suggest that the founder effect described in patients from southern England and Europe,⁹ from where our Newfoundland family may have originated, does not exist among the American patients. We did not identify any patients with *RAB7* mutation, whereas the previous investigators identified it from 24 unrelated kindreds, two with V162M and one with L129P missense mutations.⁶

Mutation analysis of the heterodimer molecule of *SPTLC1* (that is, *SPTLC2*) has been excluded as a causative factor in several families with HSAN I.⁹ Other pathways important in the regulation of sphingolipids or endosomal trafficking may be implicated in candidate gene approaches, but it is likely that linkage analysis will continue to be an important first step in the identification of new causative genes.

Authors' affiliations

C J Klein, P J B Dyck, D M Klein, P J Dyck, The Peripheral Neuropathy Research Laboratory, Mayo Clinic College of Medicine, Rochester, Minnesota, USA

Y Wu, K E Kruckeberg, S J Hebring, S A Anderson, J M Cunningham, S N Thibodeau, The Molecular Genetics Research Laboratory, Mayo Clinic College of Medicine

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Correspondence to: Dr Christopher J Klein, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA; klein.christopher@mayo.edu

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