SPTLC1 is mutated in hereditary sensory neuropathy, type 1

Hereditary sensory neuropathy type 1 (HSN1, MIM 162400; ref. 1) genetically maps to human chromosome 9q22 (refs. 2–4). We report here that the gene encoding a subunit of serine palmitoyltransferase is located within the HSN1 locus, expressed in dorsal root ganglia (DRG) and mutated in HSN1.

Starting with HSN1-linked markers, we developed a contig of 112 genomic PAC and BAC clones across the *HSN1* locus delimited by loci *D9S1841* and *D9S197* (Fig. 1*a*). This contig contains 51 markers, spans 1.85 Mb and includes 21 novel sequence-tagged sites (STSs; deposited in GenBank).

We used STS sequences to search for transcripts in three databases (http://ncbi.nlm.nih.gov/BLAST/, http://hercules.tigem.it/ and http://hercules.tigem.it/UNIBLAST/uniblast.html). This revealed expressed sequence tags (ESTs) within genes encoding seven identified proteins: faciogenital dysplasia protein-3 (*FGD3*), neurotrophic tyrosine kinase[5] (*ROR2*), ninjurin-1 (*NINJ1*; ref. 6), serine palmitoyltransferase subunit-1 (*SPTLC1*; refs. 7,8), osteoglycin (*OGN*), extracellular matrix protein-2 (*ECM2*) and osteomodulin (*OMD*). Sequencing of all exons of *ROR2* and *NINJ1* disclosed no mutations in HSN1 patients.

SPTLC1 mapped to cDNA clone Y08685 and BAC 667L20 (Fig. 1*b*). The sequence of *SPTLC1* contains an ORF of 1,422 nt and encodes a 473–amino-acid protein[7,8], designated 'long chain base 1' (LCB1). Comparison of the cDNA and genomic DNA sequences (http://www.ncbi.nlm.nih.gov/BLAST/) demonstrated that the SPTLC1 protein is encoded by 15 exons and provided intronic DNA sequences surrounding each exon.

We analyzed the full DNA sequence of *SPTLC1* in affected individuals in eight different HSN1 families using either RT–PCR and cycle sequencing of cDNA (blood leukocyte mRNA) or amplification and sequencing of the 15 exons. We detected mutations in 2 different nucleotides of codon 133 in exon 5 in affected members of 2 of 8 families. In family d1, of German origin, A replaces G at position 398 and is predicted to substitute tyrosine for cysteine at residue 133 of the protein (C133Y; Fig. 1*b*). We confirmed this change by RT–PCR on poly(A)$^+$ RNA from a lymphoblastoid cell line from the patient. Analysis of all 72 members of the family, including 27 affected individuals, showed complete linkage between the base change and the disease. We detected a second mutation in a Canadian HSN1 family[9] (n1) in which G replaces T at position 399, predicting substitution of tryptophan for cysteine (C133W). These were the only mutations in these families. We found no mutations in DNA samples from 50 unrelated normal control individuals.

Probing with IMAGE clone 133401 (encompassing *SPTLC1* ESTs A001U11 and WI-8025), we identified an approximately 3-kb *SPTLC1* transcript in northern-blot analysis of 23 adult human tissues and fetal human brain, lung, liver and kidney (data not shown). Using 5′-RACE–PCR on cDNA transcribed from adult rat dorsal root ganglion (DRG) total RNA, we amplified a predicted 320-bp product of *SPTLC1* cDNA in DRG (Fig. 1*c*), demonstrating that *SPTLC1* is expressed in DRG.

It is of interest that HSN1 arises from mutations in the gene encoding a subunit of the enzyme SPT, because this is the most frequent inherited disease affecting pain fibers and the primary defects causing hereditary axonal neuropathies have only rarely been defined[10–13]. The protein product of *SPTLC1*, LCB1, forms a complex with at least one other protein, LCB2 (ref. 7). In mammals the enzymatic SPT activity of this complex is conferred primarily by LCB2 (ref. 7), which catalyzes the pyridoxal 5′-phosphate dependent decarboxylation and

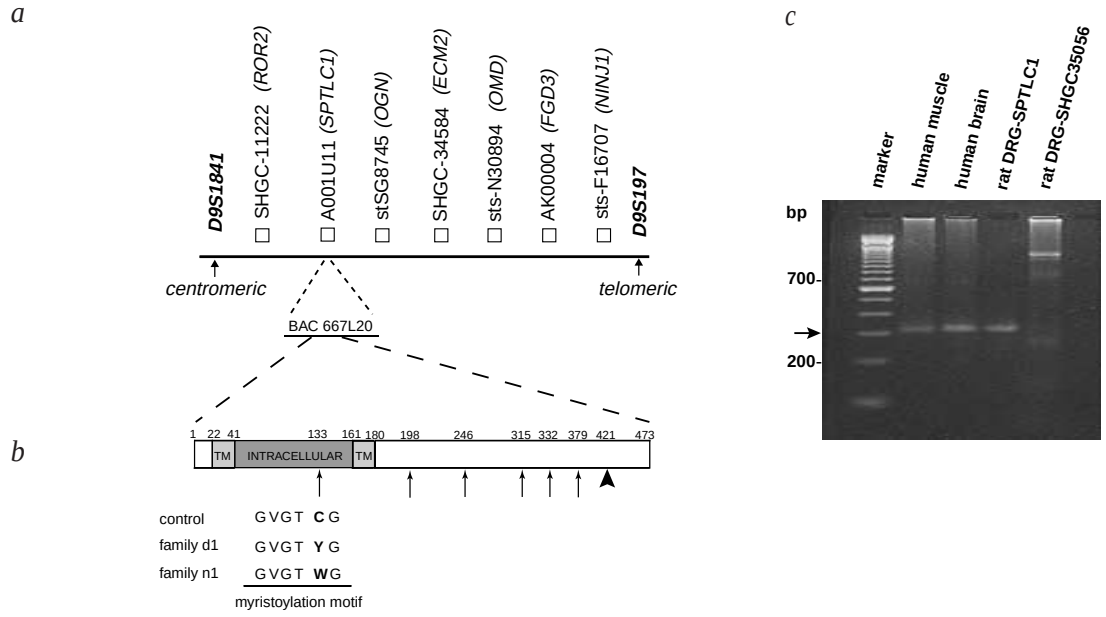

Fig. 1 The HSN1 locus. ***a***, Schematic of minimal HSN1 locus. Open squares indicate known genes. ***b***, Structure of LCB1. Transmembrane domains (TM) were predicted using the TMHMM server (http://www.cbs.dtu.dk/services/TMHMM). Arrows pointing up indicate myristoylation sites; the filled triangle indicates a possible glycosylation site. ***c***, 320-bp 5′-RACE fragment of *SPTLC1* amplified from human brain and muscle and rat DRG cDNA.

condensation of serine with palmitoyl-CoA to form 3-ketosphinganine. 3-Ketosphinganine undergoes acylation and dehydrogenation to form ceramide, a precursor of all sphingolipids. SPT is the rate-limiting, regulatory step in the synthesis of sphingolipids and may therefore be an important modulator of diverse functions downstream of *de novo* sphingolipid biosynthesis. That HSN1 is transmitted as an autosomal dominant trait argues either that the mutant LCB1 protein has acquired a novel adverse property or that it somehow alters levels of SPT activity. For example, mutant LCB1 may act as a dominant-negative inhibitor of LCB2. Alternatively, mutant LCB1 may augment total SPT activity, elevating ceramide production. This has potential implications for long-term viability of DRG, as ceramide may be a signaling factor in apoptotic death and other pathways[14]. The mutations reported here replace a cysteine within a possible consensus site for N-myristoylation (residues 129–134; ref. 15). Acylation of proteins with lipids like myristate enhances protein–membrane interactions by increasing hydrophobicity of the acylated domain[15]. It is therefore possible that the mutations in families d1 and n1 eliminate critical interactions of LCB1 with membrane binding sites.

There is heterogeneity among hereditary sensory and autonomic neuropathies (also designated HSAN). The genetic loci for four are defined: HSN1 (chromosome 9q22), HSAN3 (MIM 223900, chromosome 9q31), HSAN4 (MIM 256800, TRKA gene defects on chromosome 1q22) and HSAN-6 (MIM 310470, X-linked; ref. 16). There also is genetic heterogeneity within the HSN category. A family with an HSN1 phenotype that resembles Charcot-Marie-Tooth disease type 2B (MIM 600882) links to chromosome 3q (ref. 17). In another HSN1 family, linkage to 9q and 3q was excluded[18], implicating a third HSN locus. In our study, six cases from different HSN1 pedigrees revealed no *SPTLC1* mutations. The gene encoding the second subunit, *SPTLC2* (human chromosome 14q, GeneMap98), is therefore a candidate for mutation in HSN cases with no *SPTLC1* defects.

Acknowledgments

We thank G. Nicholson for sharing prepublication results. This work was supported by the Deater Foundation, Inc., the Neuropathy Association, Inc., ProjectALS and the Muscular Dystrophy Association. R.H.B.'s laboratory also receives support from the A.L.S. Association, the P.L. de Bourgknecht A.L.S. Research Foundation, the Myrtle May MacLellan A.L.S. Research Foundation and the C.B. Day Investment Company, Inc. Support is also acknowledged from the National Institute of Health and the National Institute for Aging.

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Received 12 December 2000; accepted 30 January 2001.

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