

Report

***NIPA1* Gene Mutations Cause Autosomal Dominant Hereditary Spastic Paraplegia (SPG6)**

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The hereditary spastic paraplegias (HSPs) are genetically heterogeneous disorders characterized by progressive lower-extremity weakness and spasticity. The molecular pathogenesis is poorly understood. We report discovery of a dominant negative mutation in the *NIPA1* gene in a kindred with autosomal dominant HSP (ADHSP), linked to chromosome 15q11–q13 (SPG6 locus); and precisely the same mutation in an unrelated kindred with ADHSP that was too small for meaningful linkage analysis. *NIPA1* is highly expressed in neuronal tissues and encodes a putative membrane transporter or receptor. Identification of the *NIPA1* function and ligand will aid an understanding of axonal neurodegeneration in HSP and may have important therapeutic implications.

Ten loci for autosomal dominant hereditary spastic paraplegia (ADHSP) have been mapped, and four ADHSP genes have been identified: *SPG4*/spastin, *SPG3A*/atlastin, *SPG13*/chaperonin 60, and *SPG10*/KIF5A (Hazan et al. 1999; Zhao et al. 2001; Hansen et al. 2002; Reid et al. 2002). (For reviews of hereditary spastic paraplegia [HSP] ["Strümpell-Lorrain syndrome," MIM 182600], see Fink and Hedera 1999; Fink 2001, 2002; Reid 2003.) Despite these advances, the molecular pathophysiology of the ADHSPs is largely unknown. Elsewhere, we identified a locus for uncomplicated ADHSP in chromosome 15q (*SPG6*) (Fink et al. 1995a; 1995b). We now report identification of disease-specific mutations in a novel gene, *NIPA1* (fig. 1a) in the original SPG6-linked kindred (Fink et al. 1995a) and in an unrelated kindred with ADHSP (fig. 1b).

The *SPG6* locus extends 6.1 cM between D15S128 and the centromere (Rainier et al. 2000) (fig. 2a), an interval often involved in deletions that result in Prader-Willi syndrome (PWS) or Angelman syndrome (AS). PWS and AS are characterized by genetic imprinting, a

phenomenon in which gene expression and the phenotype depend on the sex of the transmitting parent (Nicholls and Knepper 2001). We analyzed a large kindred—ADHSP-ARK1 (fig. 1b), in which ADHSP was linked to the *SPG6* locus—and found no evidence of genetic imprinting (Fink et al. 1995a). Therefore, we analyzed as *SPG6* candidates the four unique, nonimprinted, and highly evolutionarily conserved genes mapped proximal to the imprinted domain and within the pericentromeric region of chromosome 15q (Chai et al. 2003 [in this issue]). These candidate genes included nonimprinted in Prader-Willi/Angelman loci 1 (*NIPA1*) (NCBI accession number BK001020) and 2 (*NIPA2*) (NCBI accession number BK001120) (Chai et al. 2003 [in this issue]), *GCP5* (NCBI accession number AF272884) (Murphy et al. 2001), and *CYFIP1* (NCBI accession number NM_014608) (Koybayashi et al. 1998; Schenk et al. 2001).

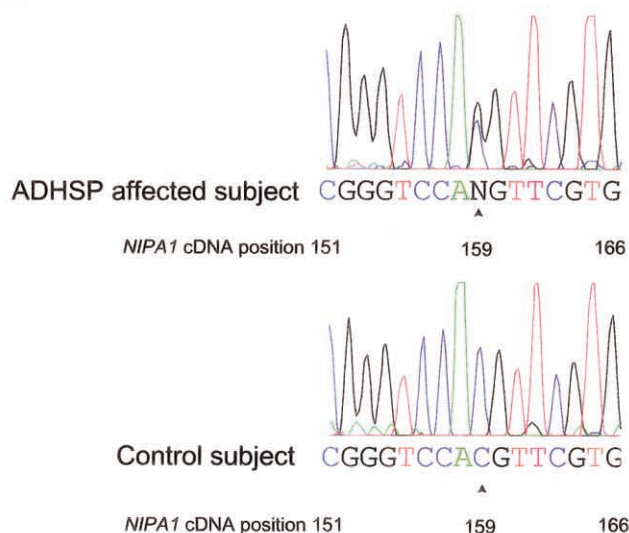
We identified a nucleotide substitution at position 159 of the *NIPA1* cDNA (159C→G; fig. 1a), which resulted in an amino acid substitution at position 45 of the *NIPA1* protein (T45R) in each affected subject ($n = 28$) in ADHSP-ARK1 (fig. 1b). In contrast, each unaffected subject ($n = 17$) had only C at this position (fig. 1b), which agrees with the known human genomic sequence (NCBI accession number NT_024668). We examined 105 control subjects (ascertained through the Elderly Subjects Program of the University of Michigan Institute of Gerontology). Each control subject had only C at position 159

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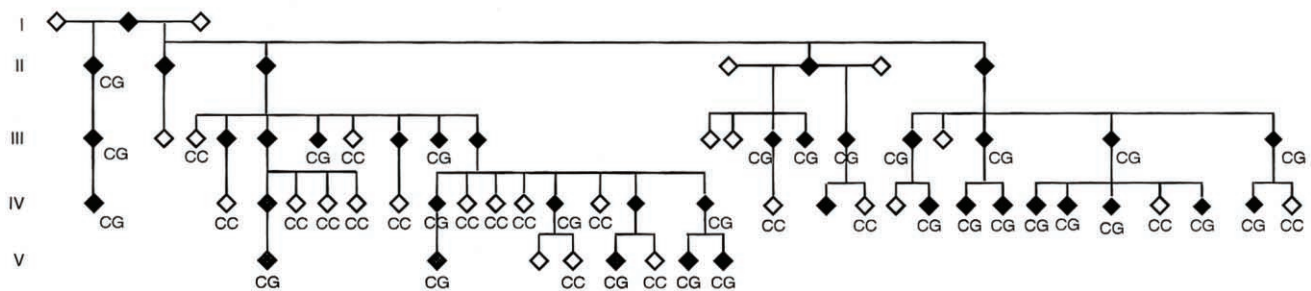
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A**B**

ADHSP-ARK1



ADHSP-IRQ1

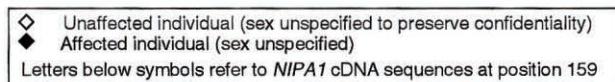
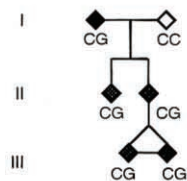


Figure 1 *a*, Representative *SPG6* sequence of normal and affected subjects from the ADHSP-ARK and ADHSP-IRQ1 kindreds. PCR products (from 50 ng of DNA) were purified through Sephadex G-50 columns, were analyzed by agarose gel electrophoresis, and were sequenced either by use of the ABI PRISM dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems) on an ABI PRISM 3100 Genetic Analyzer, according to the manufacturer's instructions, or through the University of Pennsylvania DNA sequencing facility. The arrows mark the position of the *NIPA1* mutation (cDNA nucleotide 159). *b*, Pedigrees of ADHSP-ARK1 and ADHSP-IRQ1. Letters refer to sequence at nucleotide 159 of the *NIPA1* cDNA sequence. Clinical features (Fink et al. 1995*a*), muscle biopsy (Hedera et al. 2000), and genetic features (Fink et al. 1995*b*) of ADHSP-ARK1 have been reported elsewhere. The ADHSP-IRQ1 kindred was ascertained through the University of Michigan Neurology Outpatient Clinic.

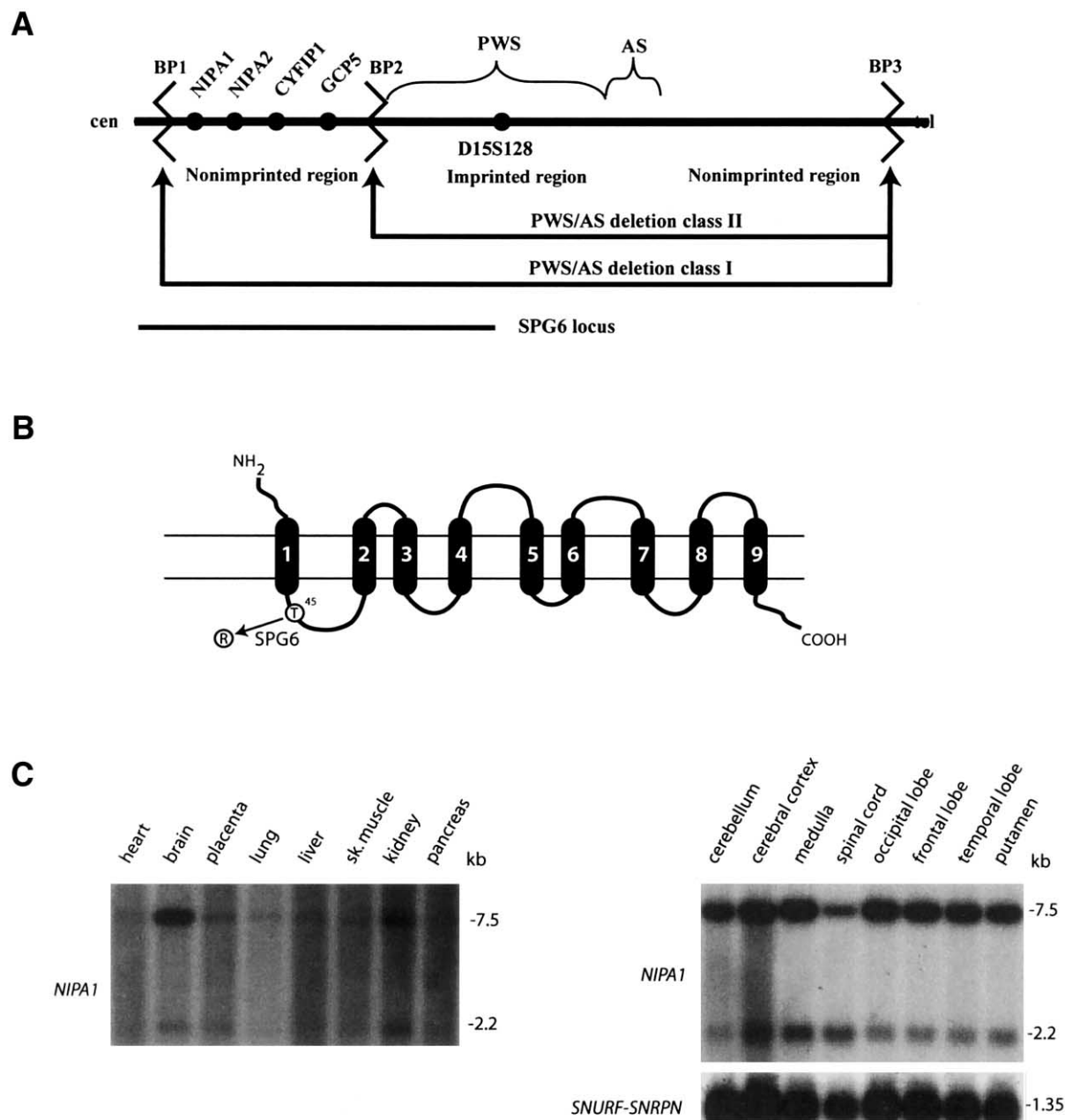


Figure 2 *a*, *SPG6* occurs in regions deleted in PWS and AS. *SPG6* is mapped to chromosome 15q11, proximal to the imprinted PWS and AS domains, within a region containing four nonimprinted genes. These genes, including *NIPA1*, map within class I deletions in patients with PWS and AS but are intact in class II deletions (Chai et al. 2003 [in this issue]). BP = breakpoint hotspot; cen = centromere; tel = telomere. *b*, *NIPA1* secondary structure analysis. Nine predicted transmembrane (TM) domains (Chai et al. 2003 [in this issue]) are shown. The *NIPA1* T45R mutation occurs at the interface of TM1 and the first putative outside loop. *c*, Expression of *NIPA1* by northern blot analysis. *NIPA1* transcripts of 2.2 kb and 7.5 kb are found in all tissues but are greatly enriched in neuronal tissues. Probes were prepared from TA-cloned RT-PCR products of human *NIPA1* (167 bp) and exons 2–3 of the control *SNURF-SNRPN* gene, were labeled with α - 32 P-dCTP, and were hybridized in ExpressHyb (Clontech) at 65°C to northern blots (Clontech), according to the manufacturer’s protocol.

of the *NIPA1* cDNA (data not shown). We analyzed the coding sequence of the other three nonimprinted genes (*GCP5*, *CYFIP1*, and *NIPA2*) in two affected members of ADHSP-ARK1 and identified no disease-specific mutations (data not shown). We then analyzed the *NIPA1* coding sequence in affected probands from 62 kindreds with ADHSP, 6 kindreds with probable autosomal recessive HSP, and 13 subjects with all the signs and symptoms of HSP but no family history ("apparently sporadic" spastic paraplegia). Affected subjects in one unrelated kindred (ADHSP-IRQ1) (fig. 1*b*) had precisely the same *NIPA1* mutation (159C→G) (fig. 1*b*) as affected subjects in the ADHSP-ARK1 kindred. Unaffected subjects from ADHSP-IRQ1 kindred showed only the normal nucleotide (159C). Whereas the ADHSP-ARK1 kindred was linked to the *SPG6* locus (Fink et al. 1995*b*), the ADHSP-IRQ1 kindred was too small for meaningful linkage analysis. Clinical features of the ADHSP-ARK1 affected individuals are typical of uncomplicated HSP of late-teenage to early-adult symptom onset (Fink et al. 1995*a*). Clinical features of ADHSP-IRQ1 were similar: onset of insidiously progressive spastic weakness in both legs that began in late teen-age years and was associated with urinary urgency and mild vibratory sensation impairment in the toes.

Kindreds ADHSP-ARK1 and ADHSP-IRQ1 are of Irish and Iraqi ancestry, respectively. Analysis of haplotypes for polymorphic markers linked to this locus (D15S541, D15S542, D15S646, D15S817, and D15S1021) showed no evidence of haplotype sharing between ADHSP-ARK1 and ADHSP-IRQ1 kindreds (data not shown). This indicates that these two kindreds with ADHSP are not closely related and suggests that the same *NIPA1* mutation arose independently in each of them.

Disease-specific *NIPA1* mutations in ADHSP-ARK1 and ADHSP-IRQ1 occur in *NIPA1* exon 1 and are predicted to change threonine to arginine at amino acid position 45 (T45R) (fig. 2*b*). This amino acid is conserved in mouse, chicken, and fish (zebrafish and *Fugu*) (Chai et al. 2003 [in this issue]; R.D.N., unpublished data) and is predicted to occur at the end of the first of nine transmembrane domains in the *NIPA1* polypeptide (fig. 2*b*). *NIPA1* does not contain an AAA domain (like that present in spastin [Hazan et al. 1999]) or a GTPase domain (like that present in atlastin [Zhao et al. 2001]) and does not bear other homology to genes that cause other forms of HSP. Although the *NIPA1* function is not known, the clear prediction based on hydrophobicity analysis of an integral membrane-associated protein (Chai et al. 2003 [in this issue]) suggests that *NIPA1* functions as a receptor or transporter. Many individuals with PWS or AS have chromosome 15q class I deletions that include *NIPA1* (fig. 2*a*) (Chai et al. 2003 [in this issue]). The fact that such individuals do not exhibit

progressive spastic paraplegia indicates that *NIPA1* haploinsufficiency does not cause progressive spastic paraplegia. Therefore, we suggest that the *NIPA1* T45R missense mutation identified in these kindreds with ADHSP is pathogenic through a dominant negative, gain-of-function mechanism.

NIPA1 mRNA is expressed constitutively at low levels with 2.2-kb and 7.5-kb transcripts in all human tissues but shows significant enrichment in the brain (fig. 2*c*). The latter expression pattern is found throughout the CNS, whereas spinal cord shows equal expression of the two *NIPA1* mRNAs (fig. 2*c*). The alternative mRNA isoforms arise from alternative polyadenylation within *NIPA1* exon 5, and equivalent expression patterns are found for mouse (Chai et al. 2003 [in this issue]).

Our observations of the same *NIPA1* gene mutation (159 C→G; T45R) in two unrelated kindreds with ADHSP that disrupts an interspecies conserved amino acid and was absent in control subjects ($n = 105$) strongly support the pathogenic significance of this mutation. Discovery of *NIPA1* mutations as the cause of SPG6-linked HSP improves the ability to diagnose HSP and to provide genetic counseling. The presence of a dominant negative *NIPA1* mutation in a putative membrane receptor or transporter suggests that SPG6 arises from either altered signal transduction or small-molecule transport through a membrane. We suggest that this property makes *NIPA1* and its unknown ligand an attractive target for therapeutic intervention in SPG6 and, perhaps, in other spastic paraplegias. Identification of the *NIPA1* cellular and subcellular localization, function, and ligand will therefore aid an understanding of axonal neurodegeneration in HSP and may have important therapeutic implications.

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Electronic-Database Information

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

National Center for Biotechnology Information (NCBI), <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/>

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