SEPT9 gene sequencing analysis reveals recurrent mutations in hereditary neuralgic amyotrophy

M.C. Hannibal, MD,
PhD
E.K. Ruzzo, BS
L.R. Miller, BS
B. Betz, BS
J.G. Buchan, BS
D.M. Knutzen, MS
K. Barnett, MS
M.L. Landsverk, PhD
A. Brice, MD
E. LeGuern, MD, PhD
H.M. Bedford, MD
B.B. Worrall, MD, MSc
S. Lovitt, MD
S.H. Appel, MD
E. Andermann, MD, PhD
T.D. Bird, MD
P.F. Chance, MD

Address correspondence and
reprint requests to Dr. Mark C.
Hannibal, Neurogenetics
Laboratory, 1959 NE Pacific St.,
Health Sciences RR236, Box
356320, Division of Genetics and
Developmental Medicine,
Department of Pediatrics,
University of Washington School
of Medicine, Seattle, WA 98195-
6320
mhanni@u.washington.edu

ABSTRACT

Background: Hereditary neuralgic amyotrophy (HNA) is an autosomal dominant disorder that manifests as recurrent, episodic, painful brachial neuropathies. A gene for HNA maps to chromosome 17q25.3 where mutations in *SEPT9*, encoding the septin-9 protein, have been identified.

Objective: To determine the frequency and type of mutations in the *SEPT9* gene in a new cohort of 42 unrelated HNA pedigrees.

Methods: DNA sequencing of all exons and intron-exon boundaries for *SEPT9* was carried out in an affected individual in each pedigree from our HNA cohort. Genotyping using microsatellite markers spanning the *SEPT9* gene was also used to identify pedigrees with a previously reported founder haplotype.

Results: Two missense mutations were found: c.262C>T (p.Arg88Trp) in seven HNA pedigrees and c.278C>T (p.Ser93Phe) in one HNA pedigree. Sequencing of other known exons in *SEPT9* detected no additional disease-associated mutations. A founder haplotype, without defined mutations in *SEPT9*, was present in seven pedigrees.

Conclusions: We provide further evidence that mutation of the *SEPT9* gene is the molecular basis of some cases of hereditary neuralgic amyotrophy (HNA). DNA sequencing of *SEPT9* demonstrates a restricted set of mutations in this cohort of HNA pedigrees. Nonetheless, sequence analysis will have an important role in mutation detection in HNA. Additional techniques will be required to find *SEPT9* mutations in an HNA founder haplotype and other pedigrees. *Neurology*® 2009;72:1755-1759

GLOSSARY

HNA = hereditary neuralgic amyotrophy; **SNP** = single nucleotide polymorphism; **STR** = short tandem repeat; **UTR** = untranslated region.

Hereditary neuralgic amyotrophy (HNA) is an autosomal dominant disorder characterized by painful, episodic, focal motor and sensory attacks, primarily affecting the nerves of the brachial plexus.¹⁻³ Associated findings in some individuals with HNA include relative hypotelorism, occasional cleft palate, and skin folds or creases on the neck or forearm.¹

A gene for HNA was localized to human chromosome 17q25.3 in several pedigrees.⁴⁻⁶ In probands of six unrelated HNA pedigrees linked to chromosome 17q25.3, we identified three different point mutations in the alternatively spliced *SEPT9* gene that encodes septin 9 proteins.⁷ One mutation was a c.-131G>C transversion in the 5' untranslated region (UTR) found in a Turkish family (nucleotide is based on *SEPT9* transcript variant 3, RefSeq NM_006640.4 in GenBank).⁸⁻¹⁰ Additionally, two mutations causing missense changes in septin-9 protein isoform c, c.262C>T (p.Arg88Trp) and c.278C>T (p.Ser93Phe), were detected in Europeans and North American descendants of Europeans (RefSeq NP_006631.2).^{8,9} Additional HNA pedigrees from Europe and North America have the c.262C>T mutation.^{11,12}

Supplemental data at
www.neurology.org

Authors' affiliations are listed at the end of the article.

Supported by funds from the NIH (National Institute of Neurological Disorders and Stroke), NS38181 (P.F.C. and M.C.H.); The Neuropathy Association, New York, NY (M.C.H. and P.F.C.); and the Allan and Phyllis Treuer Endowed Chair for Genetics and Development (P.F.C.).

Disclosure: Dr. Chance has received Speaker's Bureau honoraria from Athena Diagnostics, Inc. Dr. Bird has received licensing fees from Athena Diagnostics, Inc.

Medical Device: Gentra Puregene Blood Kit (Qiagen, Valencia, CA).

Table	Hereditary neuralgic amyotrophy <i>SEPT9</i> mutation analysis summary		
Pedigree	Cited in	Mutation position**	No. controls examined†
K4003	7		>100
K4009	This report		
K4020	This report		
K4024	11		
K4026	This report		
K4027	This report		
K4033	This report		
K4052	This report	Genomic: g.Chr17:72,909,975C>T cDNA: <i>SEPT9_v3</i> c.262C>T (NM_006640.4) Protein: SEPT9c p.Arg88Trp (NP_006631.2)	>100
K4059	This report		
K4018	7		
K4012	This report		

*Position of the *SEPT9* mutation on the genome assembly obtained from the UCSC Genome Browser, NCBI Build 36.1, March 2006 (hg18).^{35,36}

**Position of the mutation, numbered from the first nucleotide of the initiation codon of *SEPT9_v3* transcript variant and amino acid position in SEPT9c protein isoform.^{9,10}

†Additional sequence data of this region show these mutations are absent from a large number of controls.⁷

In this study, we undertook sequence analysis of the *SEPT9* gene in a large cohort of 42 pedigrees with HNA that were not included in our HNA gene identification report.⁷ This analysis was performed to confirm that *SEPT9* is the critical gene for HNA, to search for additional mutations, and to estimate the likelihood of detecting mutations in this gene given a clinical phenotype consistent with HNA.

METHODS Clinical evaluations. A diagnosis of HNA was established based on published criteria, including the presence of autosomal dominant inheritance.^{3,13} These clinical features typically included a history of one or more sudden onset, painful attacks in the neck, shoulder, or arm, followed by focal paresis, sensory disturbances, and muscle atrophy. Typically, the attacks involved the brachial plexus; however, other peripheral nerves (e.g., lumbosacral plexus, phrenic nerve) were affected in some cases. Proband and other affected and at risk subjects were evaluated by P.F.C., T.D.B., or referring physicians. Written consent was obtained from the subjects. The Human Subjects Division at the University of Washington approved this study. The clinical features for eight pedigrees included in this cohort of 42, K4001, K4004, K4006, K4012 (SAL819), K4026 (S Family), K4027 (G Family), K4028, and K4043 (classic type), have been described previously.^{1,14-17} We excluded pedigrees in our cohort that do not link to chromosome 17q25.3: K4008, K4016, and K4044 (chronic undulating type).^{17,18}

Molecular genetic analysis. Genomic DNA was isolated from peripheral blood or lymphoblastoid cell lines of subjects by standard methods using a Gentra Puregene Blood Kit (Qiagen, Valencia, CA). DNA from an affected individual from each pedigree was subjected to *SEPT9* mutational analysis by sequencing

known exons and adjacent intron-exon boundaries. Typically, the proband was chosen to be sequenced because we often had the best clinical evidence that they had features of HNA. For larger pedigrees, both clinical and genetic criteria were used to choose an affected individual who had an affected parent and, if possible, an affected offspring. The regions surrounding exons of the *SEPT9* gene were amplified by thermal cycling and subjected to bidirectional sequencing according to previously published methods.⁷ Thermal cycling primers are provided in table e-1 on the *Neurology*® Web site at www.neurology.org. In each proband where nucleotide changes that varied from the human genome reference sequence were found, the sequence was examined for known single nucleotide polymorphisms (SNPs) and segregation with disease in the pedigree. At least 100 individuals in a control population, unaffected by HNA, were also examined by direct sequencing or restriction fragment length polymorphism analysis to determine if the same *SEPT9* variant was present in a healthy, unrelated population (table).

Founder haplotype genotyping. Short tandem repeat (STR) markers, which were used previously to define the conserved shared block in the original five HNA founder pedigrees, were used to determine if pedigrees in the present cohort also possessed the founder haplotype.^{7,19} Following amplification with the STR primers, products were sized on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Segregation patterns of the alleles were determined, and haplotypes constructed in a manner to minimize crossovers.

RESULTS Identification of *SEPT9* mutations and mutation segregation. We screened a total of 42 pedigrees with HNA. *SEPT9* gene mutations are summarized in the table. The locations of the genomic changes are shown, along with the position in transcript variant *SEPT9_v3* and the corresponding protein isoform SEPT9c. Two of the previously reported *SEPT9* mutations were also identified among these 42 HNA pedigrees, the c.262C>T (p.Arg88Trp) and the c.278C>T (p.Ser93Phe) mutation.^{7,11,12} In seven pedigrees, K4009, K4020, K4026, K4027, K4033, K4052, and K4059, the c.262C>T mutation was found in affected individuals. In one pedigree, K4012, from France, the c.278C>T mutation was identified. Segregation of these mutations within HNA pedigrees is shown in the figure.

No other causal mutations were found in the other 34 pedigrees, although one coding, nonsynonymous single nucleotide polymorphism (SNP rs34587622 in dbSNP) was found to segregate with HNA in a subset of seven.²⁰ The rs34587622 SNP, c.380C>T, changes the proline at amino acid position 128 to leucine in SEPT9 isoform c (p.Pro127Leu in NP_006631.2). This SNP was found initially in a single proband, who also possessed the c.262C>T (p.Arg88Trp) mutation, and in controls during candidate gene sequencing in HNA pedigrees.²¹ The c.380T allele is present within five founder haplotype HNA pedigrees previously identified.^{7,19} This allele also segregates with HNA in 7 of 42 pedigrees in this cohort that all bear the com-

Figure Segregation of *SEPT9* gene mutations in HNA pedigrees

Females are shown as circles and males as squares. Affected persons are represented by darkened figures, presumed affected individuals have diagonal black lines, and cross-slatted figures are deceased. Question marks within figures indicate inadequate clinical data and may indicate some individuals who were obligate carriers without pain attacks. If DNA was available from an individual, the sequencing result is indicated below the identifier. (A) Pedigrees with the g.Chr17:72,909,975C>T, c.262C>T, p.Arg88Trp mutation. (B) Pedigree with g.Chr17:72,909,993C>T, c.278C>T, p.Ser93Phe mutation.

mon North American founder haplotype (data not shown). However, because the c.380C>T allele is present in 28% (29/104) of control individuals of Caucasian descent, and the dbSNP database finds it in 15.4% (4/26) of North Americans and 5% (6/120) of Europeans, we do not believe it is a causal mutation.²⁰

DISCUSSION To date, three point mutations (c.-131G>C, c.262C>T, and c.278C>T) and a ge-

netic founder haplotype have been identified in HNA pedigrees supporting a critical role for the *SEPT9* gene.^{7,11,12} Combining the results from the 42 pedigrees in this report with the 7 pedigrees previously reported from our laboratory, we detected *SEPT9* mutations in 22.4% (11/49) of our total cohort.^{7,11} A further 24.5% of these HNA pedigrees possess the founder haplotype defined by the markers previously reported.^{7,19} The moderate mutation detection rate may be due to several factors. First, our present methods may be unable to detect small *SEPT9* insertions, deletions, inversions, or other gene rearrangements. For example, it will likely be necessary to undertake complete genomic sequencing of *SEPT9* in a subject having the founder haplotype in order to detect a causal mutation in this subset of HNA pedigrees. Second, more subtle regulatory mutations may occur outside of the region of exons and intron-exon boundaries that could, in turn, influence the relative abundance of specific *SEPT9* alternative 5' exon isoforms. Third, genetic heterogeneity does exist for HNA. To date, the gene for HNA in five pedigrees has been shown to be unlinked to markers in the 17q25.3 region.^{17,18,22} In some cases, HNA pedigrees are not large enough to confirm or exclude chromosome 17q25.3 linkage. Fourth, the potential for phenocopies may exist, with familial clustering of similar, but distinct multifocal, painful neuropathies that may be caused by peripheral nerve vasculitis, an example of which can be seen in proximal diabetic neuropathy or diabetic amyotrophy.^{23,24}

HNA is the first Mendelian disorder shown to be due to mutations in a member of the septin gene family of related proteins. The septin gene family includes at least 14 members in humans.^{25,26} Septins participate in cytokinesis and cellular trafficking and have been studied for their relationship to neoplasia.²⁷ Transcriptional and potentially translational regulation of the *SEPT9* gene is complex.²⁸ *SEPT9* appears to be expressed ubiquitously, but information regarding the distribution and abundance of the various septin-9 protein isoforms in normal tissues is limited.²⁹ The longer isoforms of septin-9 have unique short N-terminal polypeptides and share a proline-rich domain that is found only in the septin-4 and septin-8 proteins.²⁵ Septin-9 has been shown to colocalize with other septins and with septin intermediate filaments that associate with actin microfilaments and microtubules.^{30,31}

At the transcriptional level, the *SEPT9* gene shows a remarkable number of alternative first exons. These multiple 5' alternative transcription start sites, as well as possible alternative splicing within the final exon, potentially create dozens of protein isoforms.²⁸ The two mutations reported here, c.262C>T

at g.Chr17:72,909,975C>T and c.278C>T at g.Chr17:72,909,993C>T, can lead to missense mutations in the translated product of the *SEPT9_v1*, *SEPT9_v2*, and *SEPT9_v3* transcripts, but are in the 5' untranslated region of *SEPT9_v4* and *SEPT9_v4** transcripts.^{28,32} This diversity of transcripts has made analysis of the functional consequences of *SEPT9* gene mutations difficult.³² Under hypoxic conditions, the c.262C>T mutation reportedly affects the level of translation of the *SEPT9_v4* transcript, encoding *SEPT9* protein isoform e (GenBank NM_001113494.1 and NP_001106966.1).³²

The HNA-associated mutations in *SEPT9_v3*, p.Arg88Trp and p.Ser93Phe, altered the colocalization of mutant septin-9 protein isoform c with septin-4 and septin-11 in mesenchymal and epithelial murine mammary gland NMuMG cells.³³ These mutations also perturb the regulation of septin-9-containing filaments by Rho/Rhotekin signaling.³³ Thus, this N-terminal proline-rich domain, in which these mutations reside, may regulate specific interactions of septin-9 with other septins or other cellular proteins as proposed by Nagata et al.^{31,34} These findings are only the first steps in determining how mutations in *SEPT9* lead to the distinctive phenotype seen in HNA. The mechanism of how *SEPT9* mutations lead to a brachial neuropathy and other features of HNA remains unknown.

In this study, we provided further evidence that mutation of the *SEPT9* gene is the molecular basis of some cases of HNA. We also found that the detection rate for *SEPT9* mutations in pedigrees consistent with this disorder is low. This is due to few identifiable mutations found by our present mutation analysis strategy of sequencing *SEPT9* exons. This strategy has not found a mutation in the significant fraction of HNA pedigrees that harbor a genetic founder chromosome. Clearly, identification of the specific *SEPT9* mutation associated with the genetic founder will greatly improve the clinical usefulness of *SEPT9* analysis as a diagnostic and predictive tool for HNA.

AUTHORS' AFFILIATIONS

From the Departments of Pediatrics (M.C.H., E.K.R., L.R.M., B.B., J.G.B., D.M.K., K.B., M.L.L., P.F.C.), Neurology (T.D.B., P.F.C.), and Medicine (T.D.B.), University of Washington School of Medicine, Seattle; Seattle Children's Hospital (M.C.H., P.F.C.); Geriatric Research Education and Clinical Center (T.D.B.), VA Puget Sound Health Care System, Seattle; Department of Genetics and Cytogenetics (A.B., E.L.), INSERM, UMR S679, Pierre and Marie Curie Paris VI University, Pitié-Salpêtrière Medical School, AP-HP, Pitié-Salpêtrière Hospital, Paris, France; Genetics Programme (H.M.B.), North York General Hospital, Toronto, Ontario, Canada; Departments of Neurology and Public Health Sciences (B.B.W.), University of Virginia Health System, Charlottesville; Department of Neurology (S.H.A.), Methodist Neurological Institute (S.L.), Houston, TX; and Departments of Neurology & Neurosurgery

and Human Genetics (E.A.), Neurogenetics Unit, Montreal Neurological Hospital and Institute, McGill University, Montreal, Quebec, Canada.

ACKNOWLEDGMENT

The authors thank Jonathan Adkins and Melissa Eckert for DNA isolation and lymphoblastoid cell line establishment and all of the clinical contributors, patients, and families for facilitating pedigree identification and obtaining research materials.

Received August 11, 2008. Accepted in final form February 13, 2009.

REFERENCES

1. Jeannet PY, Watts GD, Bird TD, Chance PF. Craniofacial and cutaneous findings expand the phenotype of hereditary neuralgic amyotrophy. *Neurology* 2001;57:1963–1968.
2. van Alfen N, van Engelen BG. The clinical spectrum of neuralgic amyotrophy in 246 cases. *Brain* 2006;129:438–450.
3. Hannibal MC, van Alfen N, Chance PF, van Engelen BGM. Hereditary neuralgic amyotrophy. Available at: <http://www.genetests.org>. Accessed August 10, 2008.
4. Pellegrino JE, Rebbeck TR, Brown MJ, Bird TD, Chance PF. Mapping of hereditary neuralgic amyotrophy (familial brachial plexus neuropathy) to distal chromosome 17q. *Neurology* 1996;46:1128–1132.
5. Wehnert M, Timmerman V, Spoelders P, Meuleman J, Nelis E, Van Broeckhoven C. Further evidence supporting linkage of hereditary neuralgic amyotrophy to chromosome 17q. *Neurology* 1997;48:1719–1721.
6. Stögbauer F, Young P, Timmerman V, et al. Refinement of the hereditary neuralgic amyotrophy (HNA) locus to chromosome 17q24-q25. *Hum Genet* 1997;99:685–687.
7. Kühlenbäumer G, Hannibal MC, Nelis E, et al. Mutations in *SEPT9* cause hereditary neuralgic amyotrophy. *Nat Genet* 2005;37:1044–1046.
8. den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum Mutat* 2000;15:7–12.
9. Pruitt KD, Tatusova T, Maglott DR. NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res* 2007;35:D61–D65.
10. Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. GenBank. *Nucleic Acids Res* 2008;36:D25–D30.
11. Laccone F, Hannibal MC, Neesen J, Grisold W, Chance PF, Rehder H. Dysmorphic syndrome of hereditary neuralgic amyotrophy associated with a *SEPT9* gene mutation: a family study. *Clin Genet* 2008;74:279–283.
12. Hoque R, Schwendemann RN, Kelley RE, Bien-Willner R, Sivakumar K. Painful brachial plexopathies in *SEPT9* mutations: adverse outcome related to comorbid states. *J Clin Neuromuscul Dis* 2008;9:379–384.
13. Kühlenbäumer G, Stögbauer F, Timmerman V, DeJonghe P. Diagnostic guidelines for hereditary neuralgic amyotrophy or hereditary familial neuritis with brachial plexus predilection. on behalf of the European CMT consortium. *Neuromuscul Disord* 2000;10:515–517.
14. Taylor RA. Hereditary familial mononeuritis multiplex with brachial predilection. *Brain* 1960;83:113–137.
15. Jacob JC, Andermann F, Robb JP. Hereditary familial neuritis with brachial predilection. *Neurology* 1961;11:1025–1033.

16. Gouider R, LeGuern E, Emile J, et al. Hereditary neuralgic amyotrophy and hereditary neuropathy with liability to pressure palsies: two distinct clinical, electrophysiologic, and genetic entities. *Neurology* 1994;44:2250–2252.
17. van Alfen N, van Engelen BG, Reinders JW, Kremer H, Gabreels FJ. The natural history of hereditary neuralgic amyotrophy in the Dutch population: two distinct types? *Brain* 2000;123:718–723.
18. Watts GD, O'Briant KC, Borreson TE, Windebank AJ, Chance PF. Evidence for genetic heterogeneity in hereditary neuralgic amyotrophy. *Neurology* 2001;56:675–678.
19. Watts GD, O'Briant KC, Chance PF. Evidence of a founder effect and refinement of the hereditary neuralgic amyotrophy (HNA) locus on 17q25 in American families. *Hum Genet* 2002;110:166–172.
20. Sherry ST, Ward MH, Kholodov M, et al. dbSNP: The NCBI database of genetic variation. *Nucleic Acids Res* 2001;29:308–311.
21. Meuleman J, Kühlenbäumer G, Audenaert D, et al. Mutation analysis of 4 candidate genes for hereditary neuralgic amyotrophy (HNA). *Hum Genet* 2001;108:390–393.
22. Kühlenbäumer G, Meuleman J, DeJonghe P, et al. Hereditary neuralgic amyotrophy (HNA) is genetically heterogeneous. *J Neurol* 2001;248:861–865.
23. Vinik A, Ullal J, Parson HK, Casellini CM. Diabetic neuropathies: clinical manifestations and current treatment options. *Nat Clin Pract Endocrinol Metab* 2006;2:269–281.
24. Said G. Diabetic neuropathy: a review. *Nat Clin Pract Neurol* 2007;3:331–340.
25. Russell SE, Hall PA. Do septins have a role in cancer? *Br J Cancer* 2005;93:499–503.
26. Peterson EA, Kalikin LM, Steels JD, Estey MP, Trimble WS, Petty EM. Characterization of a SEPT9 interacting protein, SEPT14, a novel testis-specific septin. *Mamm Genome* 2007;18:796–807.
27. Hall PA, Russell SE. The pathobiology of the septin gene family. *J Pathol* 2004;204:489–505.
28. McIlhatton MA, Burrows JF, Donaghy PG, Chanduloy S, Johnston PG, Russell SE. Genomic organization, complex splicing pattern and expression of a human septin gene on chromosome 17q25.3. *Oncogene* 2001;20:5930–5939.
29. Scott M, Hyland PL, McGregor G, Hillan KJ, Russell SE, Hall PA. Multimodality expression profiling shows *SEPT9* to be overexpressed in a wide range of human tumours. *Oncogene* 2005;24:4688–4700.
30. Surka MC, Tsang CW, Trimble WS. The mammalian septin MSF localizes with microtubules and is required for completion of cytokinesis. *Mol Biol Cell* 2002;13:3532–3545.
31. Nagata K, Asano T, Nozawa Y, Inagaki M. Biochemical and cell biological analyses of a mammalian septin complex, Sept7/9b/11. *J Biol Chem* 2004;279:55895–55904.
32. McDade SS, Hall PA, Russell SE. Translational control of SEPT9 isoforms is perturbed in disease. *Hum Mol Genet* 2007;16:742–752.
33. Sudo K, Ito H, Iwamoto I, Morishita R, Asano T, Nagata K. SEPT9 sequence alternations causing hereditary neuralgic amyotrophy are associated with altered interactions with SEPT4/SEPT11 and resistance to Rho/Rhotekin-signaling. *Hum Mutat* 2007;28:1005–1013.
34. Nagata K, Inagaki M. Cytoskeletal modification of rho guanine nucleotide exchange factor activity: identification of a rho guanine nucleotide exchange factor as a binding partner for Sept9b, a mammalian septin. *Oncogene* 2005;24:65–76.
35. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. *Nature* 2001;409:860–921.
36. Karolchik D, Baertsch R, Diekhans M, et al. The UCSC genome browser database. *Nucleic Acids Res* 2003;31:51–54.

New Guidelines Examine Safety of Women with Epilepsy and Pregnancy

New evidence-based practice guidelines developed by the American Academy of Neurology in full collaboration with the American Epilepsy Society show the relative safety for women with epilepsy to become pregnant, but caution against taking one particular epilepsy drug, which can cause birth defects. The guidelines were published in the April 27, 2009, online issue of *Neurology*® and were presented at the AAN's Annual Meeting in Seattle. The guidelines were also published electronically in *Epilepsia*, the journal of the International League Against Epilepsy. They represent an update of the 1998 guideline, "Management Issues for Women with Epilepsy."