SEPT9 Mutations and a Conserved 17q25 Sequence in Sporadic and Hereditary Brachial Plexus Neuropathy

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Background: The clinical characteristics of sporadic brachial plexus neuropathy (S-BPN) and hereditary brachial plexus neuropathy (H-BPN) are similar. During attacks, inflammation of the brachial plexus nerves has been identified in both conditions. SEPT9 mutations (Arg88Trp, Ser93Phe, 5'UTR c.-131G>C) occur in some families with H-BPN. These mutations were not found in North American kindreds with H-BPN with a conserved 500-kilobase sequence of DNA at the 17q25 chromosomal region (where SEPT9 localizes) where a founder mutation has been suggested.

Objective: To study the 17q25 sequence and SEPT9 in S-BPN (56 individuals) and H-BPN (13 kindreds).

Methods: Allele analysis at 17q25, SEPT9 DNA sequencing, and messenger RNA analysis from lymphoblast cultures were performed.

Results: A conserved 17q25 sequence was found in 5 of 13 kindreds with H-BPN and 1 individual with S-BPN. This conserved sequence was not found in the family with a SEPT9 mutation (Arg88Trp) or in 182 control subjects. SEPT9 messenger RNA expression levels did not differ between forms of H-BPN and control subjects. No known mutations of SEPT9 were found in S-BPN.

Conclusions: Rarely, individuals with S-BPN may have the same conserved 17q25 sequence found in many North American kindreds with H-BPN. Individuals with BPN with this conserved sequence do not seem to have SEPT9 mutations or alterations of messenger RNA expression levels in lymphoblast cultures and are predicted to have the most common genetic cause in North America by a founder-effect mutation.


Hereditary Brachial Plexus Neuropathy (H-BPN), also known as hereditary neuralgic amyotrophy (OMIM 162100), is an autosomal dominant, variably penetrant disease in which affected individuals experience periodic attacks of unilateral or asymmetric pain, weakness, atrophy, and sensory alterations of the shoulder girdle and upper limb. It is readily differentiated from hereditary neuropathy with pressure palsy, which tends to be painless, affects nerves at usual sites of compression, and is associated with mild generalized neuropathy. In H-BPN, the symptoms, distribution of neurological findings, and course of the attacks are difficult to differentiate from those of sporadic brachial plexus neuropathy (S-BPN), also known as idiopathic neuralgic amyotrophy or Parsonage-Turner syndrome. Both attacks of H-BPN and S-BPN may be associated with antecedent immunization or infection. Combined with descriptions of brachial plexus nerve inflammation during attacks, altered immunity has been suggested as a potential causative mechanism in BPN attacks.

Recently, mutations of the septinlike molecule SEPT9 (Arg88Trp, Ser93Phe, 5'UTR c.-131G>C) have been reported as molecular genetic abnormalities associated with H-BPN but have been found in only some kindreds. The septin protein belongs to a class of guanosine triphosphate–binding scaffold proteins thought to be important in membrane dynamics, vesicle trafficking, apoptosis, and cytoskeletal remodeling. The protein is ubiquitously expressed with greater levels of expression in lymphocytes than in neural tissues. Cell culture transfection experiments using Arg88Trp and Ser93Phe constructs in mouse mammary gland cells have suggested possible disruption of septin interactions or the Rho family of guanosine triphosphatases. Why these mu...
tations lead to intermittent attacks and a possible inflammatory immune mechanism is not understood, and a clear model for BPN has not been established.

Mutations of SEPT9 have not been identified in North American kindreds who share a conserved 500-kilobase (kb) region of DNA at 17q25 wherein SEPT9 lies. Watts et al predicted that, among North American families with H-BPN with a conserved DNA region at 17q25, a common mutation would be found originating from a shared ancestor (that is, a founder mutation). Because no mutations in SEPT9 have been identified in the expressed protein in these families, a mutation that alters expression regulation of SEPT9 could still be causative and was not studied in earlier investigations.

We tested (1) whether a conserved DNA region at 17q25 occurs in S-BPN attacks, (2) whether known mutations of SEPT9 are present in S-BPN, and (3) whether SEPT9 messenger RNA (mRNA) from lymphocyte cultures differs between North American kindreds with and those without a conserved region at the 17q25 chromosomal region.

METHODS

AFFECTED INDIVIDUALS AND CONTROL SUBJECTS

With institutional review board approval, we studied 13 individuals with H-BPN, 56 with S-BPN, and 182 healthy control subjects. Previously obtained transformed lymphoblast cell lines were available for all of these individuals for our studies. All control subjects had undergone clinical examination and nerve conduction studies in epidemiologic studies of healthy subjects in Olmsted County, Minnesota. We reported the disease features in 4 of these kindreds with H-BPN who previously had undergone nerve or brachial plexus biopsy during attacks. Probands of each family and sporadic cases had all been examined by us during attacks and had been studied with nerve conduction tests, needle electromyography, brachial plexus or cervical spine imaging, and other laboratory studies from which we judged this disorder to be H-BPN. Confirmation that the disorder was inherited was based on either information from the probands or their families who had undergone kindred evaluation.

STUDIES IN THE INDIVIDUAL WITH S-BPN AND CONSERVED ALLELES

Additional clinical attention was paid to the individual with S-BPN with the identified conserved alleles at 17q25 chromosomal region (Figure 1). That patient had experienced attacks of BPN at ages 6 and 24 years. He was from a large family with no other members who had known attacks of upper extremity pain or weakness. He was not clear dysomorphic features (eg, hypotelorism or short stature). His parents were examined and underwent DNA sampling. Multiple other family members underwent questioning and medical history review and were without known attacks of BPN. We inquired about mimickers of neuritic attacks such as rotator cuff disease (shoulder pain), anterior interosseous syndrome (index finger and thumb weakness), radial neuropathy (wristdrop), and recurrent pneumonia (diaphragm paralysis), but none were reported by family members.

SEPT9 MUTATION ANALYSIS

The 13 probands of kindreds with H-BPN, 56 individuals with S-BPN, and 182 control subjects all underwent DNA sequencing analysis (pyrosequencing) of the codons previously identified with alterations in Arg88Trp, Ser93Phe, and 5’UTR c.-131G>C [Arg indicates arginine; Trp, tryptophan; and Ser, serine]. For all probands with H-BPN, we sequenced the entire open-reading frame of SEPT9 using the polymerase chain reaction (PCR) primers previously published.

ALLELE STUDIES AT THE 17q25 CHROMOSOMAL REGION

On the basis of previously published data,9 fluorescent-labeled oligonucleotide primers 72GT1, 72GT2, D17S937, D17S939, and GT1 were created within a 500-kb region at 17q25. Using these allele markers, linkage and haplotype analysis was performed using conventional fragment analysis methods using a 3730XL DNA Analyzer and Genotyper software (Applied Biosystems, Forester City, California). Two-point LOD (logarithm of the odds [to the base 10]) scores were calculated in 6 of 13 kindreds of sufficient size to enable such analysis. Autosomal dominant inheritance was assumed, and penetrance was set at 99%. High penetrance was set to enable discovery of those not localizing to 17q25 (that is, exclusionary linkage). The frequency of mutant alleles was designated at 0.0001. Haplotypes were generated assuming least number of crossovers and previous knowledge of a disease-associated set of alleles between affected families. One hundred eighty-two healthy control subjects also underwent allele analysis of the polymorphic markers.

REAL-TIME PCR ANALYSIS OF SEPT9

Using an RNeay Mini Kit (Qiagen Inc, Valencia, California), total RNA was extracted from transformed lymphoblasts from the cell lines of 13 patients with H-BPN, 1 individual with S-BPN with conserved haplotype at 17q25, and 14 healthy controls. Their total RNA was subject to reverse transcription per instruction of Taqman Reverse Transcription Reagents.
The amplification cycle is as follows: 25°C for 10 minutes, 48°C for 30 minutes, 95°C for 5 minutes, and maintenance at 4°C. Real-time PCR was performed using TaqMan Gene Expression Assay mixes (part No. 4331182; Applied Biosystems) labeled with fluorescent color dye 5-carboxyfluorescein for targeting endogenous control glycer-aldehyde-3-phosphate dehydrogenase (assay identification: Hs00266705_g1) and target gene SEPT9 (Hs00246396_m1). Reverse transcription product (5 µL) was used for real-time PCR setup per instruction of Taqman Universal PCR Mastermix, 2X (part No. 4324018; Applied Biosystems). The amplification cycle was 50°C for 2 minutes, 95°C for 10 minutes, followed by 95°C for 15 seconds and 60°C for 1 minute, for 40 cycles. All real-time PCR data were analyzed using standard comparative ΔΔCt (cycle threshold) and compared with normal control samples. The Fisher exact test was used to compare the expression levels between individuals with and without the conserved 17q25 haplotype and between those with H-BPN and healthy controls.

**RESULTS**

Of the 13 probands of our North American kindreds with H-BPN, 1 had a SEPT9 mutation (Arg88Trp[262C→T]). The family was of German-Austrian, Pennsylvania Dutch, Irish, and Native American descent (**Figure 2**). Redundant skin folds and short stature were not identified. However, interpupillary distance was short at the second percentile in both affected and unaffected individuals. Haplotype analysis identified a conserved set of alleles (72GT1-318, 72GT2-341, 17S937-120, 17S939-210, and GT1-296) in 5 of the 13 kindreds (**Figure 3**), although

**Figure 2.** One of 13 kindreds with hereditary brachial plexus neuropathy with the SEPT9 mutation. A, Five-generation kindred affected by recurrent attacks of brachial plexus neuropathy with apparent dominant inheritance. B, Sequencing analysis identifying 262C→T transition leading to Arg88Trp in the proband. C, Complementary strand confirmatory pyrosequencing assay shows A and G heterozygous occurrence at base position 262, with kindred evaluation on top and possible combination below. Arg indicates arginine; E, enzyme; S, substrate; Ser, serine; and Trp, tryptophan.
Figure 3. Five kindreds of 13 with hereditary brachial plexus neuropathy (H-BPN) and a conserved set of alleles (vertical black bars) at the 17q25 chromosomal region analyzed using polymorphic markers 72G71, 72G72, 17S937, 17S939, and GT1. Haplotypes were generated assuming least numbers of crossovers, and previous knowledge of a disease-associated set of alleles between affected families with H-BPN. Carrier status is indicated by a dot within an open circle or square.
not in this family identified with a SEPT9 mutation (Arg88Trp) or the 182 healthy controls. In addition, these same alleles were found in 1 individual with S-BPN and his unaffected father (see “Studies in the Individual With S-BPN and Conserved Alleles” section and Figure 1). The maximal 2-point LOD scores in 5 of 6 kindreds favored linkage to the 17q25 chromosomal region, with values ranging from 0.49 to 3.7 LOD with juxtaposed markers consistent with linkage to this region. Five of these kindreds had the conserved haplotype (Figure 3). None of the 56 individuals with S-BPN or the 182 controls had known mutations of SEPT9 on the basis of the results of complete lymphoblast open-reading frame DNA sequencing analysis of all probands. Additionally, no mutations were identified in the individual with S-BPN and the conserved haplotype. The SEPT9 RNA levels by reverse transcriptase–PCR quantification were not statistically different between H-BPN kindreds. In addition, no significant difference was found between normal control SEPT9 mRNA levels and H-BPN.

The clinical and histopathologic similarities between S-BPN and H-BPN1,2,3 suggest the possibility that a shared underlying mechanism is important in both disorders. Several different genetic or epigenetic mechanisms are likely operational.10 Previously, intensive questioning and examination of family members for a history of symptoms and signs of brachial plexopathy was the mainstay of identifying hereditary predisposition to BPN. Now genetic discoveries in H-BPN5,10 permit addressing whether similar alterations at SEPT9 and the 17q25 chromosomal region might explain the similarities between individuals with S-BPN and those with H-BPN. The current molecular information suggests at least 5 genetic classifications for BPN attacks: (1) H-BPN with SEPT9 mutation, (2) H-BPN with conserved 17q25 haplotype, (3) H-BPN not localizing to the 17q25 haplotype, (4) S-BPN with conserved 17q25 haplotype, and (5) S-BPN with a known genetic abnormality.

The evidence for genetic predisposition in some individuals with S-BPN in our study is not definitive but is suggestive. Specifically, only 1 of 56 individuals with S-BPN was found to have the same conserved sequence seen at the 17q25 chromosomal region in North American kindreds with H-BPN. No known mutations of SEPT9 were identified in that individual or others with S-BPN. Genetically, it seems that the family of this individual with presumed S-BPN is at risk for attacks of brachial plexopathy. That this individual had a first episode at age 6 years and experienced recurrent attacks at older ages supports this possibility.2 The absence of attacks in other family members, including his father, who carries the same conserved chromosome region, might suggest low penetrance in this family for unclear reasons. Alternatively, we considered whether this conserved set of alleles could be found in healthy individuals not at risk but did not find a similar allele combination in 182 examined healthy controls or previous reports of such phenomenon in 50 additional controls.9

Our data support the earlier work by Watts et al,8 who reported that a shared mutation that localizes to the 17q25 chromosomal region has yet to be identified in many families with H-BPN. Specifically, in our kindreds, 5 of 13 had a conserved region of DNA at 17q25, which suggests a common mutation inherited from a shared ancestor in each of these families (ie, a founder mutation). In those 5 families, 88% of affected individuals had the conserved region of DNA, which suggests high penetrance in most families studied. The percentage of kindreds with H-BPN with the conserved 17q25 region is less than previously reported by Watts et al,9 who found the shared conserved region in 6 of 7 kindreds. Their study, however, differed from ours in that all families were preselected by linkage to have causative genetic localization at the 17q25 chromosomal region. In our study, only 6 of 13 kindreds were of sufficient size to enable linkage analysis. Of these, 3 had LOD scores suggestive of chromosomal localization to 17q25, and all but 1 shared the conserved haplotype at 17q25; that family had the SEPT9 Arg88Trp mutation. Earlier findings in the other 4 families with Arg88Trp suggest a mutation hot spot and not an additional founder effect. Overall, our observations are consistent with genetic diversity as a cause of H-BPN by finding kindreds without a SEPT9 gene abnormality or conserved 17q25 sequence.10

Whether some of the genetic diversity identified in H-BPN relates to occult SEPT9 distant promoter or alternative gene mutation(s) resulting in variable SEPT9 mRNA expression levels was also addressed. The level of mRNA expression between our kindreds with and without the conserved 17q25 haplotype and Arg88Trp mutation was not significantly different at reverse transcriptase–PCR analysis from lymphoblast cell lines. There was also no significant difference between expression levels in affected individuals compared with healthy controls. This makes SEPT9 duplication, deletion, and promoter mutations less likely. However, this does not exclude alteration of the ratio between SEPT9 protein isoforms at times of cellular stress, as suggested by McDade et al.11 The relevance of these data also depends on whether SEPT9 is still responsible in individuals with the conserved 17q25 sequence vs alternative gene cause in the identified 17q25 chromosomal region. In addition, the relevance of these mRNA studies depends on whether the primary pathogenesis of H-BPN is humoral based (that is, an inflammatory mechanism). Our data suggest no obvious expression level difference in lymphoblasts; however, SEPT9 mRNA evaluation of transcript variance in neural tissues may also be helpful in evaluation of the unknown founder mutation in North American kindreds with H-BPN.

Molecular abnormalities other than mutations of SEPT9 exons or the exon-intron boundaries sequenced herein are likely responsible for many cases of H-BPN. A single genetic mutation of H-BPN in North American kindreds with the conserved 17q25 sequence is supported by this work (ie, founder mutation) and seems to be the most common identified genetic cause in North America. Regardless of the identity of additional molecular markers in H-BPN, interrogation of individuals with S-BPN with identified abnormalities will be important in consideration of additional genetic influence in presumed S-BPN.
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Author Contributions: Dr Klein had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: C. J. Klein, Windebank, and P. J. B. Dyck. Acquisition of data: C. J. Klein, Wu, Cunningham, Friedenberg, and D. M. Klein. Analysis and interpretation of data: C. J. Klein, Windebank, P. J. B. Dyck, and P. J. Dyck. Drafting of the manuscript: C. J. Klein, Wu, and D. M. Klein. Critical revision of the manuscript for important intellectual content: C. J. Klein, Cunningham, Windebank, P. J. B. Dyck, Friedenberg, and P. J. Dyck. Obtained funding: C. J. Klein and P. J. Dyck. Administrative, technical, and material support: Windebank, P. J. B. Dyck, and D. M. Klein. Study supervision: C. J. Klein, Cunningham, and Windebank.

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