Novel Mutation in the SPG3A Gene in an African American Family With an Early Onset of Hereditary Spastic Paraplegia

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Background: Mutations in a novel GTPase gene SPG3A cause an autosomal dominant hereditary spastic paraplegia linked to chromosome 14q (SPG3), which accounts for approximately 10% to 15% of all autosomal dominant hereditary spastic paraplegia cases. The mutational spectrum of the SPG3A gene and the phenotype/genotype correlations have not yet been established.

Objective: To describe a kindred with an infantile onset of hereditary spastic paraplegia caused by a novel mutation in the SPG3A gene.

Patients: Complete neurological examination and genetic analysis were performed on 6 affected members of a small African American kindred. Linkage analysis to genetic markers near autosomal dominant hereditary spastic paraplegia loci on chromosomes 2p and 14q was performed. The coding sequence of the SPG3A gene was analyzed, and the identified change in the sequence was tested for being a benign polymorphism by sequencing 200 chromosomes from normal controls.

Results: Every affected individual had signs of uncomplicated spastic paraparesis without additional neurological abnormalities. None of the affected family members had ever walked normally. The history was consistent with an infantile onset, despite the normal acquisition of motor milestones. Genetic analysis suggested linkage to the SPG3A locus on chromosome 14q. Analysis of the SPG3A gene revealed a missense mutation C635T, predicted to result in a threonine to isoleucine substitution at codon 156. Analysis of 200 normal chromosomes did not identify the same change in healthy subjects.

Conclusion: We report a novel mutation in the SPG3A gene in an African American family with an infantile onset of autosomal dominant hereditary spastic paraplegia.

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METHODS

CLINICAL AND LABORATORY STUDIES

We identified a small African American kindred through a proband (subject IV-2) who was initially examined at age 5 years for spasticity of unknown cause. Five additional affected relatives were identified and included in this study, which was approved by the institutional review board at Vanderbilt University. A comprehensive medical history was...
obtained from every participating subject, and information about the psychomotor development, age at symptom onset, and progression of disability was collected; information about minors was obtained from their parents. Severity of the disease was categorized into grades 1 through 5 according to previously published criteria: grade 1 corresponds to a spastic gait without functional limitation; grade 2 represents an abnormal gait with functional limitations but not requiring a consistent use of an assistive device; grade 3 corresponds to a gait abnormality requiring a consistent use of a cane, crutches, or a walker or an occasional use of a wheelchair only for long distances; grade 4 corresponds to a gait abnormality requiring frequent use of a wheelchair (up to 50% of the time) but still with an ability to walk short distances using other assistive devices; and grade 5 represents a marked functional impairment with an inability to walk with crutches, requiring a wheelchair more than 50% of the time.\(^8\)

**GENETIC LINKAGE ANALYSIS**

The phenotype of each individual was determined as definitely affected or unaffected before genetic analysis. DNA was available from all affected subjects, 1 unaffected subject (IV-1), and 3 individuals who married into the family (subjects II-6, III-5, and III-7). We tested linkage to the locus on chromosome 2p using microsatellite markers D2S352 and D2S367 and to chromosome 14q using markers D14S269, D14S989, and D14S976. Genetic linkage analysis was performed as previously described, and alleles were visualized by silver staining.\(^9\) Two-point linkage analysis was performed with the FASTLINK program,\(^10\) using an AD mode of disease inheritance and disease allele frequency of 0.001. We assigned penetrance 0.90 for logarithm of odds score calculations. Marker allele frequencies were determined by genotyping 90 healthy, unrelated individuals (180 chromosomes).

**ANALYSIS OF THE SPG3A GENE**

All 14 exons were amplified using the polymerase chain reaction with published or custom-designed (exons 1, 2, and 4) primers and conditions.\(^4\) Polymerase chain reaction products were purified through Sephadex G-50 columns (Sigma, St Louis, Mo) and sequenced using an ABI PRISM dRhodamine Terminator Cycle Sequencing Ready Reaction and the ABI PRISM 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, Calif). Each exon was sequenced in both directions in 2 affected and 1 unaffected members of this family. Observed sequence changes were further analyzed for segregation with disease in every affected subject in the family. We also sequenced 200 normal chromosomes using 100 healthy, unrelated control subjects to see if the detected mutation was a benign polymorphism.

**RESULTS**

**PHENOTYPIC DESCRIPTION**

Four affected male subjects and 2 affected female subjects (age range, 5-61 years) were included in this study (Figure 1). Each affected individual had a history of timely acquisition of motor skills. However, all had always teewalked, and none had ever had a normal gait during development. All affected subjects or their parents reported a progression of gait difficulties. None of the affected individuals had sensory symptoms or urinary bladder urgency.

![Figure 1. Pedigree of the analyzed kindred. Roman numerals indicate the generation; the order of individuals in the pedigree is counted from the left. Arrow indicates the proband in this family; circles, female subjects; squares, male subjects; diamonds, sex unknown; filled symbols, affected individuals; open symbols, unaffected individuals; slash marks, deceased individuals.](image)

Findings from neurological examinations were normal except for the lower extremities, which showed bilateral and symmetrical spasticity, hyperreflexia, and extensor plantar responses. Every affected subject had bilateral pes cavus deformities and normal distal strength. Position and vibration sensation sense were normal. Deep tendon reflexes, strength, and muscle bulk were normal in the upper extremities. Gait was markedly spastic with reduced stride length and scissoring. Only 1 family member required an assistive device for walking (subject II-5) since 61 years of age, corresponding to a grade 3 disability score. The other affected individuals, including individuals younger than 12 years (subjects IV-2, 3, and 4), reported a functional limitation because of an abnormal gait, corresponding to grade 2.

The parents of subject II-5, his 4 siblings, and their offspring did not have a history of gait abnormalities.

**GENETIC ANALYSIS**

The presence of the disease in 3 successive generations and several instances of male-to-male transmission confirmed an AD mode of inheritance. Significantly, negative 2-point logarithm of odds scores were obtained for the markers from the SPG4 locus, excluding the linkage to chromosome 2p (data not shown). No recombination was detected with markers spanning the SPG3 locus, and positive 2-point logarithm of odds scores were observed, suggesting linkage to this region (maximum logarithm of odds score, 1.46; recombination fraction \(\theta=0.001\) for marked D14S989). Analysis of the SPG3A coding sequence revealed a heterozygous single nucleotide variant at position 635 (C635T), predicted to result in threonine to isoleucine substitution at codon 156 (Figure 2). This missense mutation completely segregated with the disease and was not found in 200 control chromosomes.

**COMMENT**

We describe an African American family with an infantile onset of AD HSP caused by a novel mutation in the SPG3A gene, further expanding our understanding of the mutational spectrum of this type of HSP. Every affected
individual had a missense mutation in the fourth exon of the SPG3A gene, substituting threonine with isoleucine at codon 156. Analysis of conserved domains of the atlastin protein revealed that this codon is a part of a putative phosphorylation site, and this point mutation is predicted to disrupt this conserved motif. Previously identified mutations in the SPG3A gene did not provide additional clues about a normal function of this protein or about the pathogenesis of HSP, with the exception of a point mutation identified by Muglia et al.5 They found a missense mutation G817A that was predicted to change arginine at codon 217 to aspartic acid; this substitution disrupts a highly conserved RD (arginine–aspartic acid) domain that is characteristic for all GTPases. Our data suggest that abnormal phosphorylation due to disruption of a phosphorylation site may play an important role in the pathogenesis of this type of HSP.

Five of 6 reported mutations in the SPG3A gene, including the present one, are missense mutations.4,5,7 The only other type of mutation detected so far in this gene is a single nucleotide insertion in exon 12, resulting in a frameshift and premature protein truncation.6 Affected individuals in this family had a variable age at symptom onset with a range from 5 to 39 years, and 2 subjects were functionally asymptomatic despite abnormalities revealed by their neurological examination results. This is in contrast with the phenotype of patients from kindreds with missense mutations who have quite stereotypical clinical manifestation with uniformly early age at onset of symptoms, typically less than 10 years. Mutation analysis has yet to be reported in 2 pedigrees in which the linkage to chromosome 14q was first established.11-13 Overall, their phenotype is similar to that of other kindreds with proven missense mutations, and it remains to be seen whether the emerging genotype/phenotype correlations will be confirmed in a larger group of patients with SPG3.

The progression of gait abnormalities in patients with HSP linked to chromosome 14q tends to be slow, and many elderly patients maintain an independent ambulation.5,6 However, the majority of these patients have normal motor development and a period of normal ambulation before the onset of the disease. Every affected patient in the study family had an infantile onset of gait abnormalities and never achieved a normal gait without tiptoeing and scissoring. Similar infantile onset of symptoms has been reported in one Italian pedigree with a missense mutation in the exon 12.7 However, affected individuals from this family have also experienced a delay in the acquisition of independent gait, and 2 individuals were not able to walk independently at the ages of 3 and 4 years. Patients with an infantile onset of HSP can be misdiagnosed as having cerebral palsy. Progression of symptoms is commonly used to distinguish these 2 entities, but individuals with SPG3A can have a minimal worsening of their symptoms and can be considered to have cerebral palsy. Genetic testing may be necessary to distinguish these 2 causes of gait disturbances.

In summary, we describe a family with an infantile age at onset of HSP due to a novel mutation in the SPG3A gene. This family, to our knowledge the first affected family of African American background, further expands our knowledge of the phenotype and genotype of this type of AD HSP.

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Figure 2. A heterozygous missense mutation at position 635 (C635T) (upper panel), predicted to result in threonine to isoleucine substitution at codon 156 (sense strand shown). Lower panel shows a normal sequence of the same segment.
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REFERENCES


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