A New Episodic Ataxia Syndrome With Linkage to Chromosome 19q13

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Background: Multiple episodic ataxia phenotypes and genotypes have been described.

Objective: To describe a new episodic ataxia syndrome.

Design: Genomewide linkage analysis with dense single nucleotide polymorphism arrays.

Setting: University clinic.

The episodic ataxias (EAs) are dominantly inherited disorders characterized by recurrent episodes of cerebellar ataxia. The clinical features vary with each syndrome, and there is clear genetic heterogeneity. Online Mendelian Inheritance of Man (OMIM) currently records 6 clinical EA phenotypes, each with unique genetic features. We now describe a new EA phenotype, and a genome-wide linkage scan that suggests linkage to chromosome 19q13 and rules out linkage to previously reported EA loci.

Methods

Case Material

Proband

An 88-year-old woman reported a lifelong history of recurrent episodes of ataxia, weakness, and dysarthria typically lasting a few hours, but occasionally for as long as 3 days. The episodes were triggered by excitement or exercise. She recalled attending an exciting basketball game as a young girl and being unable to walk home because of ataxia. Between attacks, she had normal balance and coordination. Her medical history included hypertension and hypothyroidism. On neurologic examination, her gait was normal for age, extraocular movements were full without nystagmus, and coordination was normal. Results of brain magnetic resonance imaging and standard vestibular testing were normal as well.

Family History

The proband’s sister, brother, nephew, niece, and grandniece also had EA beginning before age 20 years. All but the brother (II-6), who was dead, were interviewed, and 2 (II-3 and III-4) were examined. Family members reported attacks in the brother, one of which led to incarceration for suspected intoxication while in the Navy. The duration of attacks (hours to days), triggers (excitement and exercise), and associated symptoms (weakness and dysarthria) were similar in all affected members. Two individuals (II-1 and IV-1) described experiencing vertigo with the attacks. Frequency of attacks varied from monthly events in some individuals to only every few years in others. Generally, attacks decreased in frequency with age. Intercital examination results were normal in II-3 and III-4. Two affected members (III-6 and IV-1) also had migraine headaches, but these did not occur with attacks. The proband’s father and paternal aunt were reported as affected, but details of their attacks were unknown.

Genomic DNA Preparation for 10K Mapping Assay

Genomic DNA samples were extracted from peripheral blood from consenting individuals,
with the PUREGENE DNA Purification Kit (Gentra Systems Inc, Minneapolis, Minn) and diluted to 50 ng/µL with Tris-EDTA buffer (0.1 mM EDTA, 10 mM Tris hydrochloride, pH 8.0). For individuals II-1 and IV-1, the GeneChip Human Mapping 50K Array Xba 240 was used according to Affymetrix GeneChip Mapping Assay Manual (Affymetrix, Santa Clara, Calif, available at: http://www.affymetrix.com) in the DNA microarray facility at UCLA. For all other individuals, the GeneChip Human Mapping 10K Array Xba 142 2.0 was used. The 30K array contained most single nucleotide polymorphisms (SNPs) on the 10K array; the overlap in the SNP content was used for this study. This study was approved by the institutional review board at UCLA.

**LINKAGE ANALYSIS**

The SNP calls generated by GeneChip DNA analysis software were converted to different formats by scripts written in the laboratory for each program used (H. Lee, unpublished data, 2003, available on request). A total of 7000 SNPs present on both array platforms were selected. Mendelian errors were detected with PEDCHECK and non-Mendelian errors with MERLIN (Multipoint Engine for Rapid Likelihood Inference) software. The SNPs with errors were marked as “NoCall” for all the genotyped individuals in the pedigree. Multipoint parametric linkage analysis of 7000 SNPs were run with MERLIN under an autosomal dominant model with a high penetrance (95%) and a low phenocopy (0.1%). The haplotype of the regions of interest were constructed using MERLIN.

**SEQUENCING**

All exons and flanking introns of KCNC3 and SLC17A7 were amplified from 2 affected members and directly sequenced. Primer sequences and experimental conditions are available on request.

**RESULTS**

**PEDIGREE**

The extended pedigree used for linkage analysis consisted of 17 individuals (Figure 1). The 6 individuals with EA had attacks lasting hours to days, triggered by exercise and excitement. The pedigree was consistent with an autosomal dominant pattern of inheritance with near complete penetrance.

**LINKAGE ANALYSIS**

Because the family was of adequate size for providing suggestive linkage, we proceeded to determine linkage within the whole genome. A dense panel of 7000 SNPs was typed on 12 individuals in the family using 10K and 50K SNP mapping assays. The data from these microarray-based approaches were outstanding and resulted in high information content (mean information content = 0.89) throughout the genome. The mean call rate was 98.76%. All 12 DNA samples had call rates of more than 98%, and the distribution of genotype calls was consistent with AA, AB, and BB calls. The number of mendelian inheritance errors and nonmendelian errors detected were 21 (0.025%) and 78 (0.09%) of 84 000 genotype calls, respectively. Genotypes for SNPs with at least 1 error were changed to NoCall for all the family members before starting the analysis. The genome scan was run with MERLIN for parametric multipoint analysis. A maximum logarithm of odds (LOD) score of 2.95 (penetrance, 95%; gene frequency, 0.1%) was observed on chromosome 19q13 (Figure 2A). This was the only region in the genome that had the maximum expected LOD score for this family size and marker density. Haplotype analysis was constructed for the region and recombination points were determined (Figure 2B). The length of the susceptibility haplotype was 10 centimorgans (cM) (between markers rs1366444 and rs952108). The susceptibility haplotype was used for single-point linkage assuming a rare allele frequency, conservatively estimated at 1%, and high penetrance of 99%. The LOD score of this putative susceptibility haplotype was 3.28, which is at the threshold of genome-wide significance.

**SEQUENCING**

We sequenced KCNC3 and SLC17A7 within the susceptibility haplotype in the proband and 1 other affected family member, but no mutations were found in the coding regions of either of these 2 genes.
As noted earlier, to our knowledge, to date 6 EA phenotypes have been described, but only EA1 and EA2 have been reported in large numbers of families (Table). Mutations in the potassium channel gene KCNA1 lead to the EA1 phenotype, which is characterized by brief episodes of ataxia with interictal myokymia, while EA2, caused by mutations in the calcium channel gene CACNA1A, is characterized by more prolonged episodes of ataxia with interictal nystagmus. The EA3 phenotype was described in a single large Canadian family with episodic vertigo, tinnitus, and ataxia and was recently found linked to chromosome 1q42. The EA4 phenotype, also called periodic vestibulocerebellar ataxia, was described in 2 North Carolina kindreds with late-onset vertigo and ataxia, as well as interictal nystagmus. Linkage analysis ruled out the EA1 and EA2 loci, but so far no genome-wide scan has been reported. The EA5 phenotype was identified when a series of families with EA were screened for mutations in the calcium channel β₃-subunit, CACNB4, on chromosome 2q. This family had clinical features similar to those of EA2, but mutations in CACNA1A were ruled out. Complicating matters, the same mutation was found in a German family with generalized epilepsy (but no ataxia), and functional studies showed only subtle changes in calcium channel function. Finally, EA6 was described in a single child with EA, episodes of hemiplegia, and seizures in which a rare mutation was identified from a screen of the candidate gene, SLC1A3, a glutamate transporter localized to astrocytes. The mutation was de novo, and functional studies of the mutated protein showed an almost complete loss of function.

This new family with EA (which we suggest be called EA7) has clinical features similar to those of EA2, except that there are no interictal findings on neurologic examination. There also could be phenotypic overlap with EA3, but none of the patients reported tinnitus with their attacks and none of them had the usual duration of attacks as seen with EA3. Furthermore, the EA3 locus on 1q42 was ruled out with our genome-wide scan.

With the high-density SNP arrays, we were able to simultaneously genotype over 10,000 SNPs across the whole genome at a median intermarker distance of 210 KB. The mean heterozygosity of the SNP markers (0.37), coupled with the high density, provided a rapid and powerful tool for discerning linkage. Prior studies indicate advantages of the 10K SNP mapping assay over the traditional 10-cM microsatellite genome scans. Although this study was based on a single moderate-sized pedigree, the SNP array approach proved to be efficient, accurate, and rapid. This allowed us to make optimal use of linkage within the family and determine the single region of the genome that provided the maximum expected LOD score. Because all currently identified gene mutations in families with EA involve genes coding for membrane channels, we identified 2 candidate ion channels in the linked region that were highly expressed in the brain (http://www.genome.ucsc.edu). Unfortunately, sequencing of these ion channels did not identify the causative mutation in our family within the amino acid coding sequence of the genes. We plan to systematically sequence other candidate genes and the proximal promoter regions, but the number of genes in the linked region is large so identifying additional families with similar clinical phenotypes is key to narrowing the linked region.

**Table. Summary of Genetic and Clinical Features of Different EA Syndromes**

<table>
<thead>
<tr>
<th>EA Syndrome</th>
<th>Age at Onset, y</th>
<th>Duration of Attacks</th>
<th>Associated Symptoms</th>
<th>Interictal Findings</th>
<th>Gene Locus</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>EA1</td>
<td>&lt;20</td>
<td>Minutes</td>
<td>Muscle spasms</td>
<td>Seizures, myokymia</td>
<td>12p13</td>
<td>KCNA1</td>
</tr>
<tr>
<td>EA2</td>
<td>&lt;20</td>
<td>Hours</td>
<td>Vertigo, weakness</td>
<td>Ataxia, nystagmus</td>
<td>19p13</td>
<td>CACNA1A</td>
</tr>
<tr>
<td>EA3</td>
<td>&lt;20</td>
<td>Minutes</td>
<td>Vertigo, tinnitus, headache</td>
<td>Usually none</td>
<td>1q42</td>
<td>Unknown</td>
</tr>
<tr>
<td>EA4</td>
<td>20-50</td>
<td>Hours</td>
<td>Vertigo, diplopia</td>
<td>Nystagmus, abnormal smooth pursuit</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>EA5</td>
<td>20-60</td>
<td>Hours</td>
<td>Vertigo</td>
<td>Nystagmus, ataxia</td>
<td>2q22-23</td>
<td>CACNB4</td>
</tr>
<tr>
<td>EA6</td>
<td>&lt;10</td>
<td>Hours</td>
<td>Cognitive impairment</td>
<td>Seizures, ataxia</td>
<td>5p13</td>
<td>SLC1A3</td>
</tr>
<tr>
<td>EA7</td>
<td>&lt;20</td>
<td>Hours</td>
<td>Vertigo, weakness</td>
<td>None</td>
<td>19q13</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Abbreviation: EA, episodic ataxia.