Autosomal Dominant Nocturnal Frontal Lobe Epilepsy in a Spanish Family With a Ser252Phe Mutation in the CHRNA4 Gene

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Background: A large family with autosomal dominant nocturnal frontal lobe epilepsy from the south of Spain was studied. The clinical appearance of the disease in this family, which included 28 members, of whom 11 were affected and 2 were obligate carriers, was identical to that previously described in an Australian family and a Norwegian family, in which mutations in exon 5 of the CHRNA4 gene were found.

Methods: Following DNA extraction, the family was genotyped with 4 fluorescent markers flanking the locus to the CHRNA4 gene on chromosome 20q13.3, and lod score computations were performed. The exon 5 of the CHRNA4 gene was amplified between nucleotides 535 and 825 and polymerase chain reaction products were purified and sequenced directly.

Results: The same missense mutation as that found in the Australian family, C→T, which causes the replacement of a serine with phenylalanine in amino acid 252 in exon 5, was detected. This mutation segregated with the disorder in all 11 affected members, in the 2 obligate carriers, and in 1 asymptomatic sibling, and was not found in 1 spouse and 1 daughter. Neither of the 2 polymorphisms associated with this mutation in the Australian family were found, excluding a common ancestral haplotype for the 2 families.

Conclusions: These data confirm the clinical homogeneity in the phenotypic expression of autosomal dominant nocturnal frontal lobe epilepsy caused by mutation in the CHRNA4 gene, and the pathogenic role of the Ser252Phe mutation in this disorder.

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PATIENTS AND METHODS

CLINICAL DATA COLLECTION

Twenty-eight members of a Spanish family from Seville, including 11 affected individuals and 2 obligate carriers, underwent clinical, electrophysiologic, and genetic analysis. The pedigree is shown in Figure 1. Five of the patients were followed up for 10 years in the Outpatient Consultation of the Hospital Valme of Seville. All patients were reinterviewed by one of us (J.G.), using a semistructured questionnaire for epileptic disorders.17

Intercital EEG, with 19 electrodes positioned according to the international 10-20 system, using bipolar and referential montages, was performed in 9 patients (Table). Hyperventilation and intermittent photic stimulation were used as activators. In 4 patients, an EEG was obtained during diurnal sleep, without video monitoring.

Computed tomography was performed in 3 patients, and magnetic resonance images were obtained from 5 patients with the use of a 0.5-T field strength unit (Table).

GENOTYPE DETERMINATION

Blood Samples

After informed consent was obtained, venous blood samples were collected from 16 subjects (11 affected, 4 asymptomatic, and 1 relative). DNA was extracted from whole blood by standard procedures.

Genotyping

Four fluorescent markers (loci D20S120, D20S102, D20S171, and D20S173) from the Genethon map, spaced at an average distance of 15 centimorgans from each other,18 flanking the locus of the CHRNA4 gene on chromosome 20q13.3, were typed on the DNA from the 16 individuals sampled. Microsatellite primers were labeled with either 6-Fam, Hex, or Tet phosphoramides.19 Polymerase chain reaction reactions were performed in 192-well microtiter plates in a final volume of 15 mL containing 30 ng of genomic DNA, 0.16-mmol/L dNTPs and NBL:, 1 × NBL buffer, 0.33-mmol/L concentrations of each primer, and 0.03 U of Taq polymerase. After a hot-start procedure (enzyme is added at 94°C after denaturation for 5 minutes at 96°C), 26 polymerase chain reaction cycles were performed (40 seconds at 94°C, 30 seconds at 55°C). The rest of the procedure was similar to that described by Reed et al.19 Individuals 134702 and 88415 from the Centre d’Etude du Polymorphisme Humain (Paris, France) (CEPH) families18 were genotyped in each set of reactions as allele size references.

Linkage Analysis

A simulation by the SLINK program20 was performed with the use of 4 isofrequent alleles at each marker locus, assuming that the disorder is an autosomal dominant condition, with a frequency of the susceptibility allele of 0.001, a penetrance of 70%, and no phenocopies. Lod score computations were performed with the MLINK subroutine of the LINKAGE package (version 5.10).21 Allele frequencies used were based on unrelated individuals from 8 CEPH families. The analysis was also performed with the use of allele isofrequencies and/or different penetrance values, which produced no drastic modification of the results (data not shown).

DNA Sequencing

Polymerase chain reaction fragments between nucleotides 535 and 825 of the fifth exon of the CHRNA4 gene were amplified with primers 5’-GGCGAGTGGGTACTCTGTTG-3’ and 3’-GATGACCAGTGAGGTGGACG-3’, according to the previously published sequences.22 Polymerase chain reaction products were purified by means of P100 chromatography and sequenced directly by the AmpliTaq FS Dye Terminator cycle sequencing kit and ABI Prism 377 DNA sequencer.

We report a family from the south of Spain that fulfills the clinical criteria for ADNFLE and that carries the same mutation as that reported in the Australian family, confirming the high degree of clinical homogeneity in the phenotypic expression of this syndrome and the causative role of the Ser252Phe mutation.

RESULTS

CLINICAL DESCRIPTION

As shown in the pedigree (Figure 1), there were 11 affected individuals and 2 obligate carriers. The disorder was transmitted in an autosomal dominant mode with...
incomplete penetrance. The age at seizure onset ranged from 3 to 12 years (mean, 7.6 years). Only 2 patients had antecedents of febrile seizures. No history of head trauma, meningitis, encephalitis, or any other neurologic condition that was likely to have caused epilepsy was found. The pregnancy, delivery, and postnatal period had been uneventful in all patients. Results of clinical examination were normal in all of the family members examined (11 affected patients, 3 asymptomatic carriers, and 2 healthy individuals). None of the patients had evidence of intellectual disability or psychiatric conditions.

Seizures always occurred during sleep periods, usually after the beginning of sleep. None of the patients manifested diurnal attacks. Seizures generally started with a sudden awakening with malaise and suffocation, followed by motor manifestations, with dystonic posturing of limbs and myoclonic jerks. Patients remained conscious but were frequently unable to speak during the episode. Secondary generalization was rare, and there were no postictal phenomena. Seizures were brief, shorter than 1 minute, and appeared in clusters with up to 20 seizures per night, alternating with periods with sporadic attacks or complete absence of symptoms. Two children, subjects III-3 and III-7, aged 3 and 5 years, respectively, had seizures consisting of brief episodes (approximately 1-2 minutes) of crying with absence of response that occurred in clusters, several times every night during several days. One child (subject III-6) occasionally

<table>
<thead>
<tr>
<th>Clinical, Electrophysiologic, and Neuroimaging Data*</th>
<th>Patient No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-3</td>
<td>II-2</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>M</td>
</tr>
<tr>
<td><strong>Age, y</strong></td>
<td>59</td>
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<tr>
<td><strong>Febrile convulsions</strong></td>
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<tr>
<td><strong>Age at onset, y</strong></td>
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<tr>
<td><strong>Clinical features</strong></td>
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</tr>
<tr>
<td><strong>Loss of consciousness</strong></td>
<td>NA</td>
</tr>
<tr>
<td><strong>Duration of attacks</strong></td>
<td>NA</td>
</tr>
<tr>
<td><strong>Secondary generalization</strong></td>
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<tr>
<td><strong>Postictal phenomena</strong></td>
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<tr>
<td><strong>Time of attacks</strong></td>
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<tr>
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</tr>
<tr>
<td><strong>Clusters of seizures</strong></td>
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<tr>
<td><strong>Interictal EEG</strong></td>
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<td><strong>Sleep EEG</strong></td>
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<tr>
<td><strong>Response</strong></td>
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</table>

*NA indicates not available or not applicable; EEG, electroencephalogram; CT, computed tomographic; MR, magnetic resonance; VPA, valproic acid; PB, phenobarbital; PHT, phenytoin; CBZ, carbamazepine; and LMT, lamotrigine.
had isolated episodes consisting of sitting in the bed, saying a few words, and continuing to sleep, without crying or showing terror. These episodes suggested somniloquia.

The interictal EEG was normal except in 2 patients who showed epileptic activity over frontal areas. The diurnal sleep EEG performed in 4 patients was normal. Neuroimaging was normal in all of the patients studied.

Carbamazepine was the most effective antiepileptic drug used, although 2 patients responded well to valproate sodium. Some patients developed tolerance, and after a period with a good response to drugs, there was poor control of seizures, even with high doses of antiepileptic drugs or polytherapy with several drugs.

Clinical information for the patients is summarized in the Table.

GENETIC ANALYSIS

Simulation of linkage by the SLINK program gave a lod score of 2.43 at θ = 0. From the 4 fluorescent markers from the Génethon map, a suggestive linkage was found with locus D20S171 (lod score, 1.47 at θ = 0) (data not shown). Sequencing of the portion of exon 5 of the CHRNA4 gene that contains the 2 previously described mutations was undertaken. A missense mutation C→T that causes replacement of a serine by phenylalanine at amino acid position 252 was detected (Figure 2). This mutation seg-
affected. Diagnosis of this disorder in young children is difficult because the symptoms are often dismissed as nightmares.

The presence of the same mutation in 3 large families, with an almost identical clinical syndrome but with different ethnic origins, confirms the involvement of the CHRNA4 gene in ADNFLE. Two polymorphisms described previously in the Australian kindred and associated with the mutation Ser252Phe in the same exon at position 555 and 594 (consisting in both cases of a C→T transition in exon 5) were not found, excluding a common ancestral haplotype shared by the Australian and Spanish families. Quite surprisingly, no mutations in exon 5 were found in 15 families by Oldani et al. Moreover, there is no linkage to markers in the 20q13.2-13.3 region in the 5 largest families. This could be because of the small number of markers in this region, their low degree of informativity, the large distance between the available markers leading to recombination events, or the sequence analysis of only a part of the fifth exon of the gene.

Nevertheless, as reported by others, genetic heterogeneity exists in ADNFLE, reinforced by the absence of strict inclusion criteria and the difficulty of ascertaining the clinical features when no EEG monitoring data are available.

Clinical characteristics of families with ADNFLE caused by mutations in the CHRNA4 gene are slightly different from those in families not linked to chromosome 20q. In both groups, onset is in childhood or adolescence, and the disorder is characterized by clusters of brief nocturnal motor seizures with hyperkinetic or dystonic manifestations, which persist through adult life. The seizures frequently respond to antiepileptic drugs, especially carbamazepine, although recurrence on withdrawal of medication is frequent. However, the presence of enuresis, diurnal attacks, or other subjective complaints, such as tiredness on awakening or excessive diurnal somnolence, has only been reported in families without linkage to chromosome 20q. Therefore, ADNFLE is a clinically and genetically heterogeneous disorder, although some genotype-phenotype correlations exist.

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The family we describe shows a homogeneous clinical picture that is consistent from one patient to another and is in accordance with that previously described in other families with ADNFLE.

Although this syndrome is rare, it is probably more common than previously suspected. Our confirmation of the mutation in the CHRNA4 gene in a third family shows that this condition is not merely anecdotal, as has been suggested, but is a well-defined clinical entity. This knowledge is important because it can help in the diagnosis in children whose parents are also affected. Diagnosis of this disorder in young children is

| Figure 2. Sequence of a fragment of exon 5. Sequence from a healthy control is shown above the mutant sequence. Asterisk indicates the position of the mutated nucleotide, the C→T transition that replaces a Ser with a Phe. |

| Normal Sequence |
| G C A T C T C G T G |
| Mutant Sequence |
| G C A T C T N C G T G |

| G C A T C T C G T G |
| G C A T C T N C G T G |

The family we describe shows a homogeneous clinical picture that is consistent from one patient to another and is in accordance with that previously described in other families with ADNFLE.


We invite submissions to the Images in Neurology section of the ARCHIVES. We invite your submission of interesting images of patients, tissue biopsy samples, and radiographic images, including magnetic resonance imaging, positive emission tomography, and x-ray scans, etc. With your image, please send a brief summary (300 words or less) describing its uniqueness and importance. Also, indicate the magnification and stain where appropriate. Both black-and-white and color images (at no charge to the author) are welcome. Submissions should be sent in triplicate to: Roger N. Rosenberg, MD, Editor, Archives of Neurology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75235-9108.

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Correction

Error in Text. In the Observation by Säenz et al titled “Autosomal Dominant Nocturnal Frontal Lobe Epilepsy in a Spanish Family With a Ser252Phe Mutation in the CHRNA4 Gene,” published in the August issue of the ARCHIVES (1999;56:1004-1009), 2 errors occurred in the text. In the abstract on page 1004, the last sentence in the “Results” section should have read as follows: “Neither of the 2 polymorphisms found in a series of families with epilepsy were found in our sample.” Also, on page 1008, the second sentence of the third paragraph in the “Comment” section should have read as follows: “Two polymorphisms at positions 555 and 594 (consisting in both cases of a C→T transition in exon 5), described previously22 in a series of different families with epilepsy, were not found.”