A Korean Kindred With Autosomal Dominant Nocturnal Frontal Lobe Epilepsy and Mental Retardation

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Background: A Korean family had distinctive clinical and neuroimaging features and carried the same genetic mutation that was found in a previously described Japanese kindred with autosomal dominant nocturnal frontal lobe epilepsy.

Objective: To describe the first Korean family with autosomal dominant nocturnal frontal lobe epilepsy.

Methods: Members of a large family, including 9 affected individuals from 3 generations, underwent a comprehensive genetic, clinical, electroencephalographic, neuropsychological, and neuroimaging evaluation. Affected members were tested for possible mutations in transmembrane regions 1 through 3 of the neuronal nicotinic acetylcholine receptor α4 subunit (CHRNA4) by direct sequencing and subsequent restriction analysis.

Results: Seizures began in childhood, presenting as nocturnal episodes of staring, confusion, shouting, perioral movements, unintelligible speech, and hand waving. Some patients had ictal or interictal epileptiform activity in the temporal and/or frontocentral areas. Neurological examination and brain magnetic resonance imaging results showed no abnormalities, except that all patients available for testing had mild to moderate mental retardation. Fluorodeoxyglucose F 18 with positron emission tomography showed mild decreased glucose uptake in the superior and middle frontal regions, more so on the left than on the right. Patient response to carbamazepine was poor. All affected members were heterozygous for the CHRNA4 Ser252Leu mutation.

Conclusions: Disorders associated with mutations in the transmembrane region 2 of CHRNA4 are genetically and phenotypically heterogeneous. Distinctive features of this kindred include (1) mental retardation in all affected members available for testing, (2) abnormal brain findings on fluorodeoxyglucose F 18 with positron emission tomography, (3) poor response to carbamazepine, and (4) full penetrance.

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Recognition of autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) as a distinct epilepsy syndrome came after a 3-decade-long controversy regarding patients who demonstrated "abnormal nocturnal motor and behavioral phenomena." Recent genetic findings provided major insights into the nature of the illness. Autosomal dominant nocturnal frontal lobe epilepsy has been associated with 5 different mutations in the genes encoding 2 subunits of the neuronal nicotinic acetylcholine receptor (nAChR). These genes include CHRNA4 on chromosome 20q13.3 and CHRNA2 on chromosome 1q21-q22. Autosomal dominant nocturnal frontal lobe epilepsy mutations found in the former gene are amino acid exchanges (Ser248Phe and Ser252Leu) or insertions (776ins3) in the gate-forming second transmembrane domain (TM2). Within the latter gene, the TM2-located missense mutations Val287Leu and Val287Met have been identified.

We still do not know the full spectrum of electroencephalographic, clinical, and genetic features associated with ADNFLE. Most ADNFLE families described lack the known mutations. There is phenotypic variation even among members of the same family with the same genetic defect. Partial rather than generalized seizures in an autosomal dominant disease add to the complexity; it has been suggested that variability in neuronal nAChR subunit composition in different parts or layers of the frontal cortex may vary the phenotype. The most interesting finding with respect to the cause of phenotypic variability in ADNFLE is the observation that specific nAChR mutations seem to be associated with distinct

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phenotypic features. It is therefore important to identify additional families with known or new nAChR mutations. This will permit a more detailed description of the nAChR-associated ADNFLE phenotype. Furthermore, the identification of families unrelated to each other or even of different ethnic origin but carrying the same nAChR mutation may allow differentiation between phenotypic effects caused by modifying genes or environmental variability and those that are potentially mutation-specific. We have identified the first Korean family with ADNFLE caused by an nAChR mutation.

METHODS

MATERIALS

We studied a large 3-generation family from South Korea (Figure 1). There were 15 living members, with 9 affected individuals, including 2 men and 7 women, aged 20 to 76 years (median age, 23 years; mean ± SD, 34.1 ± 20.5 years). Medical histories were obtained from patients and families. The segregation pattern of the disease was consistent with autosomal dominant inheritance. The mean age at seizure onset was 11 years. Seizure frequency in all affected family members ranged from 2 to 3 per year to 4 to 5 per night. The patients had taken many different epilepsy drugs were tapered at the time of admission.

To facilitate recording of seizures during monitoring, all antiepileptic drugs were tapered at the time of admission.

POSITRON EMISSION TOMOGRAPHY

Interictal fluorodeoxyglucose F 18–positron emission tomography (FDG-PET) was performed on 6 affected family members (patients B, D, F, M, N, and O) on an ECAT EXACT HR+ scanner (Siemens Medical Systems, Knoxville, Tenn). Sixty-

three contiguous axial slices were obtained in 3-dimensional acquisition mode with a 2.46-mm thickness. The full width at half maximum of in-plane and axial resolution at the center of the field of view was 4.6 mm and 4.2 mm, respectively. Scans were performed following at least a 6-hour fast; all patients were injected with 10 mci (370) MBq of FDG. Patients’ heads were immobilized, and the scanner was oriented along the canthomeatal line. A 5-minute transmission scan was performed using a rotating germanium 68 rod source for attenuation correction. Fifteen minutes of emission data were acquired after a 40-minute uptake period. All scans were performed in the awake resting and drowsy state, with eyes patched and ears unplugged. Electroencephalography was not performed. Attenuation-corrected images were made using an iterative reconstruction algorithm (OSEM [ordered subsets expectation maximization], 6 iterations and 16 subsets). Gray-scale axial images were viewed by 3 raters (R.R., W.D.G., and W.H.T.). Individual and group data were also processed and analyzed with SPM ’99 (Statistical Parametric Map; Wellcome Department of Cognitive Neurology, London, England), using the general linear model. Images were reconstructed, realigned, and then normalized into a standard stereotactic space, conforming to the technique by Talairach and Tournoux, to allow subject-control data averaging and comparisons. Data were smoothed through an isotropic gaussian kernel filter of 12 mm. Individual comparisons were made between the 4 younger patients and 8 young control subjects (6 men and 2 women; mean ± SD age, 26 ± 7 years). Individual comparisons were also made between the 2 older patients and 8 older controls (4 men and 4 women; mean ± SD age, 49 ± 5.7 years). Individual comparisons were performed with t tests (uncorrected P < .01) and an extent threshold greater than 100 voxels. Group comparisons were made between the 6 patients and 14 controls (8 men and 6 women; mean ± SD age, 35.0 ± 10.2 years) using t tests (uncorrected P < .01) at an extent threshold greater than 100 voxels and displayed on a 3-dimensional brain rendered in Talairach and Tournoux space after transformation of fMRI for each pixel in the comparison into a standard normal distribution (z value).

NEUROPSYCHOLOGICAL EVALUATION

Affected family members, except for patients A, B, and L, were assessed using a comprehensive neuropsychological test battery that included measures of verbal and performance intelligence, verbal and visual memory, constructional praxis, and executive functions. For intelligence testing, we used the Korean–Wechsler Adult Intelligence Scale. This is a standardized Korean version of the Wechsler Adult Intelligence Scale–Revised, with linguistic and cultural modifications. Verbal memory was measured using the standardized Korean version of the Memory Assessment Scales. The testing included attention, concentration, and learning, as well as immediate, short-term, and delayed memory for verbal and nonverbal materials. Measures of recognition and intrusion during verbal learning, recall, and retrieval strategies were also provided. Constructional praxis and visual memory were measured by the Rey Complex Figure test, which has been standardized in Korea. Trail-Making Test and Pegboard Test were used to measure executive functions. The tests were administered in the same order for all subjects, following a minimum of a 2-day seizure-free period. Definitions established by the American Association on Mental Retardation were used to grade mental retardation.

MUTATION SCREENING

Genomic DNA was extracted from EDTA-anticoagulated blood samples using standard methods and resuspended in TE buffer.

Figure 1. Pedigree of the Korean autosomal dominant nocturnal frontal lobe epilepsy family. Solid symbols represent affected individuals; open symbols, unaffected individuals; squares, men; circles, women; +/−, Ser252Leu (heterozygous); and −/−, normal (homozygous). Only members assigned letters A through O were evaluated.
Polymerase chain reaction (PCR) was carried out in a total volume of 50 µL in a thermal multicycler (PTC-200; MJ Research, Inc, Waltham, Mass), containing 20 ng of genomic DNA, 10 pmol of each forward and reverse primer, 200 µmol of each deoxyribonucleotide triphosphate, 1.5 mmol of magnesium chloride, 50 mmol of potassium chloride, 20 mmol of Tris hydrochloride (pH 8.4), and 0.1 U of Taq DNA polymerase (Invitrogen Life Technologies, Karlsruhe, Germany). The PCR variables were as follows: denaturation at 95°C for 5 minutes, followed by 33 cycles at 95°C for 30 seconds, annealing at 64.3°C for 25 seconds, and extension at 72°C for 45 seconds, followed by a final extension step of 10 minutes at 72°C. The following primers were used to amplify the part of exon 5 containing TM4 through TM3: n28744 (5’ GGCAGGTGGTTCATCGTGGG) and n54624 (5’ GCCTGGGAGCAAGAGCGCGG). The obtained PCR products were sequenced on an ABI 377 sequencer (Applied Biosystems, Foster City, Calif).

Ser252Leu MUTATION DETECTION

A restriction fragment length assay was developed using primers n1415 (5’ ATGAGGAGTGAGGTCTACGGA) and n1416 (5’ TGGACAGCAGGAGAGGTTGC). Lowercase letters indicate aberrant nucleotides that were introduced to create artificial FnuDII (MBI Fermentas Inc, University of Illinois at Urbana-Champaign) restriction sites. The resulting PCR fragments included part of CHRNA4 exon 5. The fragments were amplified by a 2-step protocol in which the annealing and extension steps already described were substituted by a single step of 55 seconds at 72°C. Five microliters of the resulting PCR products was treated with 4 U of FnuDII and then run on 10% polyacrylamide gels for 2 to 3 hours at room temperature. After the electrophoresis, the bands were visualized using a standard silver-staining protocol. FnuDII restriction digest of PCR products gave the following pattern: wild-type allele, 16 base pairs (bp) + 219 bp + 20 bp, and mutant allele, 16 bp + 239 bp (Figure 2).

RESULTS

VIDEO-EEG MONITORING

Seizure symptoms did not show significant variability among the 6 patients who agreed to video-EEG monitoring. All seizures arose from sleep, and no auras were reported. All patients had multiple seizures during their hospitalizations. The episodes usually lasted about 20 to 40 seconds and typically consisted of arousal followed by arm flexion, tonic head extension, mouth movements, and unintelligible speech. During one seizure (patient M), there was head deviation to the right and right arm flexion, with no epileptiform activity on EEG. Patient D had secondary generalized tonic seizures (Table 1). Most seizures were not associated with any discernible epileptiform activity, but occasionally showed generalized spike activity (Figure 3). Patients D, G, L, (until 2000), N, and O had interictal temporal lobe spikes (Figure 4) or interictal and ictal generalized spike activity that was maximum over the frontocentral or frontotemporal areas on 1 or more occasions. One family member (patient D) had ictal onset of right frontocentral spikes, and 2 patients (N and O) had generalized spikes with maximum activity over the right temporal region.

NEUROPSYCHOLOGICAL EVALUATION

The detailed results of each patient’s neuropsychological test are shown in Table 2. All patients met all or most of the criteria for mild to moderate mental retardation. They were also at significantly low standing in terms of memory function.

NEUROIMAGING

Five of the patients had a brain MRI. The results were normal, except for a left temporal arachnoid cyst (patient N) and mild hydrocephalus (patient L). The latter had right anterior temporal spikes on EEG. No patient showed hippocampal sclerosis or other mesial temporal changes on MRI (Table 1). Six of the patients (B, D, F, M, N, and O) underwent interictal FDG-PET study. All raters read study results as normal, except for hypometabolism in the left temporal area in patient N, concordant with the previously detected arachnoid cyst. However, on SPMs, excluding the focal deficit associated with the arachnoid cyst, there were decreases in glucose uptake in the superior and middle frontal gyri (5 patients), in the left central regions (4 patients), and in the anterior parietal areas (4 patients). Four patients also showed small areas of decreased glucose uptake in the right anterior superior frontal gyrus (Table 1). The group SPM analysis (Figure 5) demonstrated decreased glucose uptake in the left middle and superior frontal gyri and the left central regions, including the anterior parietal lobe. There was also a less pronounced decrease in glucose uptake in the right anterior superior frontal gyrus.

MOLECULAR GENETICS

Sequencing of the TM2 coding part of CHRNA4 exon 5 in family members D and G revealed a C→T nucleotide substitution, causing a serine to leucine exchange at amino acid position 252 in the predicted protein sequence. Restriction analysis verified the observed Ser252Leu mutation, which was subsequently found in all 9 affected family members (A, B, D, F, G, L, M, N, and O), but not in the unaffected individuals C, E, H, I, J, and K.

COMMENT

This is the first report of an ADNFLE kindred from Korea. All affected members of this family are carriers of the same CHRNA4 mutation (Ser252Leu) that has been re-
Table 1. Clinical, Electrophysiologic, Neuroimaging, and Genetic Findings in Affected Family Members

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient</th>
<th>Patient</th>
<th>Patient</th>
<th>Patient</th>
<th>Patient</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y/sex</td>
<td>46/F</td>
<td>24/F</td>
<td>22/F</td>
<td>23/F</td>
<td>22/F</td>
<td>20/M</td>
</tr>
<tr>
<td>History of febrile seizure</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Age at onset, y ictal symptoms</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>14</td>
<td>13</td>
<td>12</td>
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<td>Loss of awareness</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Time of seizure</td>
<td>Day and night</td>
<td>Night</td>
<td>Night</td>
<td>Night</td>
<td>Night</td>
<td>Night</td>
</tr>
<tr>
<td>Seizure frequency</td>
<td>1-2 min</td>
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<td>1-2 min</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Stage 2 (100%), stage 2 (80%), stage 3 (20%)</td>
<td>2-3 Per year</td>
<td>4-5 Per night</td>
<td>5-6 Per night</td>
<td>None</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Secondary generalization</td>
<td>Occasional</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>NA</td>
<td>Occasional</td>
</tr>
<tr>
<td>Postictal phenomena</td>
<td>Confusion</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>NA</td>
<td>No</td>
</tr>
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<td>Seizure frequency taking medication</td>
<td>6</td>
<td>12</td>
<td>14</td>
<td>NA</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Intercital EEG</td>
<td>Occasional right anterior temporal spikes</td>
<td>Normal</td>
<td>Normal</td>
<td>Frequent right anterior temporal spikes</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Ictal EEG</td>
<td>Bilateral frontocentral spikes</td>
<td>Generalized spike activity</td>
<td>Generalized spikes, maximum right temporal</td>
<td>NA</td>
<td>Generalized spikes</td>
<td>Generalized spikes, maximum right temporal</td>
</tr>
<tr>
<td>Brain magnetic resonance imaging</td>
<td>Normal</td>
<td>NA</td>
<td>NA</td>
<td>Mild hydrocephalus</td>
<td>Normal</td>
<td>Left temporal arachnoid cyst</td>
</tr>
<tr>
<td>Intercital FDG-PET hypometabolism</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Intercital FDG-PET statistical parametric map analysis hypometabolism†</td>
<td>Left middle and superior frontal; left parietal</td>
<td>Left middle and superior frontal gyrus; left central and parietal; right anterior superior frontal gyrus</td>
<td>NA</td>
<td>NA</td>
<td>Left middle and superior frontal gyrus; left central and parietal; left anterior and inferior temporal lobe; right anterior superior frontal gyrus</td>
<td>PHT, PB, TPM, VGB</td>
</tr>
<tr>
<td>Previous daily medication</td>
<td>PHT, 300 mg</td>
<td>VGB, 1500 mg; ZNS, 400 mg</td>
<td>CBZ, 600 mg</td>
<td>CBZ, 600 mg</td>
<td>CBZ, 600 mg</td>
<td>CBZ, 600 mg</td>
</tr>
<tr>
<td>Current daily medication</td>
<td>CBZ, 600 mg</td>
<td>CBZ, 1200 mg; TPM, 300 mg; OCBZ, 600 mg, PRM, 750 mg</td>
<td>CBZ, 600 mg; TPM, 150 mg; LTG, 100 mg</td>
<td>Stopped since 2000</td>
<td>CBZ, 600 mg; VPA, 1000 mg; PRM, 375 mg</td>
<td>VGB, 1500 mg; VPA, 1000 mg; LTG, 200 mg</td>
</tr>
<tr>
<td>Response to therapy†</td>
<td>Significant improvement</td>
<td>Mild improvement</td>
<td>Mild improvement</td>
<td>Seizure free</td>
<td>Significant improvement</td>
<td>Mild improvement</td>
</tr>
<tr>
<td>Genetic mutation‡</td>
<td>Ser252Leu</td>
<td>Ser252Leu</td>
<td>Ser252Leu</td>
<td>Ser252Leu</td>
<td>Ser252Leu</td>
<td>Ser252Leu</td>
</tr>
</tbody>
</table>

Abbreviations: CBZ, carbamazepine; EEG, electroencephalographic; FDG-PET, fluorodeoxyglucose F18–positron emission tomography; LTG, lamotrigine; NA, not available; OCBZ, oxcarbazepine; PB, phenobarbital; PHT, phenytoin; PRM, primidone; TPM, topiramate; VGB, vigabatrin; VPA, sodium valproate; ZNS, zonisamide.
†Significant improvement, more than 50% reduction in seizure frequency; moderate improvement, 20% to less than 50% reduction in seizure frequency; and mild improvement, less than 20% reduction in seizure frequency.
‡Ser252Leu mutation in the gene CHRNA4.
trafamilial and interfamilial variability, the ADNFLE phenotype has not shown significant differences in independent families with similar genetic mutations. When clinical differences were described, they appeared to be correlated with genetic differences. So far, most family data are available for the Ser248Phe CHRNA4 mutation because of a different numbering of amino acid residues, the mutation was also named Ser252Phe. Age at onset, nocturnal clusters of hyperkinetic and dystonic movements, and favorable response to carbamazepine are among the common features in the group with 20q linkage. The phenotypic similarities between the previously described Norwegian, Spanish, and original Australian families, 2 of them with proven different genetic backgrounds, suggest that the CHRNA4 mutation Ser248Phe is the major factor determining the phenotype, rather than other background genes.

The clinical picture of ADNFLE caused by Ser252Leu is less clear. As observed in our family, the patients of the Japanese family with the Ser252Leu mutation did not have auras. Interestingly, 2 of 3 children in the Japanese family had mild mental retardation, while all 3 had behavioral problems such as hyperactivity. The phenotypic similarity between our family and the previously described Japanese family raises the possibility of common ancestry; this was denied by our family and the Japanese kindred (S. Hirose, MD, PhD, written communication, June 2003).

The possibility of subtle neurocognitive deficits in the genetically unaffected family members cannot be ruled out. Such deterioration might not be detectable without a comprehensive neuropsychological evaluation. The presence of cognitive deficits in those members would suggest that the Ser252Leu mutation alone might not be sufficient to explain the neurocognitive deficits in this family, but that additional genetic factors have to be postulated.

Five of 7 affected members of our family have been unresponsive to 600 to 900 mg of carbamazepine, which in our experience has been an adequate dose for controlling seizures in Korean patients. Patient G developed toxic signs (dizziness) when a dose higher than 600 mg was attempted. However, we cannot exclude the possibility that

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**Figure 3.** Ictal pattern (patient D) shows bilateral spike activity.
these patients' seizures might have responded better to higher doses of carbamazepine. The serum drug levels in patients D, M, N, and O ranged from 5.5 to 9.0 µg/mL (23–38 µmol/L) on doses of 600 to 900 mg.

In most described ADNFLE families, seizure treatment with carbamazepine has been successful, although unresponsiveness to antiepileptic drug treatment has been described in affected children, but not
adults, in the Japanese Ser252Leu family. The best response to carbamazepine was seen in patient L, who had prominent temporal lobe spikes (Figure 4).

The findings of temporal lobe epileptiform discharges, although not a typical feature of ADNFLE, have been reported in several European families with no known genetic mutations, as described by Picard et al. The interictal spikes recorded in 2 of our patients, although maximum in the right anterior temporal area, extend to the frontocentral regions. Therefore, further studies using additional scalp electrodes would be needed to determine the extent to which epileptiform activity in ADNFLE can occur beyond the frontal lobes.

The pattern of hypometabolism on FDG-PET is consistent with the patients' electroencephalographic and clinical picture. One previous study reported left frontopolar seizures with prominent temporal lobe spikes (Figure 4).

Further clinical and genetic studies are needed to extricate the pattern of phenotypic features found in ADNFLE families. This study was supported in part by grants Ste769/2-1 and SFB/TR3-A5 from the Deutsche Forschungsgemeinschaft, Bonn, Germany (Dr Steinlein).

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