The gene for a recessively inherited human childhood progressive epilepsy with mental retardation maps to the distal short arm of chromosome 8

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ABSTRACT A recently delineated childhood epilepsy has hitherto been observed only in a small geographic region in northern Finland, where, with the exception of one, both parents of all of the 11 siblings with affected individuals descend from one or two founding couples. The disease is characterized by generalized tonic-clonic seizures with onset at 5–10 years and progressive, severe mental retardation with onset 2–5 years after the first seizures. In this study the gene locus is assigned to the telomeric region of chromosome 8p by linkage. Analyses of recombinations place the locus in the 7-centimorgan interval between AFM185c2 and D8S262 in which three markers, D8S504, D8S264, and AFM077yg5, show no recombinations with the phenotype. Haplotypes comprising alleles at the above five loci support the hypothesis of a single founding mutation for all affected chromosomes except the one belonging to the unrelated parent, who has a very different haplotype, suggesting another mutation or a very old ancestry of a single mutation. This study raises to three the number of heritable epilepsies whose gene loci have been mapped and provides a starting point for the cloning of the gene. It also suggests the possibility that the disease might not be limited to the northern Finnish population.

Epilepsy occurs in ≈3% of the population (1), either as an isolated symptom or as a part of disease phenotypes with a Mendelian or non-Mendelian inheritance (2). Despite intensive worldwide efforts to elucidate the etiology and pathogenesis of idiopathic epilepsy, progress has been limited and slow.

One way of approaching the pathology of epilepsy is to focus on well-delineated disease entities in which epilepsy is the main or primary, as opposed to a minor or secondary, feature of the phenotype. Several such syndromes exist, many of which show undiscutable heredity (3). It is potentially important to identify the genes whose mutations cause these disorders. However, to date, the genes of only two epilepsies with clear-cut Mendelian inheritance have been mapped—namely, two loci for benign familial neonatal convulsions (4, 5) and one for progressive myoclonic epilepsy of Unverricht–Lundborg type (6)—but none of these genes has been cloned. A third epilepsy, juvenile myoclonus epilepsy, is probably clinically and genetically heterogeneous, as shown by inconsistent and contradictory linkage results (7–9).

As a recently delineated childhood epilepsy phenotype (progressive epilepsy with mental retardation, EPMR; also called Northern epilepsy) (10) had indicated recessive inheritance in the Finnish population, we decided to approach its

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gene by first attempting its mapping by linkage. Here the assignment of the gene to a small interval in the telomeric region of chromosome 8p is reported, confirming the recessive mode of inheritance and the accuracy of the clinical diagnostic criteria. Moreover, haplotype analysis raises the possibility that two different mutations are present in the small northern Finnish population, where all patients diagnosed so far originate. This should prompt investigations to clarify whether the disease might also occur elsewhere.

MATERIALS AND METHODS

Disease Phenotype. So far there is only one published account of the disease, focusing mainly on the genealogic aspects (10). A brief summary of the clinical features follows. The patients are normal at birth and develop normally up to school age. The onset is at the age of 5–10 years (mean, 6.7 years) with generalized tonic-clonic seizures. These increase in frequency reaching a maximum of approximately one or two seizures per week at puberty. After puberty the frequency of seizures begins to decline unrelated to changes in medication. In early adulthood the frequency typically is between 6 and 25 epilepsy attacks per year, and after 35 years of age many patients are virtually seizure-free. In electroencephalogram (EEG), focal and nonfocal initiation of paroxysms is seen. Serial EEG recordings show progressive slowing of the background activity, impaired reactivity to eye opening, and a disappearance of specific sleep patterns. In adulthood the background activity is theta to slow alpha with markedly reduced delta activity as compared to the findings at puberty. Constant spike foci are not seen. Photic stimulation does not cause discharges or seizures. Of several antiepileptic drugs tried so far, clonazepam appears to be the most beneficial one.

The mental development of most patients is normal until 2–5 years after the onset of seizures. A rapid mental decline occurs during the period when seizures are most frequent, resulting, for example, in failure to complete compulsory education. Of 19 presently living patients, 4 are borderline, 4 mildly, 5 moderately, 5 severely, and 1 profoundly mentally retarded. Two patients are cared for in institutions and many of them need assistance in daily routines. The oldest living patient is 57 years of age.

Patients and Samples. Nineteen presently living patients were included in the study. All were diagnosed by one of us (A.H.) according to the clinical criteria described above. The patients belonged to 11 kindreds; the pedigrees have been published (10). In 9 kindreds all parents but one descended from a genealogically verified couple 12 generations ago, who

Abbreviations: EPMR, progressive epilepsy with mental retardation; cM, centimorgan(s); lod score, logarithm of odds score. To whom reprint requests should be addressed.

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in the early 17th Century lived in the same region where most of the patients live today. One of the remaining 2 kindreds has a genealogic link to that same couple. Moreover, these 2 kindreds could be traced to another couple born in 1758 and 1762 six generations ago. They had lived in a village located only 30 km from that of the earlier founding ancestral couple but could not be genealogically linked to them. Though with one exception all parents of affected individuals were thus related, close consanguinity (closer than third cousins) was not the rule.

Thirty milliliters of venous blood was collected from each consenting individual to extract DNA and to establish a lymphoblastoid cell line for future use.

**Strategies in the Search for Linkage.** This study was part of a project designed to map three disease genes simultaneously as efficiently as possible. In the case of EPMR, a panel consisting of 12 affected individuals, 6 parents, and 2 healthy sibs of 4 multiplex kindreds was designed by linkage simulation studies to be used in the primary screening. The total number of samples (61 plus one control) from the three diseases were amplified in the same microtiter plates and resolved on single gels having 62 lanes.

**Markers.** All markers were (CA)x-repeat polymorphisms from the Genethon collection (11, 12). For the initial screening a set of markers located ≈40 centimorgans (cM) apart was utilized, followed by another set filling each 40-cM gap with one to three markers. Whenever possible, three to five, carefully chosen markers were co-amplified in a 10-μl reaction volume using published protocols (13).

**Linkage and Linkage Disequilibrium Analysis.** Linkage analyses were performed by computer programs of the LINKAGE program package (14). All results were obtained assuming complete penetrance. The simulation program SLINK (15, 16) was used to define a minimum number of studied individuals in the initial screening. For multipoint analysis marker genotypes were reduced to three-allele loci. The analysis (LINKMAP) based on the given fixed order telomere-\(AFM185xb2\-D8S504-D8S264-AFMO77yg5-D8S262\-centromere and fixed sex-average recombination fractions (0.01, 0.01, 0.03, 0.02 cM) between the five marker loci (12). Based on the analysis of three telomeric loci, \(D8S277\), \(D8S201\), and \(D8S17\), from the Centre d'Etude du Polymorphisme Humain (Paris) data base version 6.0 (17), a constant female/male sex recombination ratio 0.278 was used in the analysis. Because the allele frequencies in a small and isolated population may not coincide with the overall Caucasian frequencies, we approximated the allele frequencies from the study population. Multipoint logarithm of odds scores (lod scores) were calculated as \(\log_{10}(L(x)/L(\infty))\), where \(L(x)\) stands for the likelihood of the disease locus at a given map location \(x\), and \(L(\infty)\) stands for the likelihood of the disease locus being unlinked to the map. To test whether the allele and haplotype frequencies differed significantly between unaffected and affected persons we used Fisher's exact test.

**RESULTS**

After testing 153 polymorphic DNA markers, linkage was observed with marker \(D8S264\). Thereafter the panel was enlarged by six affected persons and all available parents and unaffected sibs to comprise 50 individuals. The observed linkage was confirmed with four additional markers. The results of the pairwise analysis are shown in Table 1. No recombinations were observed between \(EPMR\) and markers \(D8S504\, D8S264\, and \(AFMO77yg5\), with maximum two-point lod scores (\(Z_{\text{max}}\)) of 1.01, 4.45, and 1.95, respectively. The closest flanking markers that showed recombination were \(D8S262\) on the centromeric and \(AFM185xb2\) on the telomeric side.

The results of a six-point linkage analysis are shown in Fig. 1. The maximum multipoint lod score was 7.03 at 2.7 cM distal to marker \(AFMO77yg5\) and 1.8 cM proximal to \(D8S264\). Linkage disequilibrium was readily apparent to alleles of markers \(AFM185xb2\ (P < 0.001), D8S264\ (P < 0.01), and \(D8S262\ (P < 0.01\) (Table 2). Markers \(D8S504\) and \(AFMO77yg5\) showed the same allele in the majority of the disease chromosomes, but as that allele was also the most common one in the normal chromosomes a possible linkage disequilibrium was not statistically significant. Haplotypes around the disease locus were constructed using the allele data of all five markers studied (Table 3). Of 16 disease-associated haplotypes, 13 were identical and none occurred on any of the 18 normal chromosomes studied. Two disease haplotypes deviated only little from the main one. However, one was very different and, interestingly, this haplotype belonged to the

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**Table 1.** Pairwise lod scores between \(EPMR\) and five marker loci

<table>
<thead>
<tr>
<th>Locus</th>
<th>0.00</th>
<th>0.01</th>
<th>0.05</th>
<th>0.10</th>
<th>0.20</th>
<th>0.30</th>
<th>0.40</th>
<th>(Z_{\text{max}})</th>
<th>(\theta_{\text{max}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(AFM185xb2)</td>
<td>-(\infty)</td>
<td>6.10</td>
<td>6.90</td>
<td>6.73</td>
<td>5.92</td>
<td>3.99</td>
<td>2.07</td>
<td>0.58</td>
<td>6.99</td>
</tr>
<tr>
<td>(D8S504)</td>
<td>1.01</td>
<td>1.01</td>
<td>0.98</td>
<td>0.88</td>
<td>0.74</td>
<td>0.47</td>
<td>0.23</td>
<td>0.06</td>
<td>1.01</td>
</tr>
<tr>
<td>(D8S264)</td>
<td>4.45</td>
<td>4.44</td>
<td>4.35</td>
<td>3.93</td>
<td>3.39</td>
<td>2.25</td>
<td>1.15</td>
<td>0.31</td>
<td>4.45</td>
</tr>
<tr>
<td>(AFMO77yg5)</td>
<td>1.95</td>
<td>1.95</td>
<td>1.91</td>
<td>1.72</td>
<td>1.48</td>
<td>1.01</td>
<td>0.56</td>
<td>0.17</td>
<td>1.95</td>
</tr>
<tr>
<td>(D8S262)</td>
<td>-(\infty)</td>
<td>1.93</td>
<td>2.83</td>
<td>3.11</td>
<td>2.86</td>
<td>2.04</td>
<td>1.11</td>
<td>0.33</td>
<td>3.12</td>
</tr>
</tbody>
</table>

*The 90% confidence limits are based on the ±1 lod unit method.

**FIG. 1.** Six-point linkage analysis in \(EPMR\) pedigrees with regard to a fixed order of five marker loci on chromosome 8. A male map is represented assuming Kosambi interference function computed by the LINKMAP program. Marker \(AFM185xb2\) was chosen as the starting point (zero). The telomere is to the left.
only parent who could not be genealogically connected with any of the two founding couples.

**DISCUSSION**

Our results show that homozygosity for a mutated gene located in chromosome 8p causes the clinical phenotype typical of EPMR in Finland. We had previously excluded the EPM1 locus for progressive myoclonus epilepsy in chromosome 21q (10) as well as the two loci for benign neonatal convulsions in chromosomes 20q and 8q (4, 5) as candidate loci for EPMR. Our data confirm the recessive type of inheritance of EPMR suggested by previous pedigree studies (10). The patients chosen for the study were clinically homogeneous with little intra- and interfamilial variation. As they also shared one or two ancestral couples, the absence of linkage heterogeneity and the finding of marked linkage disequilibrium were not surprising. Our findings amply confirm the validity of the established diagnostic criteria (10) and the specific identity of this epilepsy syndrome.

By constructing haplotypes of loci closely linked to the disease locus the presumptive ancestral haplotype can be deduced. Individuals in whom a disease chromosome shows deviations from this haplotype may provide information comparable to recombinations that one can observe in larger study populations. In our series of 16 disease chromosomes, 13 had the ancestral haplotype 2-1-3-2-4 that did not occur in any of the 18 normal chromosomes studied. Two other haplotypes, 2-4-3-2-4 and 4-1-3-1-4, differed somewhat from the ancestral haplotype. If the changes represent ancestral crossovers rather than new mutations of the marker loci, then the region proximal of the fourth marker and distal of the second marker can be excluded as the site of the mutation. This would limit the region of EPMR to between D8S504 and AFM077yg5, and EPMR would thus lie very close to locus D8S264, the only locus having the same allele in all disease chromosomes except the one discussed below.

Interestingly, one disease chromosome had the haplotype 4-4-11-2-1 that is very different from the ancestral one and that was also not seen in any of the normal control chromosomes. As this chromosome occurred in the only parent who could not be genealogically linked to the founding ancestral couples it lends itself to two alternative explanations. First, the different haplotype might indicate a mutation different from the main one. The existence of more than one mutation in an isolated population from a limited geographical area might be somewhat unexpected. However, another autosomal recessive disorder, nonketotic hyperglycinemia, which occurs with a high incidence in northern Finland, has been shown to be caused by at least two mutations (18). Moreover, in the case of choreoaridemia, a rare X chromosomal eye disorder, very surprisingly, three different mutations occur in the same limited northern Finnish population (19, 20). However, an alternative second explanation of the one very different disease-associated haplotype is that it is genealogically far removed from the others—in other words, that the ancestral mutation might be much older than the 12 generations indicated by the main pedigree. In either case we conclude that the mutation causing this epilepsy syndrome might be more widespread than one might assume from it having been detected exclusively in this isolated population. We hope that publication of the present findings and a previous paper (10) on this disorder will prompt other investigators to search for a similar phenotype among their patients.

Marker loci D8S264 and D8S262 are anchored through locus D8S201 to the region 8pter-8p22 (21, 22). Moreover, marker AFM185xb2 that is closely linked to EPMR is the most distal Genethon marker so far mapped on 8p. Thus the EPMR locus can be assigned to the telomorphic region of the short arm of chromosome 8. One way of approaching the EPMR gene is to identify genes that map to the region and that are compatible with having a pathophysiological role in epilepsy. Several genes localized in this chromosomal area are already known. We point out cathepsin B, a potential Alzheimer disease candidate gene (23), that has been localized physically to 8p23.1-p22 (24). Another interesting gene is the α2 subunit of neuronal nicotinic cholinergic receptor that has been localized to chromosome 8, but whose precise localization is not known (25). The role of these two and other potential candidate genes can be tested in our patients. If this approach is not successful in defining the EPMR gene, positional cloning is now easier than before thanks to improved tools such as yeast artificial chromosomes that have already been defined for this region (26).

Our results provide a basis for one immediate application: presymptomatic or prenatal testing can now be offered to Finnish families with at least one child affected with EPMR. Given the existence of several highly polymorphic markers showing no recombinations in Finnish families, these predictions should be highly accurate.

As a conclusion, EPMR is a progressive inherited disorder affecting the central nervous system and caused by a gene in distal 8p, whose pathogenesis is still unknown. It is one of a few conditions that are rare, show a Mendelian inheritance pattern, have epilepsy as their main manifestation, and only affect the central nervous system. In a longer perspective, the characterization of such genes may help in understanding the pathophysiology of epilepsy in general.

We thank Sinikka Lindh for collecting the samples, Matti Eerola for laboratory work, and the patients and their families for excellent

### Table 2. Distribution of alleles in normal/disease chromosomes at the five marker loci

<table>
<thead>
<tr>
<th>Locus</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFM185xb2</td>
<td>1/0</td>
<td>2/14</td>
<td>1/0</td>
<td>8/2</td>
<td>3/0</td>
<td>2/0</td>
<td>1/0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D8S504</td>
<td>13/14</td>
<td></td>
<td>1/0</td>
<td>1/2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D8S264</td>
<td>2/0</td>
<td>7/16</td>
<td>1/0</td>
<td></td>
<td>1/0</td>
<td>1/0</td>
<td>3/0</td>
<td>2/0</td>
<td>0/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFM077yg5</td>
<td>2/1</td>
<td>11/15</td>
<td>4/0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D8S262</td>
<td>6/1</td>
<td></td>
<td>1/0</td>
<td>9/15</td>
<td>1/0</td>
<td>1/0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tests for allelic association: *, P < 0.01; †, P < 0.001.

### Table 3. Haplotypes associated with EPMR and normal chromosomes

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>AFM185xb2-D8S504-D8S264-AFM077yg5-D8S262</th>
<th>Chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>%</td>
</tr>
<tr>
<td>2-1-3-2-4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-4-3-2-4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4-1-3-1-4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4-4-11-2-1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>100</td>
</tr>
</tbody>
</table>
cooperation. This study was supported by the Academy of Finland and the Paulo Foundation. Part of the study was done at the Folkhälsoan Institute of Genetics.