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RESEARCH

Genetic and Physical Mapping of the Progressive Epilepsy with Mental Retardation (EPMR) Locus on Chromosome 8p

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Progressive epilepsy with mental retardation (EPMR) is an autosomal recessive disorder discovered recently from an isolated region in Finland. The disorder is characterized by normal early development, generalized tonic-clonic seizures with onset at 5–10 years of age, and progressive mental retardation beginning 2–5 years after the onset of seizures. We recently mapped the *EPMR* locus to a 7-cM region on chromosome 8p between markers AFM185xb2 and D8S262. A recombination detected with a new microsatellite marker AFMa054td9 narrows the region to 4 cM. A yeast artificial chromosome (YAC) contig containing 22 YACs was constructed across the disease gene region. The YAC contig is characterized by a collection of 19 YAC-end sequence-tag sites together with seven microsatellite markers. The entire YAC contig spans a minimum of 3 Mb. Moreover, the distal end of the contig contains a subtelomeric YAC yRM2205 that anchors the contig to the telomere. Construction of a YAC contig across the disease gene region is an essential step toward the isolation of the *EPMR* gene.

Epilepsy is a common symptom that occurs in ~2%–3% of the population either as an isolated phenomenon or as a cosymptom of a large and diverse set of disease phenotypes (Scheuer and Pedley 1990; Hauser et al. 1993). The most common forms of inherited epilepsies do not display straightforward Mendelian inheritance patterns and are considered to be multifactorial (Gardiner 1990). Among the multifactorial forms, only the locus for juvenile myoclonic epilepsy has been genetically mapped; a subset of these families appear to be linked to DNA markers on chromosome 6p (Greenberg et al. 1988; Durner et al. 1991; Weissbecker et al. 1991; Delgado-Escueta et al. 1994). In contrast, monogenic inherited epilepsies are rare, but already six loci have been genetically mapped to specific chromosomal regions: two loci for benign familial neonatal convulsions (BFNC) on chromosomes 20q (*EBN1*; Leppert et al. 1989) and 8q (*EBN2*; Lewis et al. 1993); the *EPM1* locus for progressive myoclonus epilepsy of Unverricht–Lundborg type on chromosome 21q (Lehesjoki et al. 1991); an *EPMR* locus for progressive epilepsy with mental retardation locus on 8p (Tahvanainen et al. 1994); a partial epilepsy locus on 10q (Ottman et al. 1995); and an autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) locus on 20q13.2 (Phillips et al. 1995). Recently, a missense mutation in the α4 subunit of the neuronal nicotinic acetylcholine receptor (*CHRNA4*) gene on chromosome 20q was reported to be associated with ADNFLE in a large Australian pedigree (Steinlein et al. 1995). It now appears that a putative mutation in the *CHRNA4* gene detected in one BFNC family (Beck et al. 1994) may have been an artifact resulting from a DNA sequencing error (Steinlein et al. 1995).

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Progressive epilepsy with mental retardation (EPMR), also called Northern epilepsy, has, so far, been found only in a small rural region in northern Finland. To date, 25 patients have been identified. Genealogical records show that, with one exception, all parents of affected children are distant relatives. Children descending from the unrelated parent have a haplotype distinct from the "common" haplotype shared by 15 of 19 disease chromosomes. This suggests two different mutations segregating in this Finnish pedigree and may indicate that the disorder is not necessarily as rare as originally anticipated.

The main clinical features of EPMR are normal early development, childhood-onset epilepsy, and mental retardation that starts to develop 2–5 years after the first seizures. All patients have generalized tonic-clonic seizures. One-third of the patients also have complex partial seizures during childhood. Epileptic activity decreases during young adulthood, but complete remission does not occur. Mental retardation is most rapid prior to adulthood, during the period with most frequent seizures. Of the antiepileptic drugs used, only clonazepam has proven effective; it reduces dramatically the number of seizures and can even normalize the EEG pattern when administered early in the disease process (Hirvasniemi et al. 1994, 1995).

To facilitate the isolation and identification of the *EPMR* gene, we sought to physically map and clone the ~7-cM region encompassing the disease locus. Several microsatellite markers that always segregate with the disease locus were chosen along with markers flanking the *EPMR* region to screen physical mapping data bases and yeast artificial chromosome (YAC) libraries. A total of 22 YAC clones were identified that span the region between the flanking markers and that are anchored to genomic DNA by seven polymorphic DNA markers. A new microsatellite marker (AFMa054td9), recently positioned on the genetic map by scientists at Genethon, maps to the YAC contig and detects a meiotic recombination in one EPMR family, thereby reducing the minimal genetic region to ~4 cM.

RESULTS

Genetic Linkage Analysis

The *EPMR* locus has been mapped previously to an approximate 7-cM region defined by the flanking telomeric and centromeric markers, *AFM185xb2* and *D8S262*, respectively (Tahvanainen et al. 1994). We have genotyped a recently discovered microsatellite marker, AFMa054td9, in the EPMR extended pedigree and show that this marker recombines with the disease locus in a single healthy individual who has three affected siblings (Fig. 1). This recombination narrows the *EPMR* disease gene region to about 4 cM. Because markers *D8S504* and *D8S264* are uninformative in this branch of the family, the recombination may occur telomeric to *AFMa054td9*, leaving the possibility that additional markers will help to narrow the region still further. The maximum two-point logarithm of odds (lod) score for *AFMa054td9* is 5.28 ($\theta = 0.03$) in the extended pedigree. The results of the multipoint linkage analysis are shown in Figure 2. The maximum lod score is 10.70 at a position 0.4 cM distal to *D8S264* and 0.6 cM proximal to D8S504. The one-unit lod support interval extends from a point 0.4 cM distal to *AFMa054td9* to a point 0.2 cM proximal to *AFM185xb2* and covers a total genetic distance of 3.4 cM.

It has been hypothesized that cathepsin B may play a role in Alzheimer's disease (Tagawa et al. 1991). Because this gene has been mapped physically to 8p22–p23.1 (Fong et al. 1992), we considered it a candidate gene for EPMR. To exclude cathepsin B as a potential *EPMR* candidate gene, we analyzed a restriction fragment length polymorphism (RFLP) detected by a cathepsin B intron probe in one of the Finnish families. Several enzymes (*Eco*RI, *Pvu*II, *Rsa*I, *Stu*I, and *Taq*I) detected a novel polymorphism in intron 8 (see Methods for details). The *Taq*I polymorphism revealed recombinations between the disease locus and cathepsin B allowing us to exclude this gene as a candidate for EPMR (data not shown).

Physical Mapping of the *EPMR* Locus

Library Screening

Fifteen of the YAC clones shown in Figure 2 were ascertained from computerized data bases accessed through the World-Wide Web (see Methods). These are clones characterized as "positive" for markers in the *EPMR* region or clones that appear to overlap with such clones. To fill a gap between markers *D8S264* and *D8S504*, we screened the Centre d'Etude du Polymorphisme Humain (CEPH) Midi YAC library with both YAC ends from 858E12 (Fig. 3) but found no positive clones. We next screened with D8S264 and the right YAC-end STS from 241d10 and obtained three new YAC clones in both screenings. Probe

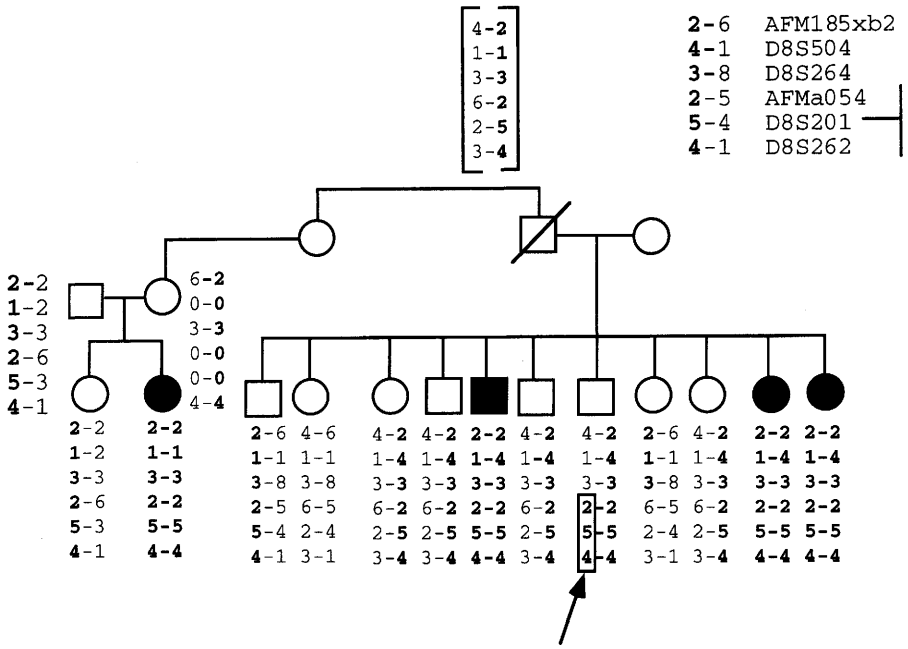

Figure 1 Refinement of the *EPMR* locus by meiotic breakpoint mapping. DNA marker genotypes across the *EPMR* locus in an affected family reveal a key recombination event that reduces the *EPMR* critical region to 4.0 cM. The recombination occurs in the paternal chromosome of a healthy sibling and is marked by an arrow. "Disease homologs" are in boldface type. The boxed region marks the site of chromosomal recombination. (0) Untyped alleles. The inferred haplotype of the deceased father is shown in brackets.

D8S201 was screened at CEPH against the Mega YAC library (Albertsen et al. 1990; Dausset et al. 1992). Several positive clones were identified (see next sections).

Characterization of YAC-end Fragments

Ligation-mediated PCR yielded YAC-end DNA sequences in 32 of 40 cases where it was attempted. Because some of these sequences were Alu repeat or nonchromosome 8 sequences, characterization of these YAC ends yielded a total of 17 chromosome 8 STSs. We were not able to generate a PCR product for the YAC ends of clone 810F8 with the ligation-mediated PCR but were successful with the vectorette PCR method (see Methods).

Construction and Characterization of the YAC Contig

Three of the critical microsatellite markers (*D8S504*, *D8S264*, and *D8S262*) from the *EPMR* region were queried in the CEPH data base for homology to known YAC clones. Preliminary characterization of 12 positive addresses yielded a total of seven YAC clones (796G6, 810F8, 858E12, 931B2, 679D3, 787C11, and 930A2) that, as shown here, unambiguously map to the disease gene region and contribute to the genomic coverage of the region. Marker *D8S201* identified several clones from the Mega YAC library, four of which (787C11, 797C11, 808E6, and 930A2) were also positive for DNA markers *D8S262*, *AFM077yg5*, and *AFMa054td9*. These four markers span an estimated 3 cM. One of the YAC clones identified by this screening (808E6) overlaps with a *D8S264* positive clone (931b12), thus closing the gap between markers *D8S264* and the centromeric YACs. Then, YAC-end STSs for three YAC clones (931B2, 858E12, and 796G6) were used to PCR amplify YAC clones obtained from the CEPH data base. Alu-PCR fingerprinting by scientists at CEPH indicated that these clones were members of the same contig with YAC clones known to be positive for markers *D8S264* and *D8S504*. Two YAC clones (972C9 and 926F11) could be unambiguously mapped to the contig by this procedure and one more, 781D11, was later shown positive for YAC-end STSs 463B10R and 679D3L. To fill the gap between markers *D8S264* and *D8S504*, we screened the CEPH Midi YAC library (Albertsen et al. 1990; Green and Olson 1990) with DNA marker D8S264 and identified YAC clones 463B10, 241D10, and 42F7. We then screened the library with YAC-end 241D10R and identified clones 225G1, 374E8, and 485C11. None of these clones extended to *D8S504* and thus did not fill the gap between D8S264 and D8S504. Then, we queried the data base for contigs using YAC 858E12 that contains locus D8S504 and found two, WC-148 and WC-1335 (Center for Genome Research at Whitehead Institute for Biomedical Research, Cambridge, MA). From these contigs we identified three additional overlapping clones, 808B12, 795E11, and 772C1.

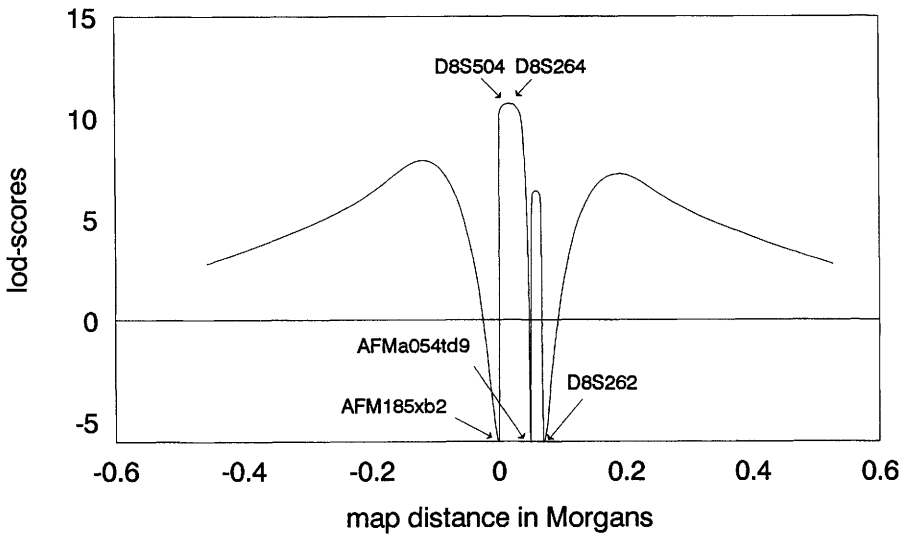

Figure 2 Six-point analysis in EPMR pedigrees assuming a fixed order of five marker loci on chromosome 8 as described in Methods (Linkage). The telomere is to the *left*.

Three unique YAC-end clones (42F7L, 463B10L, and 808B12R) identify similar sets of overlapping YAC clones. Two lines of evidence indicate that these end clones encode low-copy repeat DNA sequences (indicated by "X" in Fig. 3). First, over half of all the CEPH Midi YAC library pools were positive when PCR-amplified with primers specific for 463B10L and 808B12R. Second, Southern blot analysis of *Taq*I-digested YACs from the contig revealed multiple homologous restriction fragments for the radiolabeled PCR products of 42F7L and 808B12R. Also, hybridization of digested total genomic DNA with radiolabeled PCR product of 42F7L resulted in a smear instead of discrete bands. Unlike YAC clones 42F7 and 808B12, clone 463B10 does not contain the end clones 485C11R and 374E8R, suggesting that it does not extend as far in the centromeric direction as the overlapping clones.

The *Eco*RI cloning site of the YAC yRM2205 maps within 350–450 kb of the 8p terminus by RecA-assisted restriction endonuclease cleavage mapping (H. Riethman, pers. comm.). The placement of our most distal marker, *AFM185xb2*, in this YAC clone thereby positions the contig at the very distal region of the short arm of chromosome 8. Somewhat surprisingly, YAC-end STS 772c1L appears to map telomeric to yRM2205 (Fig. 3). 772c1L does map to chromosome 8 because it amplifies chromosome 8-only cell hybrid DNA, but it does not amplify other YACs in the contig. It seems most likely, therefore, that this STS maps either telomeric to YAC yRM22055, or it maps to a deleted portion of the subtelomeric YAC clone. In Figure 3 we have based the degree of overlap between YAC clones strictly on the number of STSs shared between YACs, and thus the size of the overlaps are not representative of the actual size of the homologous regions. We estimate that the minimum distance between loci AFM185xb2 and AFMa054td9 is the sum of the sizes of the nonoverlapping, nonchimeric YACs 810F8 and 931B2, which is 2.1 Mb. The minimum distance spanned by the entire contig is 3 Mb. Genetic mapping indicates that the relative order of Genethon markers (telomeric to centromeric) is *AFMa054td9, AFM077yg5,* and *D8S262*. Because *D8S201* cosegregates with these three markers in the EPMR pedigree, we are unable to position it within the cluster.

DISCUSSION

The epilepsies are characterized by recurrent convulsive and nonconvulsive seizures resulting from partial or generalized epileptogenic discharges in the cerebrum (Delgado-Escueta et al. 1983). Clinically and etiologically, the epilepsies are a heterogeneous group of diseases. Etiological causes range from predominantly environmental as in the case of posttraumatic epilepsies to predominantly genetic as exemplified by EPMR. In humans there are >140 Mendelian traits (including disorders of amino acids, enzymes, hormones, and vasculature) that include epilepsy as part of a more complex phenotype. These patients, however, account only for a small fraction of all epilepsy patients. Most cases of heritable epilepsy are believed to be multifactorial (Gardiner 1990). Patients with EPMR are distinguished from the majority of epilepsy cases in that they suffer mental deterioration following the onset of seizures. The degree of mental deterioration correlates with the severity of cerebral atrophy. The cause of epilepsy and the generalized atrophic process in the brain are unknown.

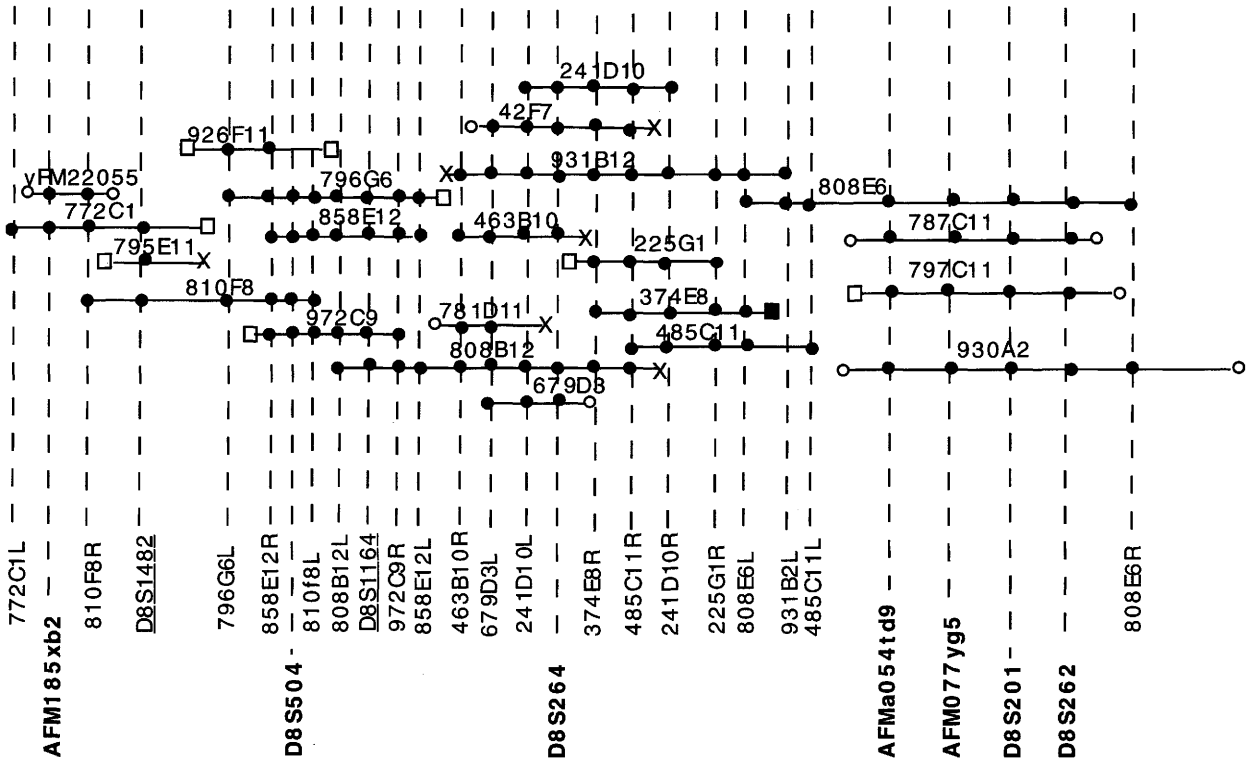

Figure 3 YAC contig of the *EPMR* region. The contig spans the region between the telomeric marker *AFM185xb2* and the centromeric locus *D8S262*. Microsatellite markers are shown in boldface type. The STSs developed in this study are shown in plain type. STSs derived from computerized data bases (see Methods) are underlined. (●) An STS within a YAC; (□) chimeric YAC ends, with the exception of one (■) that maps to chromosome 8 by PCR analysis but that maps outside the *EPMR* region. (X) Repetitive YAC ends. (○) Sequence information is not available for these YAC ends. The order of microsatellite markers is determined by genetic and physical mapping.

It is not known whether or to what extent the frequency of seizures or the dose and duration of medication are correlated with cerebral atrophy. In any event, the association between cerebral atrophy and the duration of epilepsy or mental deterioration distinguishes EPMR patients from those with general chronic epilepsy (Hirvasniemi and Karumo 1994).

Recently, a missense mutation in the α4 subunit of the neuronal nicotinic acetylcholine receptor was reported to be associated with the chromosome 20q form of ADNFLE (Steinlein et al. 1995). It is relevant in this regard that neither of the two nicotinic acetylcholine receptor subunits recently mapped to chromosome 8 (Koyama et al. 1994; Spurr et al. 1995) are located near the *EPMR* locus. We considered the cathepsin B gene to be a reasonable candidate for EPMR based on its physical location and function. Tagawa et al. showed in 1991 that cathepsin B is an intracellular protease with amyloid precursor protein (APP) secretase activity. Because APP secretase cleaves the amyloid β peptide found in senile plaques of Alzheimer patients, we reasoned that a mutant cathepsin B might conceivably lead to neuronal dysfunction in EPMR patients. This possibility was, however, excluded by the identification of recombinations between a cathepsin B RFLP and the *EPMR* locus. One consequence of the construction of a YAC contig across the *EPMR* disease locus is that we can now eliminate as candidates any genes that fail to map to YACs in this 3-Mb region. For example, we have already eliminated the squalene synthetase gene that maps to 8p22–p23.1 and is involved in cholesterol synthesis (Shechter et al. 1994) as a possible candidate gene.

We describe the physical mapping and cloning of the minimal genetic region encompassing the *EPMR* locus. A key recombination event has been identified that reduces the minimal genetic region encompassing the disease locus to 4 cM. Haplotype analysis indicates that a single founder effect mutation accounts for 18 of 21

Table 1. YAC-end STSs Listed by the YAC Address

STS NAME	PRIMERS 5'-3'
225G1R	1 ATT CCT TTG CCC TGT ACA CG 2 GTG CAG ATA AAC CAG TGT CTG C
241D10L	1 GGG TAA GGA AAG ATG CCA CA 2 AAA CAG GAC GGA CTC CAG G
241D10R	1 ATT CCG CTT TGG TTT CCC 2 CCA ACA CAT TCC CAG TGC TA
374E8R	1 ATT CGC TCT GTA CTG AAA GGT G 2 TCA CAG GCC CCA ATT CTC
463B10R	1 CAC TTC CCA GCA GAC ATT CC 2 TAT GAG CAA GCC TGG AGT CC
485C11L	1 AAA GGA TGC TGG GCC TTA TT 2 CCA GGT CCC TGG TGT TTT AA
485C11R	1 TTC AGT TGC TTC TGT ATT TCC A 2 TGG CTG CAT CAG TAG CTC TG
679D3L	1 CTT TGA CTT CCC GTA CTG GG 2 CTG GAA TTT GTA TTT AGG GCA
772C1L	1 TCT CAC CTG ACC CAT GAT GA 2 TTT TAC TCA GCC TGG CGT G
796G6L	1 TTT CAC TCT CTC TGT TTT C 2 TGT TCG ATC ACA TAG CTC
808B12L	1 CAC AGG TGT GTA GAG CTC AGA A 2 CTC CCA GAG ACA GAA CCT GG
808E6L	1 TCA TCT CGG TCC TTC TCA GA 2 CAT TTT GCT ATT CCT TTT AGC C
808E6R	1 TTC GTC GAT TTT CCT GCT CT 2 AGA AAT GGG GGA AAG GAG AA
810F8L	1 AGA AAC TTC CGA GTC CAG TCC 2 TTT CTG TGA GTT CCC GAG CT
810F8R	1 TGA GGT GCT TCT GTT TGT GG 2 GAA TTC TGG GAA GGT GGT AGC
858E12L	1 ATG AAA CGC AGC GGA AAC 2 GGC AGC AAT TCC ATC AAG
858E12R	1 CCA GTC TAC ACA ATC AGA AAA C 2 ACC TGT GTC GCT TAG ATG
931B2L	1 ACT TGA AGT TCC GAT GCC C 2 TTC TGC TGA GTG GGA GCC
972C9R	1 AGG GTC AGT TTC TGG AGA TTT G 2 TGG GAA GGG AAA GAA TTG G

(R) Right and (L) left arm of the vector. Primer sequences are shown.

affected individuals within the extended EPMR pedigree, excepting only the 3 affected offspring-fected offspring of a married-in father who clearly harbors a disease chromosome with a distinct haplotype (Tahvanainen et al. 1994). Among the DNA markers in the *EPMR* region, only marker D8S264 is homozygous (allele 3) in the 18 affected individuals who contain identical (15 of 18) or very similar (3 of 18) haplotype and heterozygous for allele 3 in the remaining 3 affected individuals. D8S264 allele 3 occurs with a frequency of ~20% in the general population. Given the strong evidence for a founder effect in this large pedigree, we expect that this marker detects a region of homozygosity across the disease locus and that further delineation of this region will expedite the disease gene search. On the basis of the marked homozygosity of *D8S264*, we consider the 3-cM chromosomal region bounded by *D8S504* and *AFMa054td9* to be the most likely site for the *EPMR* gene. The YAC clones spanning this region can now be used as DNA sources for gene isolation and as probes to identify shorter genomic (P1, BAC, or cosmid) clones or cDNA clones. Our immediate goals are to identify physically mapped, polymorphic markers (Petrukhin et al. 1993) that span the minimal genetic region. These markers will be used to define the minimal region of marker homozygosity across the disease locus. We are hopeful that this strategy will continue to narrow the disease locus to a sufficiently small genomic region that gene-coding segments can be systematically identified and characterized as potential disease genes. We have begun to identify cDNA sequences that map to the minimal genetic region using immobilized YAC DNA and selective hybridization conditions as described previously (Bonaldo et al. 1994; Soares et al. 1994)

METHODS

Linkage

Two-point and multipoint analyses were carried out using MLINK and LINKMAP, respectively, of the FASTLINK computer program package (Cottingham et al. 1993; Schäffer et al. 1994). To reduce the computation time required for

Table 2. Characteristics of YAC Clones in the EPMR Contig

YAC address	YAC size[a]	Right end[b]	Left end[b]
225G1	280	+	Chi
241D10	470	+	+
374E8	360	+	Chi
42F7	350	N.D.	Rep
463B10	270	+	Rep
485C11	360	+	+
679D3	740*, 360/280	N.D.	+
772C1	1770*, 1300	Chi	+
781D11	580*, 650	N.D.	Rep
787C11	1740*, 1600	N.D.	N.D.
795E11	1800	Rep	Chi
796G6	1280*, 950	Chi	+
797C11	1670*, 1250/950	Chi	N.D.
808B12	1230*, 900	Rep	+
808E6	1780*, 1200	+	+
810F8	1180*, 1000	+	+
858E12	810*, 800	+	+
926F11	950	Chi	Chi
930A2	1480*	N.D	N.D.
931B2	1230*, 1100	Rep	+
972C9	850	+	Chi
yRM2205	250*	N.D.	N.D.

[a]YAC sizes were determined in our laboratory, except those marked by an asterisk (*), which were derived from computer data bases (see Methods). In cases where the YAC contained more than one insert, the sizes of each insert are separated by a / symbol.
[b](+) Unique sequence DNA mapping to chromosome 8; (Chi) chimeric, indicating that the YAC end does not map to chromosome 8; (Rep) repetitive DNA sequence; (N.D.) not determined.

lod-score calculations, alleles were down-coded. For the multipoint analysis, we used the following fixed order of markers and respective sex-averaged map distances: telomere–AFM185xb2–D8S504–D8S264–AFMa054td9–D8S262–centromere (1.0, 1.0, 2.0, 3.0 cM). To evaluate linkage between the potential candidate gene, cathepsin B, and the *EPMR* locus, primers were designed to amplify intronic sequence between exons 8 and 9 (primer sequences: 5′→3′ forward-GGG AGA TAC CCC CAA GTG TAG C, reverse-CCT TCT CGC TAT TGG AGA CGC) (Chan et al. 1986; Gong et al. 1993). Radiolabeled PCR product was used to probe Southern blot filters containing restriction digested DNA (*Bam*HI, *Bgl*II, *Bst*I, *Eco*RI, *Hae*III, *Hin*dIII, *Kpn*I, *Msp*I, *Pst*I, *Pvu*II, *Rsa*I, *Stu*I, *Taq*I) from a panel of unrelated individuals to detect RFLPs for linkage analysis.

YACs and YAC Library Screening

YAC clones were isolated by PCR screening of the CEPH Midi and Mega YAC libraries (Albertsen et al. 1990; Dausset et al. 1992), or they were identified from two of several physical mapping data bases available through the World-Wide Web. One YAC clone, yRM2205 [Genome Data Base (GDB) designation D8S596, D8Z5], was isolated from a telomere-enriched library and kindly provided to us by Dr. Harold Riethman (Wistar Institute, Philadelphia, PA). Marker D8S264 and several YAC-end sequence-tagged sites (STSs) (858e12R, 858e12L, and 241d10R) were used to screen the CEPH Midi YAC library by PCR amplification and combinatorial pooling strategies (Albertsen et al. 1990; Green and Olson 1990). Positive YACs were picked from glycerol stocks, colonies were amplified, and the result was verified by PCR with the original marker before YAC-end isolation was attempted. The Mega YACs positive for markers D8S504, D8S264, D8S201, and D8S262, as well as a number of YACs placed in the region by Alu-PCR fingerprinting, were ascertained from the CEPH data base (Cohen et al. 1993). Three YAC clones (808B12, 772C1, 795E11) were selected from YAC contigs WC-148 and WC-1335 (Center for Genome Research at Whitehead Institute for Biomedical Research, Cambridge, MA). YAC DNA was separated from the yeast chromosomal DNA by pulsed-field gel electrophoresis using a Pharmacia LKB apparatus with a hexagonal electrode and alkali blot filters were prepared. The filters were hybridized with radiolabeled total human genomic DNA to determine the size of each clone.

YAC-end Isolation

YAC ends were isolated by ligation-mediated PCR ampli-

fication with minor modifications of published protocols (Kere et al. 1992). YAC DNA from single clones was prepared in agarose beads and digested in separate 15-μl reactions with *Rsa*I, *Alu*I, *Pvu*II, and *Eco*RV. A short linker sequence (2 pmoles), T4 DNA ligase (2 units), and buffer recommended by the enzyme supplier were added, and the reactions were continued for 1–2 hr at 37°C. One microliter of the ligated mixture was amplified by PCR in 20-μl reactions using the linker primer (3 pmoles) and a YAC vector arm-specific primer (30 pmoles). The 5 × PCR buffer contained 20 mM $MgCl_2$, 335 mM Tris-HCl (pH 8.8), 80 mM $(NH_4)_2SO_4$, 50 mM β-mercaptoethanol, and 0.5 mg/ml of bovine serum albumin. Temperature cycling conditions were as follows: 94°C for 4 min followed by 35 cycles of 94°C for 1 min, 64°C for 2 min, and 72°C for 2 min. One microliter of the reaction mixture was diluted to 500 μl with water and reamplified using the linker primer and an internal YAC vector arm-specific primer in 50-μl reactions under the same concentrations and temperature conditions as above. The primer sequences were as follows: YAC vector arm, left, 5′-CACCCGTTCTCGGAGCACTGTCCGACCGC-3′; internal, left, 5′-TCTCGGTAGCCAAGTTGGTTTAAGG-3′; YAC vector arm, right, 5′-ATATAGGCGCCAGCAACCGCACCTGTGGCG-3′; internal, right, 5′-TCGAACGCCCGATCTCAAGATTAC-3′; linker primer 5′-GCGGTGACCCGGGAGATCTGAATTC-3′; linker 5′-GAATTCAGATC-3′. The reamplification products were run on a 1.5% low-melting-point agarose gel, excised, and sequenced. Both ends of the YAC 810F8 were isolated by the vectorette PCR method (Riley et al. 1990; Wang et al. 1995). The primer pairs specific for the YAC insert end genomic DNA were selected using the PRIMER program (M.J. Daley, S. Lincoln, and E. Lander, Massachusetts Institute of Technology). Each STS was used to amplify the parent clone, DNA from a chromosome 8-only hybrid cell line, and other YACs within the contig. The oligonucleotide primers used to detect STSs are presented in Table 1. Some characteristics of the YACs are shown in Table 2.

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