Myofibrillogenesis Regulator 1 Gene Mutations Cause Paroxysmal Dystonic Choreoathetosis

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Background: Paroxysmal dystonic choreoathetosis (PDC) is characterized by attacks of involuntary movements that occur spontaneously while at rest and following caffeine or alcohol consumption. Previously, we and others identified a locus for autosomal dominant PDC on chromosome 2q33-2q35.

Objective: To identify the PDC gene.

Design: Analysis of PDC positional candidate genes by exon sequencing and reverse transcription–polymerase chain reaction.

Setting: Outpatient clinical and molecular genetic laboratory at a university hospital.

Patients: Affected (n=12) and unaffected (n=26) subjects from 2 unrelated families with PDC and 105 unrelated control subjects.

Results: We identified missense mutations in the myofibrillogenesis regulator gene (MR-1) in affected subjects in 2 unrelated PDC kindreds. These mutations were absent in control subjects and caused substitutions of valine for alanine at amino acid positions 7 and 9. The substitutions disturb interspecies conserved residues and are predicted to alter the MR-1 gene's amino-terminal α helix. The MR-1 exon containing these mutations (exon 1) was expressed only in the brain, a finding that explains the brain-specific symptoms of subjects with these mutations.

Conclusions: Although MR-1 gene function is unknown, the precedence of ion channel disturbance in other episodic neurologic disorders suggests that the pathophysiologic features of PDC also involve abnormal ion localization. The discovery that MR-1 mutations underlie PDC provides opportunities to explore this condition's pathophysiologic characteristics and may provide insight into the causes of other paroxysmal neurologic disorders as well as the neurophysiologic mechanisms of alcohol and caffeine, which frequently precipitate PDC attacks.

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P A ROXYSMAL DYSTONIC CH OREATHETOSIS (PDC) (Mendelian Inheritance in Man No. 11880; also known as paroxysmal nonkinesigenic dystonia) is an episodic movement disorder in which attacks of dystonia, chorea, and athetosis begin in childhood through early adulthood; involve the extremities, trunk, and face; and may cause dysarthria or dysphagia. These episodes last from several minutes to more than an hour and may occur several times each day.1-6 The PDC attacks occur both spontaneously while at rest and following provoking factors that include alcohol or caffeine consumption and to a lesser extent fatigue, hunger, and emotional stress.

Previously, we7 and others8-13 identified a locus for autosomal dominant PDC on chromosome 2q33-2q35. These studies yielded a consensus PDC locus interval spanning approximately 2.7 cM between DNA polymorphisms D2S295 and D2S163. This region included a cluster of ion channel genes7 and other candidate genes, some of which were analyzed and excluded.14-16 In this article, we report the discovery of disease-specific myofibrillogenesis regulator 1 gene (MR-1) mutations in 2 unrelated PDC kindreds.

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METHODS

SUBJECTS

Subjects participated according to the University of Michigan (Ann Arbor) institutional review board–approved protocol. They were diagnosed as either affected with PDC or unaffected prior to genetic linkage and candi-
date gene analysis. Diagnosis was based on normal developmental milestones, observers’ descriptions of episodes consistent with nonkinesigenic, nonhypnogenic paroxysmal dyskinesia lasting longer than 5 minutes, normal interictal neurologic examination results, and witnessed episodes (in person or on videotape) by J.K.F. of subjects PDC-Det IV-2 and PDC-Det V-7 (Figure 1) that were consistent with PDC. Control subjects were older than 60 years and were determined by neurological examination and structured psychiatric interview to have no personal or family history of neurologic or psychiatric disorders.

GENETIC LINKAGE ANALYSIS

The DNA was extracted from peripheral blood leukocytes, microsatellite DNA polymorphisms, and 2-point lod scores calculated as previously described. Genetic linkage analysis of the PDC-Det kindred (substitution of valine for alanine at amino acid position 9) (Figure 1) was reported previously. For the PDC-Pa kindred (substitution of valine for alanine at amino acid position 7) (Figure 1), genetic linkage between the disorder and the PDC locus was assessed by the examination of markers D2S295, D2S2210, D2S434, D2S249, D2S94, D2S173, D2S2179, D2S104, D2S2250, D2S433, D2S2244, D2S2151, and D2S163 using an autosomal dominant mode of inheritance, applying an assumed disease allele frequency of 0.001, and assigning genetic penetrance to 0.90. Allele frequencies were assumed to be equal because there were too few marrying-in spouses to calculate allele frequencies accurately.

PHYSICAL MAPPING OF THE PDC LOCUS INTERVAL

We created a physical map across the consensus PDC locus interval (D2S295-D2S163) consisting of 22 overlapping bacterial artificial chromosome elements (data not shown). Subsequently, the Human Genome Project created overlapping contigs (NT_005337 and NT_005289, that were combined into contig NT_005403; http://www.ncbi.nlm.nih.gov/Entrez) that included the 2.4-Mb PDC locus and for which DNA sequences were made publicly available. We confirmed the sequence tagged site (STS) content of these contigs by a combination of STS amplification from individual bacterial artificial chromosome elements and Basic Local Alignment Search Tool analysis to determine if the DNA sequences of given STSs were contained in the annotated contig sequence (http://www.ncbi.nlm.nih.gov/).

IDENTIFYING, PRIORITIZING, AND ANALYZING PDC CANDIDATE GENES

We identified 116 potential candidate genes (http://www.med.umich.edu/neuro/MR1.htm) in the PDC contig by analysis of expressed sequence tags and complementary DNA (cDNA) sequences listed with annotated contigs from the National Center for Biotechnology Information (Bethesda, Md) and by Pipeline analysis of contig and individual bacterial artificial chromosome DNA sequences (http://compbio.ornl.gov/).

Involuntary movements in PDC involve (but do not necessarily originate in) the extrapyramidal motor system. Therefore, to prioritize the analysis of 116 positional candidate genes, we used reverse transcription–polymerase chain reaction (RT-PCR) to determine which candidate genes were expressed in the brain. For this we used a Superscript RT-PCR kit (Invitrogen, Carlsbad, Calif) to amplify candidate genes from adult brain messenger RNA (mRNA) (Stratagene, La Jolla, Calif). Whenever possible, we designed exonic primers to amplify across small introns so that we could determine by amplification fragment size whether the template consisted of cDNA or contaminating genomic DNA. When intronic sequences were less than 3 kilobases (kb), we compared RT-PCR amplification products with those obtained from the genomic DNA template.

ANALYSIS OF MR-1 GENE EXPRESSION IN MULTIPLE TISSUES BY RT-PCR

This analysis was performed using a Superscript RT-PCR kit (Invitrogen) to amplify a fragment of the MR-1 gene from the adult brain, liver, kidney, skeletal muscle, heart, and lung mRNA (Stratagene). Placement of the forward primer (5’- ATTGAAACTGGCGCGGCGTGTTAG-3’) in MR-1 NM_015488 exon 1 and the reverse primer (5’-AGTGGCCCGTAAGGGTAGCAGGATTCC-3’) in MR-1 NM_015488 exon 3 resulted in a 333-base pair (bp) cDNA amplification product. Inclusion of introns 1 and 2 allowed size discrimination of amplification products from mRNA (333 bp) and genomic DNA templates (>50 kb). The RT-PCR amplification of a 626-bp β-actin mRNA fragment served as a control and was performed with previously described primers and methods. The conditions were the same for both MR-1 and β-actin amplification (50°C for 30 minutes, denaturation for 2 minutes at 94°C, followed by 34 cycles each at 94°C for 15 seconds, 55°C for 30 seconds, and 72°C for 1 minute, followed by 10-minute elongation at 72°C).

PROTEIN SEQUENCE ANALYSIS

The MR-1 homologues were found using Tblastn, the protein query of the translated database (http://www.ncbi.nlm.nih.gov/BLAST/). The secondary structure prediction was per-
formed using Protext sequence analysis software (DNASTAR, Madison, Wis).

RESULTS

CLINICAL FEATURES

Clinical features and (+)-\(\alpha\)-[\(\text{[1]}\text{C}\)]dihydrotetrabenazine positron emission tomography for the PDC-Det kindred have previously been reported.\(^{20,21}\) The PDC-Det kindred is of Polish ancestry, and the PDC-Pa kindred is of English and mixed European ancestry. The clinical syndrome of early childhood-onset nonkinesigenic dyskinesia in affected subjects in the PDC-Pa family was very similar to that previously described for the PDC-Det family.\(^{20}\) Affected subjects in the PDC-Pa kindred experienced episodes (ranging from once a week to several times a day) of involuntary movements involving the face and all extremities that lasted from 5 minutes to more than 1 hour. Episodic movements occurred spontaneously while at rest and following caffeine or alcohol consumption. These involuntary movements did not occur during sleep, when falling asleep, or when waking up and were not provoked by exercise or sudden movement. Developmental milestone and interictal neurological examination results were normal in all subjects with the exception of 1 individual who had childhood-onset polio and 1 subject who had facial tics (blinking), both of whom were in the PDC-Det kindred and have been described previously.\(^{20}\)

LINKAGE OF THE DISORDER IN PDC-DET AND PDC-PA KINDREDS TO CHROMOSOME 2q33-2q35

Previously, we reported linkage of this disorder to chromosome 2q33-2q35 in the PDC-Det kindred (maximum 2-point lod score, 4.77 at \(\theta\)=0 for marker D2S173 [AFM249wg9]).\(^7\) Analysis of the PDC-Pa kindred (Figure 1) was also consistent with linkage to this locus (maximum 2-point lod score, 2.41 at \(\theta\)=0 for D2S163).

HAPLOTYPE ANALYSIS

We analyzed extended haplotypes for linked markers in PDC-Det and PDC-Pa families and found no evidence of haplotype sharing (data not shown). Therefore, there was no evidence that these 2 families were closely related.

IDENTIFYING AND ANALYZING PDC POSITIONAL CANDIDATE GENES

The RT-PCR analysis (described previously) provided evidence that 45 of the 116 known and putative genes in the PDC locus interval were transcribed in the brain. Intron-exon boundaries were identified, and candidate gene sequencing was performed as previously described.\(^{17,18}\) Analysis of 17 of these genes did not disclose PDC-specific, nonconserved coding sequence mutations.\(^{15,16}\) (See http://www.med.umich.edu/neuro/MR1.htm for a list of candidate genes analyzed, candidate genes shown to be expressed in the brain, primer sequences, and RT-PCR conditions.)

IDENTIFICATION AND ANALYSIS OF MR-1 GENE MUTATIONS IN SUBJECTS WITH PDC

The MR-1 gene was identified as a positional candidate gene of unknown (unpublished) function and was expressed in the brain. The National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/Entrez) lists 2 MR-1 transcripts (NM_015488 and NM_022572). These transcripts have identical sequences for their last 8 exons but differ in their first 2 exons. NM_015488 is the larger transcript (3032 bp) and encodes a 385 amino acid protein with a predicted molecular weight of 42.9 kDa. NM_022572 is a slightly smaller transcript (2918 bp) that encodes a 361 amino acid protein with a predicted molecular weight of 40.7 kDa. Exons 1 and 2 (284 bp combined) of the larger transcript contain 47 bp of a 5’ untranslated sequence and encode 79 amino acids that are not present in the smaller MR-1 transcript. Conversely, exon 1 (165 bp) of the smaller transcript encodes 55 amino acids that are absent in the larger MR-1 transcript (GenBank accession numbers: NM_015488 and NM_022572 for human MR-1 splice variants, AY299972 for mouse MR-1, and NT_005403 for the contig containing the MR-1 gene).

We sequenced each MR-1 exon (from NM_015488 and NM_022572) in affected and unaffected subjects from the PDC-Det and PDC-Pa kindreds (Figure 2). Sequencing MR-1 NM_015488 exon 1 in samples from the PDC-Det kindred revealed heterozygosity (both C and T) at MR-1 NM_015488 cDNA nucleotide 72 in each affected subject (n=8) (Figure 1). With the exception of 2 previously identified nonpenetrant subjects\(^1\) discussed as follows, each unaffected subject (n=17) and each control subject (n=105) had only C at this position, which agreed with the DNA sequence of contig NT_005289 and the MR-1 gene’s published cDNA sequence (NM_015488). Sequencing MR-1 NM_015488 exon 1 in samples from the PDC-Pa kindred revealed heterozygosity (C and T) (Figure 1) at MR-1 NM_015488 cDNA nucleotide 66 (Figure 1 and Figure 2) in each affected subject (n=4). This mutation was absent in unaffected relatives (n=7) and control subjects (n=105). Sequencing each remaining MR-1 NM_015488 exon and each MR-1 NM_022572 exon in 2 affected subjects from the PDC-Det and PDC-Pa kindreds revealed no other mutation.

Previously, we reported that unaffected subject PDC-Det IV-2 (Figure 1) contained the affected haplotype for the D2S173 locus markers.\(^7\) The MR-1 NM_015488 exon 1 sequence analysis confirmed that this unaffected individual also had the C-to-T substitution at MR-1 nucleotide 72. She is thus a nonpenetrant individual as is her unaffected sibling (subject PDC-Det IV-3) (Figure 1), who along with her affected child has the substitution of T for C at MR-1 nucleotide 72.

The MR-1 NM_015488 exon 1, the exon containing the C66T and C72T mutations described previously, is found only in 1 MR-1 transcript variant of NM_015488. We created primers specific for this exon
to examine MR-1 gene expression by RT-PCR. These experiments detected the expression of MR-1 NM_015488 exon 1 only in the brain (Figure 3). We did not detect MR-1 NM_015488 exon 1 in the liver, kidney, skeletal muscle, heart, or lung (Figure 3).

**COMMENT**

We discovered 2 missense MR-1 mutations, each of which segregates with the disorder in 2 unrelated PDC kindreds and is absent in both unaffected relatives and unrelated control subjects. These mutations predict substitutions of valine for alanine at residue 9 (A9V) in subjects in the PDC-Det kindred and at residue 7 (A7V) in those in the PDC-Pa kindred. These alanine residues are part of an amino-terminal α helix that becomes disrupted by either substitution (Figure 4). The 7Ala and 9Ala residues are conserved in the 2 species (human and mouse) for which the MR-1 cDNA sequence is known and in the amino acid sequence deduced from rat genomic DNA (contig NW_047816).

The MR-1 protein contains a conserved glyoxylase domain (amino acid residues 130-380). There is no published information about MR-1 gene function. The only glimpse into MR-1 function can be gleaned from the Human Genome Project database entries that state that MR-1 is the “likely ortholog of mouse brain protein 17, transactivated by hepatitis C virus core protein 2” (http://www.ncbi.nlm.nih.gov/Entrez; gene identification No. 2593).
and that “MR-1, a myofibrillogenesis regulator, is associated with the myofibril contractile apparatus” (http://www.ncbi.nlm.nih.gov/Entrez; locus AAL08573).

As noted previously, MR-1 sequences are present in 2 transcripts, NM_015488 and NM_022573, that share 8 exons but differ in the first 2 exons. The MR-1 transcript NM_015488 (derived from 10 exons spanning 76.3 kb of genomic DNA) encodes a 385 amino acid protein of 42.9 kDa (predicted). The MR-1 transcript NM_022573 (derived from 9 exons spanning 23.5 kb of genomic DNA) encodes a 361 amino acid protein of 40.7 kDa (predicted).

The 2 MR-1 transcripts could reflect either variable transcription initiation or alternative mRNA splicing. Only MR-1 transcript NM_015488 contains exon 1. Our studies indicate that the MR-1 NM_015488 transcript containing exon 1 is expressed only in the brain. The PDC-specific MR-1 NM_015488 mutations C66T and C72T are present in exon 1. That this exon is expressed only in the brain could explain why PDC symptoms are restricted to this area.

Given the scarcity of information about MR-1 gene function, it is difficult to speculate about the mechanisms by which MR-1 causes episodic extrapyramidal disturbance. The observation that 13 other paroxysmal neurologic disorders are due to ion channel gene mutations supports the possibility that PDC is also caused by abnormal ion localization.

The presence of disease-specific MR-1 mutations in subjects with PDC permits the use of MR-1 sequence analysis for laboratory confirmation of PDC diagnosis and prenatal counseling. It will be important to determine whether MR-1 mutations underlie other extrapyramidal and paroxysmal neurologic conditions, particularly episodic movement disorders.23,24

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