Presence of Alanine-to-Valine Substitutions in Myofibrillogenesis Regulator 1 in Paroxysmal Nonkinesigenic Dyskinesia

Confirmation in 2 Kindreds

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Background: Paroxysmal nonkinesigenic dyskinesia (PNKD) is a rare disorder characterized by attacks of involuntary movements brought on by stress, alcohol, or caffeine, but not by movement. An autosomal dominant form of this disorder was mapped to chromosome 2q33-36, and different missense mutations in exon 1 of the myofibrillogenesis regulator 1 (MR1) gene were identified recently in 2 kindreds.

Objectives: To describe studies on a new pedigree with PNKD, to explore the possibility of locus heterogeneity, and to further delineate the spectrum of mutations in MR1 in 2 families with PNKD.

Design, Setting, and Patients: All 10 exons of MR1 were sequenced in DNA from members of 2 pedigrees with autosomal dominant PNKD.

Results: Different missense mutations in exon 1 of MR1 that cosegregate with disease were identified in each multiplex family. These single-nucleotide mutations predicted substitution of valine for alanine in residue 7 in one family and residue 9 in the other. The same mutations were found in the only 2 families previously published. Family history and haplotype analysis make it unlikely that the families with the same mutations are related.

Conclusions: The function of MR1 is unknown, but the 2 mutations identified in the 4 families with PNKD studied to date are predicted to disrupt the amino terminal α-helix suggesting that this region of the gene is critical for proper gene function under stressful conditions. Study of additional families will be important to determine whether analysis of a single exon (MR1 exon 1) is sufficient for genetic testing purposes.

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Power and 2-point linkage analyses were per-
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LINKAGE software package, version 5.1.10,11 Multipoint analy-
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ED03 were collected and genomic DNA was isolated from leuko-
cytes as previously described.6 A sample was also collected
from an apparently isolated case of PKC (ED04).

LINKAGE ANALYSIS

Genotyping of polymorphic markers D2S128, D2S2359, D2S126,
and D2S130, D2S344, D2S163, D2S377, and D2S2148 (Research
Genetics, Huntsville, Ala), which flank the PNKD criti-
cal region, were performed in family ED03 with radiolabeling
as previously described.6 Power and 2-point linkage analyses were per-
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were performed with GENEHUNTER, version 1.2,12 and
VITESSE.13 (Linkage analysis of family ED01 has been de-
scribed by Raskind et al.) The statistical analyses for ED03
were performed under the assumptions of autosomal dominant
inherence, a disease frequency of 0.0001, and 90% penetrance.

MUTATIONAL AND HAPLOTYPING ANALYSES

To analyze MR1, fragments encompassing each of the 10 cod-
ing exons and corresponding splice junctions were amplified
using the following forward and reverse primer pairs, all given
in 5’ to 3’ direction: exon 1, TGTAGGCAGGACGGAGGAAGG
and TGAGAAGGTAGTGAGGAAGACC; exon 2, CTCCCTC-
CACAGGGCTTACT and ACGCTCAGCCCCTCAGAC; exon 3,
AGGCCAGCTAGGAGAAAG and GTGGCCGCGGTAAAGG;
exon 4, CGGCTCTGCTCCCTTACAC and CCGCTCTGCTCCCTTAC
and exon 5, CTGATCTCTCGTCTCATC and CTCCTGCTCCCTTAC
and exons 6, CTGATCTCGTCTGTGGTT; and AGAGAAGGCAGCAG
AGGAAAGGAAGAGGAAAGT and GTGGGGGCTGGTGGTGT
Exon fragments were amplified with Fast-
Start Taq polymerase (Roche Diagnostics Corporation, India-
napolis, Ind), in a total volume of 20 µL containing polymerase
chain reaction (PCR) buffer provided with the enzyme, 0.5M 2-
(trimethylammonio) ethanoic acid (Betaine; Sigma-Aldrich Corp,
St Louis, Mo), 0.5 µmol/L of each primer, 800 µmol/L of deoxy-
nucleotide triphosphate mixture, and 60 µg of DNA. The PCR
amplification profile contains an initial denaturation step at 95°C
for 5 minutes, 33 cycles at 95°C for 45 seconds, 64°C (or 54°C
for the exon 7 fragment) for 45 seconds, and 72°C for 1 minute,
with a final extension at 72°C for 10 minutes. By methods pre-
viously described,14 direct DNA sequencing was performed us-
ing the forward primers, and electrophoresis was carried out on a
 genetic analyzer (ABI PRISM 3100 Avant Genetic Analyzer; Ap-
plied Biosystems, Foster City, Calif). To confirm the sequence
alterations, exon 1 was also sequenced in reverse.

The nucleotide substitutions 66C→T and 72C→T elimi-
nated restriction endonuclease recognition sites for Tsel and HaeII,
respectively. Restriction fragment length polymorphism (RFLP)
analyses with these enzymes (New England Biolabs, Beverly, Mass)

were performed in affected individuals and control subjects on
271-base pair (bp) exon 1 PCR fragments, under conditions sug-
gested by the manufacturer. Restriction fragments were sepa-
rated on 3% agarose gels. Tsel digestion of the wild-type allele
generated fragments of 165 bp and 106 bp, and HaeII digestion
of the wild-type allele generated fragments of 153 bp and 118
bp. The mutant allele is not digested by either of these enzymes.

To address the possibility that the families bearing the same
MR1 mutations are genetically related, genotypes for fluore-
cent markers D2S325, D2S382, D2S338, D2S333, D2S362, and
D2S396, which flank the gene, were analyzed as previously de-
scribed.15 In addition, haplotypes were constructed manually for
members of families ED01 and ED03 and for 2 affected mem-
bers of families PDC-Det and PDC-PA, who were previously
described by Rainier et al.8

RESULTS

PEDIGREES

A description of family ED01 (Figure 1A), of German
background, was previously published.8 The pedigree of a
newly ascertained family of predominantly French and
Irish background, ED03, is shown in Figure 1B. The neu-
rologic disorder could be traced back 4 generations to

French Canadian family members living in New Bruns-
kirk, who were previously described by Rainier et al.8

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ditional neurologic symptoms or signs. Some affected adults in this family have found that relaxation techniques lessen the intensity of the symptoms and sometimes stop an attack. Carbamazepine was of no benefit, but benzodiazepines have been somewhat helpful. The phenotype of individual ED04 differs from that of families ED01 and ED03 in that the episodes are shorter (seconds to minutes) and precipitated by movement but not caffeine. The disorder in individual ED04 is therefore consistent with PKC rather than PNKD.

POWER AND LINKAGE ANALYSES

Our group previously confirmed linkage of PNKD to chromosome 2q in a 3-generation family, ED01 (Figure 1A), for which a maximum pairwise logarithm of odds (LOD) score of 4.19 at \( \theta = 0.001 \) was obtained for the marker D2S120.6 Samples were available from 8 members of family ED03. Power analysis using all 8 subjects and 8 markers spanning the critical region suggested that these samples could yield average and maximum 2-point LOD scores of 0.819 and 1.28, respectively, at \( \theta = 0.001 \). A maximum multipoint LOD score of 1.46 at \( \theta = 0.08 \) was obtained with both GENEHUNTER12 and VITESSE.13 Although not sufficient to confirm linkage, these data are consistent with linkage of disease in family ED03 to the chromosome 2q PNKD locus.

IDENTIFICATION OF MR1 MUTATIONS IN FAMILIES WITH PNKD

The 10 exons and splice junctions of MR1 were amplified with the use of PCR and sequenced in members of families ED01 and ED03 and in individual ED04. No sequence alteration was detected in ED04, the subject with PKC. Heterozygous C-to-T transitions were identified in nucleotide 72 (affecting residue 9) and nucleotide 66 (affecting residue 7) in affected members of ED01 and ED03, respectively (Figure 2). These sequence changes both predict substitution of valine for alanine in the respective residues. No other sequence alterations were found. The mutation segregated with the disease in all 8 affected individuals tested and in an obligate carrier in family ED01. The 6 at-risk, unaffected members had the wild-type sequence. The spouse of the obligate carrier also had the wild-type sequence. In ED03, the mutation was also found in all affected individuals tested and in neither of the unaffected individuals. The RFLP analyses with TseI and HaeII in affected individuals confirmed the 66C→T and 72C→T mutations, respectively. Paroxysmal nonkinesigenic dyskinesia is a rare disorder, and these are the same nucleotide changes seen in the 2 families described by Rainier et al.8 To investigate the possibility that the families bearing the same mutation are genetically related, 3 markers proximal to the gene—D2S325 (204.53 centimorgans [cM]), D2S2382 (213.49 cM), and D2S1338 (215.78 cM; 219 082 kilobase pairs [kbp])—and 3 markers distal to it—D2S433 (216.31 cM; 219 968 kbp), D2S126 (221.13 cM), and D2S396 (232.9 cM)—underwent genotyping, and haplotypes were compared in the relevant pairs of families. The MR1 gene is at 219 337 to 2 194 140 kbp on the National Center for Biotechnology Information map, build 34.3. The 72C→T mutation was present in the PDC-Det kindred (of Polish ancestry) described by Rainier et al8 and in the ED01 kindred (of German ancestry) from the present study. The 66C→T mutation was present in the PDC-Pa kindred (of English and mixed European ancestry) described by Rainier et al8 and in the ED03 kindred (of French ancestry) from the present study. The disease-related haplotypes in families PDC-Det and ED01 were completely different, with the exception of D2S433, the distal marker closest to MR1. This marker has a relatively low polymorphic information content of 0.6, and in all 4 families the common 191-bp allele (57.6%) was shared by all 11 persons tested, including those who were unaffected as well as those who

![Figure 2](https://jamanetwork.com/)

**Figure 2.** Reverse-strand chromatograms for portions of exon 1 of the myofibrillogenesis regulator 1 gene that show heterozygous mutations in affected individuals from 2 families with paroxysmal nonkinesigenic dyskinesia as compared with control subjects. The G→A changes shown correspond to C→T transitions in the coding strands in nucleotide 72 in family ED01 (A) and nucleotide 66 in family ED03 (B).
were affected. In families PDC-Pa and ED-03, the disease-related haplotypes proximal to MR1 were dissimilar, but they also shared the common 191 allele of D2S433, and a recombination event that occurred in family ED03 distal to this marker precluded comparison of the distal portion of the region in these 2 families. Haplotypes based on intragenic single nucleotide polymorphisms rather than flanking markers would be more convincing if they were informative in these families, but the observation of such distinct haplotypes in at least 1 of the 2 sets of families argues for independent mutation events.

The detection of mutations in 2 additional families confirms that MR1 is responsible for PNKD. The MR1 gene encodes 2 isoforms (NM_015488 and NM_022572) that differ in the first 2 exons and that have different expression patterns.\(^8\) The mutations identified in PNKD are located in exon 1 of the brain-specific transcript NM_015488. The function of MR1 is unknown, but the observation that the 2 recurrent mutations identified to date disrupt the amino terminal α-helix suggests that this region of the gene is critical for proper gene function under certain stressful conditions. The haplotype analysis suggests that the A9V mutation occurred independently in 2 families, and this may also be true of the 2 A7V mutations. In the 4 families with identified MR1 mutations to date, there are now 2 instances of decreased penetrance, both in females (families PDC-Det and ED01).

The possibility that there may be a very limited spectrum of mutations that result in the phenotype of PNKD has implications for genetic testing approaches. The amino terminal portion of the MR1 protein is very rich in alanine: 10 of the first 30 residues are alanines. The Protein Sequence Analysis (PSA) Protein Structure Prediction Server\(^16\) predicts that 5 of these 10 alanines, all located among the first 10 amino acids of the protein, have at least a 60% probability of participating in an α-helix structure. Changing any of these 5 alanines to valine markedly reduces the probability. The 2 alanine-to-valine mutations identified to date in patients with PNKD are detectable by RFLP analysis. It will be important to study additional families to determine whether RFLP or sequence analysis of only a single exon in MR1 is sufficient in molecular screening for PNKD.

The gene for the kinesigenic form, PKC, has been mapped to chromosome 1p11.2-q12.1.\(^1\) Because few families with either PNKD or PKC have been studied, it is not known whether there is overlap in the phenotypes or whether these disorders are genetically completely distinct. It is not possible to determine a chromosomal location for the gene responsible for PKC in individual ED04 because this is an isolated case. However, the absence of an MR1 mutation in ED04 is consistent with separate causes and locus heterogeneity for these 2 clinically similar disorders.

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### References