Genetic heterogeneity in paroxysmal nonkinesigenic dyskinesia

Abstract—Paroxysmal nonkinesigenic dyskinesia (PNKD) is characterized by attacks of dystonia or chorea lasting minutes to hours. Recently, mutations in the myofibrillogenesis regulator 1 gene (*MR-1*) have been identified in 10 unrelated PNKD kindreds. The authors describe a Canadian PNKD family who does not have mutations in the *MR-1* gene and links to a separate locus at 2q31. This indicates that there are at least two different genes responsible for PNKD.

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Paroxysmal nonkinesigenic dyskinesia (PNKD) can be sporadic and in familial forms is autosomal dominant with incomplete penetrance. PNKD is clinically characterized by attacks of dystonia or chorea lasting minutes to hours. Attacks can be triggered by alcohol, caffeine, fatigue, and excitement but not by sudden movement. Onset is variable, ranging between 1 and 77 years.[1] A locus for PNKD has been mapped to a 2-cM region on chromosome 2q33-35.[2,3] To date, PNKD has proven to be genetically homogeneous because families from diverse geographic areas have all mapped to this region.[4,5] Recently, researchers have successfully identified the myofibrillogenesis regulator 1 gene (*MR-1*), on chromosome 2q33-35, as a gene associated with PNKD in 10 unrelated kindreds.[4,5] We present a Canadian family of European descent who suffers from PNKD but does not have a mutation in the *MR-1* gene. Additional linkage studies in this family indicate that the gene locus links to a second distinct region on chromosome 2q31. These results are the first to suggest that there are at least two different genes responsible for PNKD, implying genetic heterogeneity for PNKD.

Methods. We identified the family through the University of British Columbia (UBC) Neurogenetics Clinic. Ethics approval for this study was obtained through the UBC Clinical Research Ethics Board. Written informed consent was obtained from all participating family members, who were examined in person by a neurologist (S.D.S.).

Genomic DNA was isolated from blood using standard techniques. Each of the 10 exons of the *MR-1* gene and the 17 exons of the *GAD67* gene, plus their flanking intron/exon boundaries, were PCR amplified and sequenced from the genomic DNA of affected family members III:2, III:4 and the unaffected family member III:5. Sequence results were compared to the human genomic sequence of the *MR-1* gene and the GAD67 gene using NCBI's online nucleotide-nucleotide BLAST program.[6]

DNA and linkage analysis. We analyzed 277 highly polymorphic microsatellites spanning all 22 autosomes with an average distance of 10 cM (Linkage Mapping Set version 2.5 MD10, PE Applied Biosystems). Microsatellite markers were amplified from genomic DNA using the PCR technique as specified by the manufacturer and electrophoresed on a denaturing acrylamide gel using a 377 DNA Sequencer (PE Applied Biosystems). Genotypes were determined by DNA fragment size analysis, performed semiautomatically using the Genescan and Genotyper software (PE Applied Biosystems).

Parametric and nonparametric LOD scores were generated using the Genehunter program (V2.1) (UK HGMP Resource Centre), with an assumption of autosomal dominant inheritance, age-related reduced penetrance (0.8), a gene frequency of 0.0001, and equal allele frequencies for each marker. Haplotypes were constructed to detect recombination events, assigning phase based on the minimum number of recombinants. Marker order and genetic distances are based on Genethon map (1996) and CEPH database.

Results. We have identified a Canadian family of European decent with PNKD. This is a three-generation, 14-member family (figure) in which affected family members present with episodes of dystonia primarily affecting hands and feet symmetrically. These episodes were not triggered by movement or exercise. Alcohol, caffeine, and excitement were not obvious triggers either. Only one family member pursued medical treatment for the attacks (III:4); her attacks would occur three to four times per day, but they did not respond to adequate trials of phenytoin, carbamazepine, clonazepam, gabapentin, or acetazolamide. The clinical features are summarized in table 1.

We performed a mutation analysis of the *MR-1* and *GAD67* genes. No polymorphisms or mutations in the affected family members III:2 and III:4 were identified.

A genome-wide screen generated positive LOD scores at chromosome 2q31. Multipoint parametric LOD scores between the gene locus in this family and marker loci are given in table 2. Maximum positive LOD scores by parametric analysis were >2.0 for markers D2S324, D2S2310, and D2S364. A multipoint nonparametric analysis generated a maximum LOD score of 7.00 for the same markers (table 2). All affected individuals in the family shared a common haplotype between D2S2188 and D2S364 (figure). The lower extent was defined by crossover events between markers D2S364 and D2S152 in individuals III:4 and III:6. The upper extent was defined by a crossover in individual III:2 between markers D2S335 and D2S2188. One unaf-

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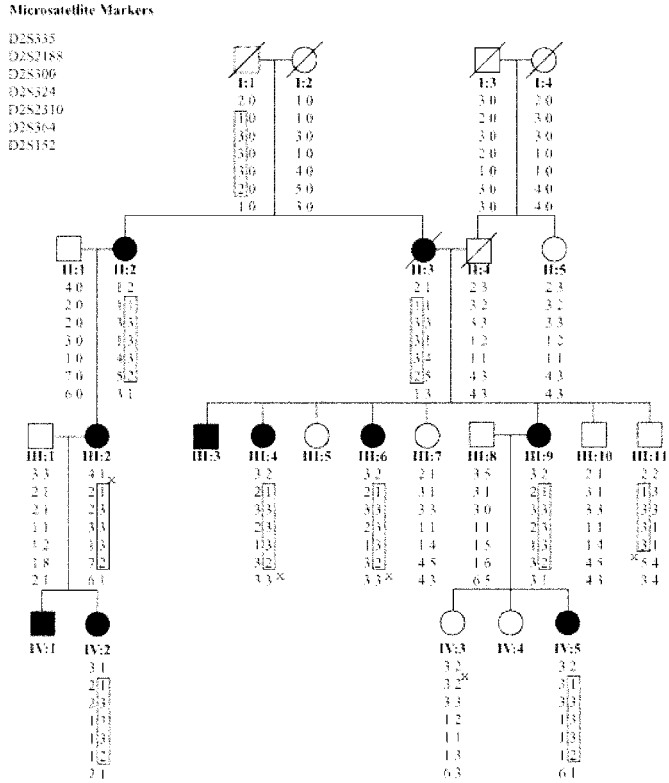

Figure. Pedigree of a paroxysmal nonkinesigenic dyskinesia (PNKD) family. The circles represent females and the squares represent males. The solid black symbols denote individuals affected with PNKD. The arrows indicate crossovers. The boxed haplotypes are deduced based on haplotypes of the offspring. The x indicates that the affectation status is unknown.

fected family member (III:11) did carry the disease haplotype; however, this is consistent with the reduced penetrance seen in this disease.

Based on LOD scores and haplotype analysis, no other loci were identified on the genome screen; in particular, there was no evidence of linkage to the PNKD locus previously described,[2,3] (LOD less than −2.65), the PKD locus (LOD less than −2.97), or the paroxysmal choreoathetosis/spasticity locus (LOD less than −3.0) based on multipoint parametric and nonparametric analyses.

Discussion. The phenotype of this PNKD family shares many characteristics seen in other previously described PNKD families. The main clinical distinction is that caffeine and alcohol do not trigger attacks in our PKND family; these triggers appear to be consistent in other PNKD pedigrees associated with *MR-1* gene mutations.[2,3]

PNKD was generally thought to be genetically homogeneous because all 10 reported families from around the world have linked to the chromosome 2q32-36 locus. It was therefore reasonable to assume that the newly identified *MR-1* gene was responsible for PNKD in our pedigree. We sequenced the exons and intron/exon boundaries of the *MR-1* gene in our PNKD pedigree and did not identify any mutations or polymorphisms. Subsequent linkage analysis in this pedigree did not link the causative gene to chromosome 2q32-36 (LOD less than −2.65) and instead identified a novel gene locus at chromosome 2q31 (parametric LOD = 2.03, nonparametric LOD = 7.00) (table 2). These results are the first to indicate that there are two different genes responsible for

Table 1 *Clinical features of paroxysmal nonkinesigenic dyskinesia pedigree*

Pedigree no.	Age at onset	Age at remission	Nature	Duration of episodes	No. of attacks/d	Trigger	Associated disorders
II:2	30s	No remission	Dystonic feet	3 min	1/d initially, now decreased to 1/mo	None	None
II:4*	?	?	Dystonic hands, feet	Minutes	?	?	None
III:2	Teens	20s	Dystonic feet	2–3 min	Daily	None	None
III:3	50s	No remission	Dystonic hands, feet	5 min	Daily	None	None
III:4	50s	No remission	Dystonic hands, feet, abdomen	5–10 min	3–4/d	Fatigue	None
III:6	50s	No remission	Dystonic hands, feet	3–4 min	1/wk	None	None
III:9	40s	No remission	Dystonic hands, feet	4–5 min	Daily	None	Migraines, single seizure as an infant
IV:1	Childhood	No remission	Dystonic feet	2–5 min	1–3/d	None	None
IV:2	Childhood	No remission	Dystonic feet	2–5 min	1–3/d	None	Migraines, grand mal seizure age 11
IV:5	Teens	No remission	Dystonic hands, feet	2–3 min	1–3/mo	None	Migraines

*Patient deceased and history provided by relatives.

Table 2 *LOD score table for paroxysmal nonkinesigenic dyskinesia locus chromosome 2q31*

Markers	Multipoint LOD score	Two-point LOD score
D2S335	0.5	−2.69
D2S2188	1.72	1.81
D2S300	6.00	1.97
D2S324	7.00	2.03
D2S2310	7.00	2.03
D2S364	7.00	2.03
D2S152	0.5	−2.59
D2S118	−2.59	−2.59

Two-point and multipoint LOD scores were generated for each marker using the using the Genehunter program (V2.1) (UK HGMP Resource Centre) with an assumption of autosomal dominant inheritance, age-related reduced penetrance (0.8), a gene frequency of 0.0001, and equal allele frequencies for each marker.

PNKD. From this point on, this putative second gene will be referred to as *PNKD2*.

The PNKD2 locus has been mapped to a 10-cM interval on chromosome 2q31, between markers D2S335 and D2S152. Haplotype analysis in this PNKD2 family demonstrated a disease penetrance of 89%. A couple of candidate genes lie in this region; the most interesting is the glutamate decarboxylase gene (*GAD-1*)[7] (OMIM 605363), which codes for glutamic acid decarboxylase (GAD67). Glutamate decarboxylase is expressed in the mammalian brain and catalyzes the conversion of glutamic acid to γ-aminobutyric acid (GABA). The GABAergic neurons represent a major neuronal inhibitory population in the basal ganglia. A dyskinetic transgenic mouse model (R6/2) has demonstrated decreased GAD67 in the basal ganglia (globus pallidus and substantia nigra pars reticulata),[8] supporting the hypothesis that dyskinesia might result from a deficiency of GAD67. However, after sequencing all 17 exons and the intron/exon boundaries including exon 1, which contains the 5′ untranslated region of the mRNA, and exon 17, which encodes the protein's C terminus and part of the mRNAs 3′ untranslated sequence, no mutations or polymorphisms were identified in the DNA of our pedigrees. However, this does not eliminate a role for this gene in the pathogenesis of PNKD2 because there may be an unidentified mutation in an intron, alternative splice site, or promoter region of this gene.

Other potential candidate genes are the distal-less homeobox 1 and 2 genes (*DLX1/DLX2*)[9] (OMIM 600029/126255). These genes are expressed in the two lineages of neocortical GABAergic neurons. It has been hypothesized that modifications of its expression pattern in the brain may alter neocortical GABAergic local circuit neurons and be implicated in genetic and acquired diseases of the human brain.[10] Alterations in GABAergic systems offer a viable hypothesis for the underlying mechanism in PNKD2.

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