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A Gene for Familial Paroxysmal Dyskinesia (FPD1) Maps to Chromosome 2q

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Summary

Dyskinesias are hyperkinetic and involuntary movements that may result from any of a number of different genetic, infectious, and drug-induced causes. Some of the hereditary dyskinetic syndromes are characterized by paroxysmal onset of the abnormal movements. The classification of the familial paroxysmal dyskinesias (FPD) recognizes several distinct, although overlapping, phenotypes. Different forms of the disorder include attacks that are (1) induced by sudden movement (kinesiogenic); (2) spontaneous (non-kinesiogenic); and (3) induced by prolonged periods of exertion. Linkage analysis was pursued in a family segregating an autosomal dominant allele for non-kinesiogenic FPD. The disease allele was mapped to a locus on chromosome 2q31-36 (LOD score 4.64, $\theta = 0$). Identification of distinct genetic loci for the paroxysmal dyskinesias will lead to a new genetic classification and to better understanding of these disorders.

Introduction

Dyskinesia is a term used to describe hyperkinetic involuntary movements. These include movements that are brisk, small in amplitude, and "dancelike" (chorea); slower and writhing (athetosis); large in amplitude and flailing (ballismus); and fixed posturing (dystonia). These abnormal movements may always be present or may occur only during wakefulness, but in some patients occur paroxysmally.

Paroxysmal dyskinesias can be classified clinically as either familial or acquired conditions (Goodenough et al. 1978). Familial paroxysmal dyskinesias include choreoathetotic, dystonic, and mixed forms. Within the familial form, both kinesiogenic (movement-induced) and non-kinesiogenic types have been noted (Mount and Reback 1940; Kertesz 1967; Goodenough et al. 1978; Fahn 1994; Demirkiran and Jankovic 1995). These disorders can be further classified on the basis of attack length, short (seconds to minutes) or long (5 min to hours). Both autosomal dominant and recessive forms are seen. Despite the clinical heterogeneity, the phenotype in any particular family generally breeds true. The classification of these disorders is confusing; whether this clinical heterogeneity reflects underlying genetic heterogeneity is not known. Characterization of this classification awaits genetic approaches that will help to characterize underlying pathogenetic mechanisms resulting in these diseases.

A genetic linkage approach was undertaken in a large Italian family with familial paroxysmal dyskinesia (FPD) in order to localize the disease-causing gene. The strategy was to pursue a general linkage search with particular attention to regions of the genome harboring known ion channel genes. Ultimately, localization of FPD loci will allow identification and characterization of the disease-causing genes. This knowledge will eventually lead to a genetic classification of the familial dyskinesias, availability of molecular diagnosis, and, it is hoped, to better treatments for patients with these diseases.

Subjects and Methods

Family Studies

Observations are based on an Italian family (K2688) of five generations with 40 members (see fig. 1). Twenty-seven of these family members were examined by two of the authors (S.S. and E.B.). Family members underwent a complete history and physical and neurological exam, with particular attention to the nature of the dyskinetic movements, age at onset, precipitating factors, and response to therapies. A detailed family history was obtained through personal interviews with available family members. Anticoagulated venous blood samples

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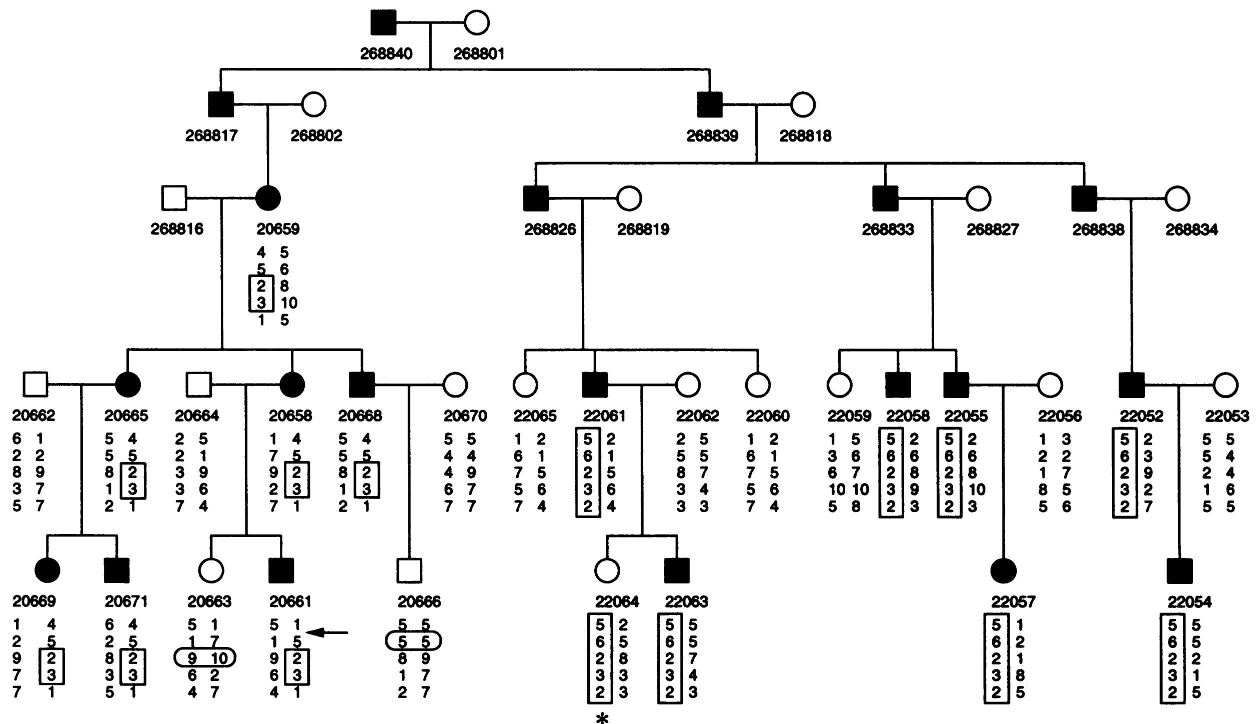

Figure 1 Pedigree of kindred 2688, with shaded symbols representing affected individuals. Each patient was examined by two of the authors (S.S. and E.B.). The pedigree structure has been altered by changing birth order and sex of selected individuals to protect patient confidentiality. Genotypes are listed under each individual in the following order: D2S128, UT6232, UT1459, D2S102, D2S126. The haplotype segregating with the disease allele in the right branch of the family is enclosed in a rectangle. Two recombinations that flank the disease allele occur, such that the segregating haplotype in the left branch is shorter (*small rectangles*). An additional recombination can be noted between markers D2S128 and UT6232 in individual 20661 (*arrow*). Segregation of nearly 100 markers in the family support reported paternity. Ovals mark two genotypes where misinheritance is suggested. However, these appear to represent a spontaneous mutation in individual 20663 (an expansion of one of the "9" alleles to a "10," for example), and either a null allele or expansion of the repeat (4 to 5) in individual 20666. An asterisk (*) marks one asymptomatic individual (22064) who carries the disease haplotype.

obtained from the examined individuals were used for direct DNA preparations and to establish lymphoblastoid cell lines by Epstein-Barr virus transformation as described elsewhere (Ptáček et al. 1991). All human tissue samples used in this project were obtained with the approval of the institutional review board at the University of Utah Health Sciences Center.

DNA Isolation and Marker Analysis

High-molecular-weight genomic DNA was isolated from whole blood lysate with a phenol/chloroform extraction followed by isopropanol precipitation. Genetic evaluation was undertaken in kindred 2688 by using markers that either flanked candidate genes or were appropriately spaced throughout the genome for a general linkage search. End-labeled primers were prepared as follows: 25 pmol of primer, 50 mM Tris HCl, 10 mM $MgCl_2$, 5.0 mM DTT, 8.4 U of T_4 polynucleotide kinase, and 6.0 μl of [γ-^{32}P] ATP (5 mCi/ml), in a total volume of 10 μl. This mixture was incubated at 37°C for 1 h and was then heated to 94°C for 3 min to inactivate the T_4 polynucleotide kinase.

PCR was used to amplify total genomic DNA by using primers flanking polymorphic regions. The reaction mixture contained 50 ng of genomic DNA, 10 pmol of each primer, 1 pmol of the end-labeled primer, 2.5 nmol of each deoxynucleoside triphosphate, 10 mM Tris HCl (pH 8.4), 40 mM NaCl, 1.5 mM $MgCl_2$, and 0.5 U *Taq* DNA polymerase in a volume of 25 μl. PCR was carried out under the following conditions: (1) one cycle at 94°C for 3 min; (2) 30 cycles, each at 94°C for 1 min, T_{anneal} for 1 min, and 72°C for 1 min; and (3) cooling to 4°C. After PCR, 10 μl of stop dye (98% formamide, 0.05% bromphenol blue, 0.05% xylene cyanol, and 20 mM EDTA) were added. Four microliters of each sample were then loaded on a 7% acrylamide gel that contained 5.6 M urea, 32% formamide, 90 mM Tris borate (pH 7.5), and 2 mM EDTA. Pre-electrophoresis of gels (1 h prior to loading) and electrophoresis were performed at room temperature and at constant power (80 W/gel)

with 90 mM Tris borate (pH 7.5) and 2 mM EDTA running buffer. Gels were placed on filter paper and exposed to x-ray film overnight at −20°C. Autoradiograms were analyzed for genotypes of the polymorphic alleles.

Statistical Analysis

Using maximum-likelihood methods, we performed pairwise linkage analysis with the MLINK program of the LINKAGE system (Lathrop et al. 1985). A gene frequency of .001 was assigned to the disease allele, and .999 was assigned to the normal allele. We calculated two-point analysis of the markers to the disease locus at a penetrance of .95. In addition, LOD scores were also calculated with penetrances ranging from .7 to .99.

Results

Families

Kindred 2688 includes 40 members, of whom 27 were examined. Of these, 14 have had paroxysmal attacks with onset between birth and the 7th year of life. In untreated infants, the attacks occur several times per week (minimum, one per year; maximum, several per day) and last from 5 min to 12 h but taper in frequency into old age. The episodes begin with dystonic posturing of upper and/or lower extremities, face, neck, and oromandibular muscles and are usually followed by choreoathetosis. The episodes are frequently precipitated by stress, fatigue, coffee, chocolate, and alcohol, but not by sudden movement. Treatment with carbamazepine and phenobarbital did not alter the frequency or severity of episodes in these patients and valproic acid had only modest effects, but low doses of clorazepate led to nearly complete resolution of attacks. The penetrance in the kindred appears to be high, although expression is variable. Two individuals (20669 and 22054) have a history of only four or five mild attacks each but no longer experience such episodes. Interictal neurological exam was normal in all patients. Several patients underwent electroencephalography (EEG) between attacks. All were normal except in one patient (22057); the abnormal EEG showed 4 Hz bilateral and synchronous spike-wave complexes with prevalence in the central brain region.

Linkage to Chromosome 2

Genotypes were generated in kindred 2688 for 99 markers uniformly distributed throughout the human autosomes. Approximately 25% of the genome was excluded before positive scores were noted for marker D2S102 on the distal long arm of chromosome 2 (table 1). Additional markers in the region were genotyped in the family to define the smallest possible region within which the FPD gene must reside (table 1). A maximum LOD score (4.64) was calculated for marker D2S102 at $\theta = 0$ by using an assumed penetrance of .95. There were no obligate recombinants with this marker, but one asymptomatic individual (22064) is an 8 year old who carries the disease haplotype. When analysis was performed with the penetrances of .7 and .99, the LOD scores were 4.86 and 4.31, respectively. Obligate recombinations were noted with markers D2S128 (proximal) and D2S126 (distal), thereby limiting the FPD1-containing region to ~10 cM (table 1, fig. 2).

Discussion

Paroxysmal dyskinesias are a clinically heterogeneous group of movement disorders that may be acquired, sporadic, or familial. Genetic mapping of these disorders will lead to identification of disease genes and a classification based on genetic and molecular defects. We report the identification of the FPD1 locus on chromosome 2q in a family with infantile-to-early childhood onset of a non-kinesiogenic paroxysmal dystonia with choreoathetosis that responds dramatically to very-low-dose benzodiazepines. Penetrance appears high, although one individual (22064) carries the disease haplotype but has had no episodes suggestive of this disorder. This individual is only 8 years old, and so it is not possible to discern whether she is a nonpenetrant gene carrier or is too young to manifest the phenotype.

Paroxysmal disorders include such rare familial disorders as the periodic paralyses, episodic ataxias, long QT syndrome, startle disease, and FPD. More common paroxysmal diseases such as epilepsy also have genetic components. It is interesting that an increased incidence of epilepsy is present in families with FPD (Fahn 1994; Demirkiran and Jankovic 1995), but the question of whether paroxysmal dyskinesias represent forms of epilepsy has never been resolved.

Work in the periodic paralyses has elucidated the genetic and molecular basis of these disorders and has served as a paradigm for other paroxysmal disorders such as long QT syndrome (cardiac dysrhythmia) and periodic ataxia. The periodic paralyses were found to be caused by mutations in voltage-gated sodium (Ptáček et al. 1991, 1992, 1993; Rojas et al. 1991; McClatchey et al. 1992) and calcium (Jurkat-Rott et al. 1994; Ptáček et al. 1994) channels. Subsequently, one form of periodic ataxia and the long QT syndrome were shown to be caused by mutations in voltage-gated potassium and sodium channels (Browne et al. 1994; Curran et al. 1995; Wang et al. 1995, 1996). Startle disease is a disorder of excessive startle in response to sudden, unexpected stimuli and results from mutations in the glycine receptor α-1 subunit (Shiang et al. 1993).

Table 1

LOD Scores for FPD Kindred 2688 with Chromosome 2q Markers

	LOD Score at θ =								
	.00	.01	.05	.10	.20	.30	.40	Z_{max}	θ_{max}
D2S128	$-\infty$	.07	1.12	1.30	1.06	.61	.21	1.30	.10
UT6232	$-\infty$	.39	.87	.91	.73	.46	.20	.92	.09
UT1459	4.22	4.20	4.02	3.69	2.83	1.83	.80	4.22	.00
D2S102	4.64	4.62	4.42	4.06	3.15	2.08	.94	4.64	.00
D2S126	$-\infty$	2.25	2.76	2.73	2.20	1.41	.57	2.79	.07

On the basis of this knowledge, voltage-gated ion channels in the CNS are good candidates as the site of defect in FPD. Ligand-gated ion channels such as γ-aminobutyric acid (GABA) and glutamate receptors are important for modulation of neuronal excitability and also need to be considered as potentially having a role in causing these disorders.

Notably, movement-induced choreoathetosis can sometimes be seen in the patients with episodic ataxia and mutations in the KCNA1 potassium channel gene (Browne et al. 1994). Furthermore, a locus for a form of paroxysmal choreoathetosis with spasticity has recently been mapped to a potassium channel gene cluster on chromosome 1p (Auburger et al. 1996).

Genes encoding ion channels and other proteins that modulate membrane excitability are excellent candidates for the pathogenesis of FPD. One such gene maps near the FPD1 locus (fig. 2). AE3 encodes a sodium-independent anion exchanger that functions as an alkali extruder (Kopito et al. 1989; Linn et al. 1992; Su et al. 1994; Yannoukakos et al. 1994). It is widely expressed with above-average hybridization in the deep pontine gray matter, tegmentum of the midbrain, and the medulla (Kopito et al. 1989). Despite the role of $GABA_A$ receptors as the principle mediators of synaptic inhibition in the brain, dendritic $GABA_A$ receptors depolarize neurons when intensely activated. Under these conditions, there is greater movement of HCO_3^- out of a cell than inward Cl^- current. Staley et al. (1995) proposed that this GABA-mediated, activity-dependent depolarization could account for collapse of the chloride gradient, depolarization of the cell, and modulation of synaptic N-methyl-D aspartate–receptor activation. In such a model, mutations in AE3 might alter the regulation of this phenomenon and lead to hyperexcitability of neurons.

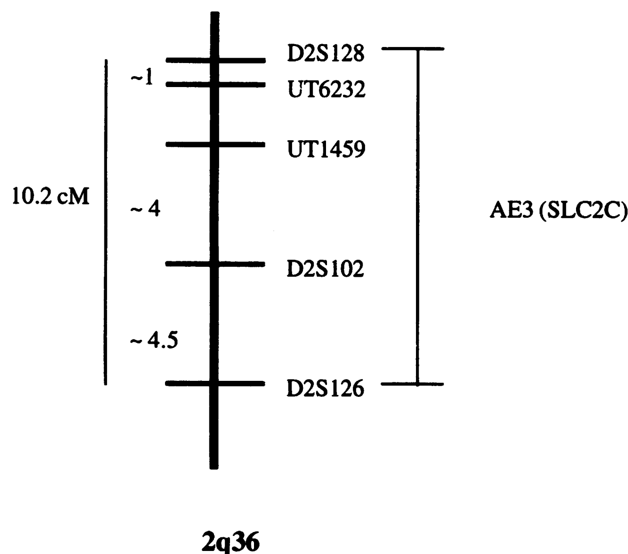

Figure 2 Genetic map of the region containing the FPD1 locus. Recombination distances between markers are given in centimorgans. The candidate gene AE3 (SLC2C) has been mapped between markers D2S128 and D2S126.

No consensus exists on the subject of whether paroxysmal dyskinesias represent a form of epilepsy (Goodenough et al. 1978; Fahn 1994; Demirkiran and Jankovic 1995). Abnormalities have been noted on EEG of patients with these disorders (Hirata et al. 1991; Lombroso 1995), but whether this represents a causal or coincidental relationship remains unknown. It is of note that one patient in K2688 demonstrated EEG abnormalities interictally.

Further study of kindred 2688, and other chromosome 2q– linked families will lead to identification of this FPD gene and the potential for molecular diagnosis. Characterization of the mutant protein may then shed light on the pathogenesis of these disorders and their relationship to epilepsy, and suggest new avenues for treatment of these patients.

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