Am. J. Hum. Genet. 59:140–145, 1996

Paroxysmal Dystonic Choreoathetosis: Tight Linkage to Chromosome 2q

John K. Fink,[1,2] Shirley Rainier,[1] Jodi Wilkowski,[1] Sandra M. Jones,[1] Akito Kume,[1] Peter Hedera,[1] Roger Albin,[1,2] Joan Mathay,[1] Lynette Girbach,[1] Tena Varvil,[3] Brith Otterud,[3] and Mark Leppert[3]

[1]Department of Neurology, University of Michigan, and [2]Geriatric Research, Education, and Clinical Center, Veterans Affairs Medical Center, Ann Arbor; and [3]Howard Hughes Medical Institute and the Eccles Institute of Human Genetics, University of Utah, Salt Lake City

Summary

Paroxysmal dystonic choreoathetosis (PDC) is characterized by attacks of involuntary movements that last up to several hours and occur at rest both spontaneously and following caffeine or alcohol consumption. We analyzed a Polish-American kindred with autosomal dominant PDC and identified tight linkage between the disorder and microsatellite markers on chromosome 2q (maximum two-point LOD score 4.77; recombination fraction 0). Our results clearly establish the existence of a locus for autosomal dominant PDC on distal chromosome 2q. The fact that three other paroxysmal neurological disorders (periodic ataxia with myokymia and hypo- and hyperkalemic periodic paralysis) are due to mutation in ion-channel genes raises the possibility that PDC is also due to an ion-channel gene mutation. It is noteworthy that a cluster of sodium-channel genes is located on distal chromosome 2q, near the PDC locus. Identifying the PDC locus on chromosome 2q will facilitate discovery of the PDC gene and enable investigators to determine whether PDC is genetically homogeneous and whether other paroxysmal movement disorders are also genetically linked to the PDC locus.

Introduction

Paroxysmal dystonic choreoathetosis (PDC) (MIM 11880; also known as "paroxysmal nonkinesiogenic dyskinesia") is characterized by attacks of involuntary movements (dystonia, chorea, and athetosis) occurring at rest (Mount and Reback 1940; Richards and Barnett 1968; Lance 1977; Nakano et al. 1982; Fahn 1987; Demirkiran and Jankovic 1995). In contrast to paroxysmal kinesiogenic, exertional, and hypnogenic choreoathetosis, PDC is not precipitated by sudden movements, exertion, or sleep. Beginning with early childhood through early adulthood, PDC subjects experience paroxysms of involuntary movements affecting the extremities, trunk, and face and often associated with dysarthria and dysphagia. Although mild episodes consist of tightening of muscles in the extremities, followed by choreoathetosis and involuntary postures (dystonia), involuntary movements may be severe and prevent walking or functional use of the arms and hands. Involuntary movements affecting oral and facial muscles may cause facial grimacing and difficulty speaking, chewing, and swallowing. Episodes last from minutes to hours and may occur several times each day. Although episodes occur spontaneously at rest, they typically are precipitated by caffeine and alcohol and, to a lesser extent, by fatigue, hunger, and emotional stress.

The cause of PDC is unknown, and treatment is often unsatisfactory. Neurological examination between attacks is normal. Neuroimaging of the brain is normal, as are two published postmortem brain analyses. Genetic linkage analysis and positional cloning are important approaches to finding the molecular basis of PDC, since the disease has no known biochemical markers that can be monitored. We report the results of a genomewide search for the PDC locus in one kindred and the observation of tight linkage of PDC to a locus on chromosome 2q.

Subjects and Methods

Subjects

We evaluated 28 members of a nonconsanguineous, North American kindred of Polish descent (fig. 1). Symptom onset occurred by age 2 years and continued throughout life. Paroxysmal dyskinesia began as a sense of muscle tightening, typically in an extremity, followed by dystonic postures and choreoathetoid movements of that extremity. Symptoms either began in many regions simultaneously or appeared to spread from one region to another. Involuntary movements also affected the face, jaw, and tongue. Spells varied in duration (<30 min to more than several hours) and occurred up to several times each week, at rest, both spontaneously and

Received April 3, 1996; accepted for publication May 3, 1996.

Address for correspondence and reprints: Dr. John K. Fink, Department of Neurology, University of Michigan Medical Center, Medical Science Research Building III, 1150 West Medical Center Drive, Room 6301, Ann Arbor, MI 48109-0642.

0002-9297/96/5901-0020$02.00

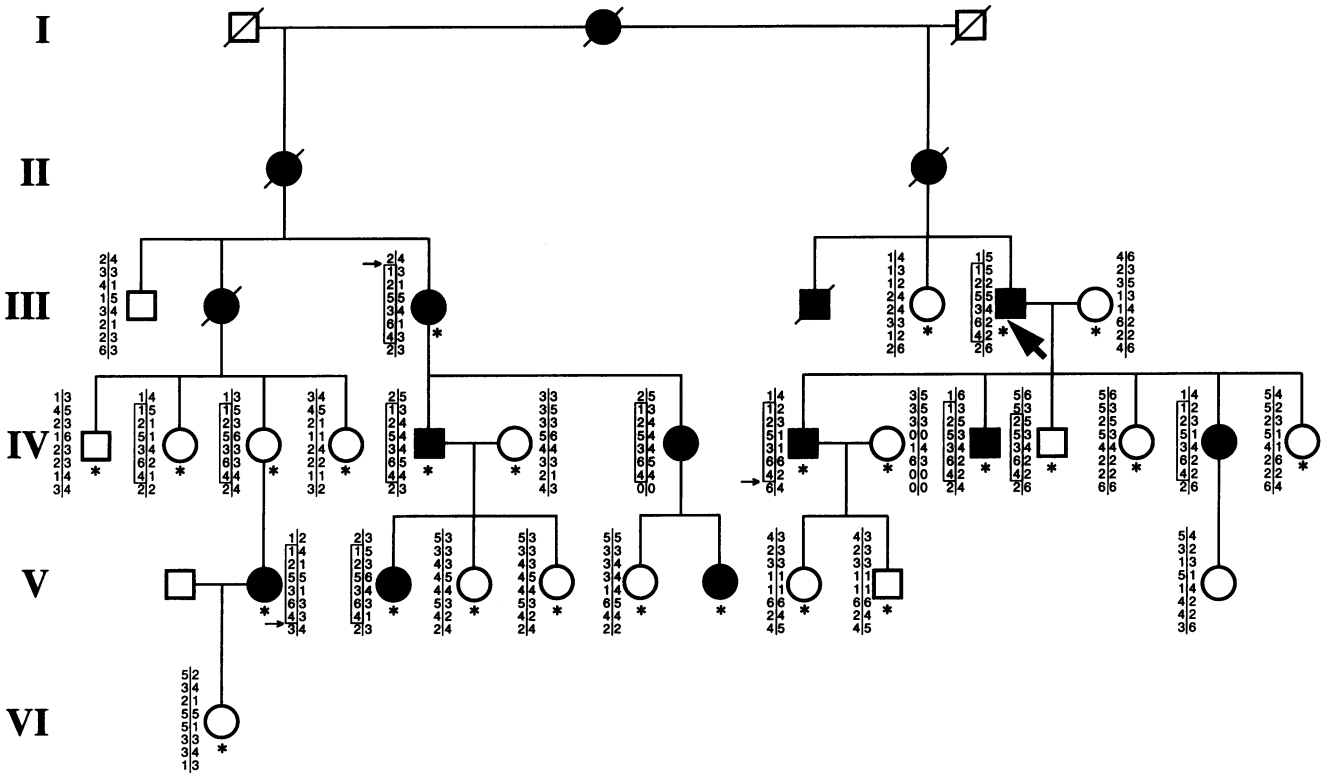

Figure 1 Paroxysmal dystonic choreoathetosis: genotypes for linked and flanking markers. Genotypes are shown for the following markers (in descending order): D2S164, D2S173, D2S434, D2S163, D2S339, D2S130, and D2S159. Disease alleles are enclosed by boxes. □ = Unaffected male >5 years of age; ○ = unaffected female >5 years of age; ■ = affected male; and ● = affected female. An asterisk (*) denotes the subject examined; and a smaller, horizontal arrow (→) denotes an obligate recombinant.

following caffeine and alcohol consumption. Several patients reported that diazepam or clonazepam, taken prophylactically each day or at the first signs of an attack, was moderately effective in preventing PDC episodes or lessening their severity. Neurological examinations between attacks were entirely normal, with the exception of the proband who had childhood polio and one subject who had facial tics (blinking). PDC in this family is transmitted as an autosomal dominant trait with incomplete penetrance (subject IV-3 [fig. 1] was entirely asymptomatic and did not abstain from caffeine or alcohol, which are known to precipitate PDC attacks). Her daughter (V-1 [fig. 1]) had typical attacks of PDC (including one videotaped spell that was reviewed).

We performed genetic linkage analysis using 28 subjects: 9 living affected subjects, 16 living unaffected subjects, and 3 spouses of descendants. "At-risk" subjects were not included in genetic linkage analysis. Unaffected subjects were neurologically asymptomatic, had normal neurological examinations, and were >5 years old. The spouses of descendants were asymptomatic and had normal neurological examinations, and there was no evidence of similar neurological disorders in their families.

Methods

Patients.—Pedigree information was obtained from many relatives. Of these 28 subjects, 25 were interviewed, examined, and videotaped. Samples were also obtained from two subjects who were interviewed but not examined and from one subject who, according to his sister, was entirely asymptomatic but who was not interviewed. Informed consent was obtained from each subject, as specified by the University of Michigan institutional review board. Subjects were diagnosed at the time of blood collection and prior to genotyping. Subjects were diagnosed as follows: (1) *unaffected*—subject was neurologically asymptomatic, had a normal neurological examination, and was >5 years old; (2) *affected*—subject reported (and family members corroborated) recurrent episodes of dystonic-choreoathetosis occurring at rest, without disturbance of consciousness; interictal examination was normal for all affected subjects, except for the subject who had childhood polio and the subject who had occasional facial tics; or (3) *at-risk*—the subject was <5 years old and a neurologically asymptomatic first-degree relative of a PDC patient. Deceased subjects were diagnosed as "affected" or "unaffected," on the basis of the descriptions by at least two

relatives. Age at symptom onset was obtained by interviewing nine living affected subjects. Estimated age at symptom onset in deceased subjects was not used in this calculation. Electroencephalograms obtained prior to this study in the proband and in one other affected subject during paroxysmal dyskinesia were reportedly normal. Episodes in two subjects were witnessed; a third subject's videotaped spell was reviewed.

Genotyping.—DNA was extracted from peripheral blood leukocytes, as described elsewhere (Bell et al 1981). Microsatellite DNA polymorphisms used to genotype subjects were amplified by the PCR, according to standard procedures Amplifications were performed in 25-μl volumes in 96-well trays by using Coy thermocyclers for 35 cycles One primer was labeled with 32P-dATP by using T4 polynucleotide kinase Amplified DNA was electrophoresed on 7% polyacrylamide/6 M urea-formamide gels, and alleles were scored from autoradiographs

Linkage analysis.—Two-point linkage analyses (table 1) were performed with the MLINK subroutine of the LINKAGE program (Lathrop et al 1985), by using an autosomal dominant model of disease inheritance with genetic penetrance of .90 and disease-allele frequency of .001. The LOD score was calculated with marker-allele frequencies assumed to be equal

Results

Exclusion Analysis

We analyzed 265 microsatellite polymorphisms, on chromosomes 1–22 and spaced approximately every 10–15 cM, for linkage with the disorder in our PDC kindred. Included among these loci were markers near other inherited neurological disorders, including dopa-responsive dystonia (Nygaard et al. 1993), paroxysmal ataxia with myokymia (Litt et al. 1994), and paroxysmal ataxia without myokymia (Teh et al. 1995; Vahedi et al. 1995; von Brederlow et al. 1995). Our results (data not shown) strongly excluded the PDC locus from loci associated with these potentially related neurological disorders.

Linkage to Chromosome 2q

After exclusion of candidate regions and a thorough genomewide search, genetic linkage to a set of contiguous genetic markers on chromosome 2q33-35 was established. The maximum pairwise LOD score obtained in this family, under the assumptions of .90 penetrance and an autosomal dominant gene, was 4.77 at a recombination fraction of 0, for the marker D2S173 (AFM249wg9). An additional three markers—D2S434 (GATA4G12), D2S377 (AFM319zf9), and D2S339 (AFM277vb9)—also yielded LOD scores >3.0 (table

Table 1

Results of Two-Point Linkage Analysis for Microsatellite Polymorphisms on Chromosome 2q

Marker and Penetrance	LOD Score at Recombination Fraction of								Maximum LOD Score	Maximum Recombination Fraction
	.0	.001	.01	.05	.10	.20	.30	.40		
D2S173:										
.70	4.30	4.29	4.22	3.89	3.46	2.53	1.53	.57	4.30	.001
.80	4.58	4.57	4.50	4.18	3.74	2.78	1.72	.66	4.58	.001
.90	4.77	4.76	4.71	4.42	4.01	3.05	1.93	.76	4.77	.001
D2S434:										
.70	3.11	3.11	3.06	2.82	2.52	1.88	1.21	.57	3.11	.001
.80	3.31	3.30	3.25	3.01	2.70	2.03	1.33	.63	3.31	.001
.90	3.49	3.48	3.43	3.20	2.89	2.20	1.46	.70	3.49	.001
D2S163:										
.70	2.41	2.41	2.37	2.20	1.98	1.50	1.00	.51	2.41	.001
.80	2.51	2.51	2.47	2.32	2.10	1.62	1.10	.56	2.51	.001
.90	2.50	2.50	2.48	2.38	2.20	1.75	1.21	.63	2.50	.001
D2S377:										
.70	3.30	3.29	3.24	3.00	2.67	1.94	1.15	.41	3.30	.001
.80	3.38	3.37	3.33	3.12	2.82	2.11	1.29	.48	3.38	.001
.90	3.23	3.23	3.22	3.13	2.91	2.26	1.43	.55	3.23	.001
D2S339:										
.70	3.00	2.99	2.93	2.67	2.32	1.60	.90	.34	3.00	.001
.80	3.17	3.17	3.11	2.85	2.50	1.75	1.00	.38	3.17	.001
.90	3.25	3.25	3.20	2.98	2.65	1.91	1.11	.43	3.25	.001
D2S130:										
.70	2.78	2.78	2.73	2.51	2.21	1.59	.92	.29	2.78	.001
.80	2.85	2.84	2.80	2.59	2.30	1.67	.98	.32	2.85	.001
.90	2.82	2.82	2.79	2.63	2.37	1.76	1.05	.35	2.82	.001

1). Marker D2S434 was placed on the Généthon map (Gyapay et al. 1994) at the D2S173 locus by using the CLODSCORE subroutine of the LINKAGE program (Lathrop et al. 1985). Marker D2S173 is fully informative in this family. The genotypes for all markers with no recombination and for D2S164 (AFM234xb8) and D2S159 (AFM218zg3), the two adjacent flanking markers showing obligate recombinants, are shown with the pedigree (fig. 1). Inspection of the genotypes indicated that unaffected subject IV-2 had the disease haplotype (allele numbers 1, 2, 5, 3, 6, and 4 for markers D2S173, D2S434, D2S163, D2S377, D2S339, and D2S130, respectively). This 43-year-old individual was entirely asymptomatic and did not refrain from consuming alcohol or caffeinated substances known to precipitate PDC attacks. Subject IV-11 is an entirely asymptomatic 41-year-old male. Analysis of his genotypes indicates that he has disease-allele genotypes distal to D2S173 (genotypes 2, 5, 4, 6, and 4 for markers D2S434, D2S163, D2S377, D2S339, and D2S130, respectively) and normal allele genotypes for the proximal linked marker D2S173. In view of his clinically unaffected status, he represents either genetic nonpenetrance (in which case the disease locus is between D2S434 and D2S159) or a nonobligate recombinant (in which case the disease locus is between D2S173 and D2S164). Recognizing that subject IV-2 and possibly IV-11 represent additional, asymptomatic carriers, we repeated pairwise LOD-score analyses using varying estimates of penetrance from .70–.90. Even at a lowered penetrance of .7, all four markers with LOD scores >3.0 remained statistically significant. The most informative marker, D2S173, gave a calculated LOD score of 4.30 when penetrance of .7 was assumed.

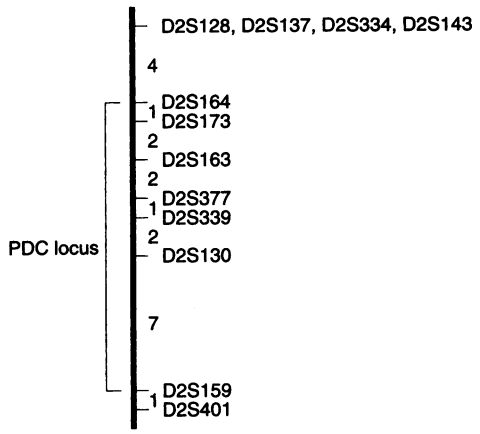

Figure 2 Paroxysmal dystonic choreoathetosis locus on chromosome 2q33-35.

Discussion

Our data clearly establish the existence of a locus for autosomal dominant PDC, in the distal region of chromosome 2q. Previous physical mapping (Spurr et al. 1994) of microsatellite markers allows us to assign the PDC locus to chromosome 2q33-35. At present, our data localize this region to an ~15-cM interval lying between the two flanking markers D2S164 and D2S159 (fig. 2). Analysis of additional telomeric flanking markers may increase the precision of this localization.

Our patients differ, in two important respects, from those with autosomal dominant paroxysmal choreoathetosis/spasticity (CSE), recently mapped (Auburger et al. 1996) to a 2-cM region on chromosome 1p. First, episodic dyskinesia in our patients occurs spontaneously at rest; physical exercise was a precipitating factor for CSE patients. Second, none of our patients had spasticity; 5 of 18 CSE patients had constant spastic paraplegia. We consider the disorder in our kindred to be "pure PDC," insofar as (*a*) paroxysmal extrapyramidal disturbance is not accompanied by ataxia or other neurological impairments and (*b*) patients are neurologically normal and asymptomatic between attacks. Although the description of involuntary movements and dystonic postures in their CSE patients is similar to those in our PDC kindred and in those recently reviewed elsewhere (Demirkiran and Jankovic 1995), Auberger et al. (1996) considered the disorder in their kindred to be a variant of episodic ataxia (even though ataxia was not seen in the episodes observed). In this circumstance, it seems appropriate to consider autosomal dominant PDC to be a clinically heterogeneous group of disorders with loci identified for pure PDC (chromosome 2q33-35) and "PDC/spasticity" (also known as CSE; chromosome 1p) (Auburger et al. 1996). Autosomal dominant episodic ataxia refers to a similarly heterogeneous group of disorders; a locus for "pure" episodic ataxia exists (Teh et al. 1995; Vahedi et al. 1995) on chromosome 19p13; episodic ataxia/myokymia is due to potassium-channel KCNA1 gene mutations (Browne et al. 1994; Litt et al. 1994) on chromosome 12p13. This clinico-genetic classification undoubtedly will be modified as loci for additional paroxysmal cerebellar and extrapyramidal syndromes (such as paroxysmal kinesiogenic choreoathetosis) are discovered.

There is precedent for other paroxysmal neurological disorders affecting cerebellum and muscle to be associated with mutations in the following ion-channel genes: sodium-channel SCNA4 (hyperkalemic periodic paralysis, paramyotonia congenita, and acetozolamide-responsive myotonia congenita), potassium-channel KCNA1 (periodic ataxia with myokymia), and calcium-channel CACNLIA3 (hypokalemic periodic paralysis) (Rojas et al. 1991; McClatchey et al. 1992; Browne et al. 1994; Fontaine et al. 1994; Jurkat-Rott et al. 1994; Ptacek et al. 1994*a*, 1994*b*). This observation raises the possibility that PDC is also due to an ion-channel gene mutation. Unlike PDC, paroxysmal neurological disorders due to ion-channel gene mutations have significant neuromus-

cular involvement. Nonetheless, there is one reported kindred in which some patients had myokymia in addition to PDC (Byrne et al. 1991). It is noteworthy that the clinically similar disorder CSE, described above, maps (Auburger et al. 1996) near a cluster of potassium-channel genes on chromosome 1p. These observations raise the possibility that PDC is due to mutation in an ion-channel gene whose expression is limited to the brain.

It is intriguing that a cluster of ion-channel genes exists on chromosome 2q, near the PDC locus. Sodium-channel genes (SCN1a, SCN2a, and SCN3a, on chromosome 2q24; and SCN6a, on chromosome 2q21-24) and the potassium-channel gene (KCN3a, on chromosome 2q21) are cytogenetically mapped proximal to the PDC locus (chromosome 2q33-35) (Litt et al. 1989; Malo et al. 1994*a*, 1994*b*; Stoffel et al. 1994). This makes SCN1a, SCN2a, SCN3a, SCN6a, and KCN3a unlikely candidates for PDC. It is possible that PDC is related to disturbance in a novel ion-channel gene also located in this region. Following identification of the PDC locus on chromosome 2q33-35, we can now determine whether "pure" PDC is genetically hetero- or homogeneous and the extent to which other paroxysmal movement disorders (such as paroxysmal kinesiogenic dystonia) are genetically related to PDC. In addition to providing insight into PDC's pathophysiology and treatment, identification of the PDC gene will advance our understanding of the complex neurophysiological effects of alcohol and caffeine, both of which induce episodes of PDC.

Acknowledgments

This research is supported by grants from the Muscular Dystrophy Association, American Paralysis Association, National Association for Research in Schizophrenia and Depression (NARSAD), and National Institutes of Health (NINDS grant 1R01NS33645) (all to J.K.F). We gratefully acknowledge the support of the Technology Access Section of the Utah Center for Human Genome Research and the technical assistance of Ms. Aubrey Lesicki, Mr. Todd Reinglass, Mr. Nickesh Pahade, and Ms. Daina Druva. This research would not be possible without the participation of PDC patients and their family members.

References

Auburger G, Ratzlaff T, Lunkes A, Nelles HW, Leube B, Binkofski F, Kugel H, et al (1996) A gene for autosomal dominant paroxysmal choreoathetosis/spasticity (CSE) maps to the vicinity of a potassium channel gene cluster on chromosome 1p, probably within 2 cM between D1S443 and D1S197. Genomics 31:90–94

Bell GJ, Karam JH, Rutter WJ (1981) Polymorphic DNA region adjacent to the 5′ end of the human insulin gene. Proc Natl Acad Sci USA 78:5759–5763

Browne DL, Gancher ST, Nutt JG, Brunt ERP, Smith EA, Kramer P, Litt M (1994) Episodic ataxia/myokymia syndrome is associated with point mutations in the human potassium channel gene, KCNA1. Nat Genet 8:136–140

Byrne E, White O, Cook M (1991) Familial dystonic choreoathetosis with myokymia; a sleep responsive disorder. J Neurol Neurosurg Psychiatry 54:1090–1092

Demirkiran M, Jankovic J (1995) Paroxysmal dyskinesias: clinical features and classification. Ann Neurol 38:571–579

Fahn S (1987) Paroxysmal myoclonic dystonia with vocalisations. J Neurol Neurosurg Psychiatry 50:117–118

Fontaine B, Vale-Santos J, Jurkat-Rott K, Reboul J, Plassart E, Rime CS, Elbaz A, et al (1994) Mapping of the hypokalaemic periodic paralysis (HypoPP) locus to chromosome 1q31-32 in three European families. Nat Genet 6:267–272

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, et al (1994) The 1993–94 Généthon human genetic linkage map. Nat Genet 7:246–339

Jurkat-Rott K, Lehmann-Horn F, Elbaz A, Heine R, Gregg RG, Hogan K, Powers PA, et al (1994) A calcium channel mutation causing hypokalemic periodic paralysis. Hum Mol Genet 3:1415–1419

Lance JW (1977) Familial paroxysmal dystonic choreoathetosis and its differentiation from related syndromes. Ann Neurol 2:285–293

Lathrop GM, Lalouel JM, Julier C, Ott J (1985) Multipoint linkage analysis in humans: detection of linkage and estimation of recombination. Am J Hum Genet 37:482–498

Litt M, Kramer P, Browne D, Gancher S, Brunt ER, Root D, Phromchotikul T, et al (1994) A gene for episodic ataxia/myokymia maps to chromosome 12p13. Am J Hum Genet 55:702–709

Litt M, Luty J, Kwak M, Allen L, Magenis RE, Mandel G (1989) Localization of a human brain sodium channel gene (SCNA2) to chromosome 2. Genomics 5:204–208

Malo MS, Blanchard BJ, Andresen JM, Srivastava K, Chen X-N, Li X, Jabs EW, et al (1994*a*) Localization of a putative human brain sodium channel gene (SCN1A) to chromosome band 2q24. Cytogenet Cell Genet 67:178–186

Malo M, Srivastava K, Andresen JM, Chien X, Korenberg JR, Ingram VM (1994*b*) Targeted gene walking by low stringency polymerase chain reaction: assignment of a putative human brain sodium channel gene (SCNA3A) to chromosome 2q24-31. Proc Natl Acad Sci USA 91:2975–2979

McClatchey AI, McKenna-Yasek D, Cros D, Worthen HG, Kuncl RW, DeSilva SM, Cornblath DR, et al (1992) Novel mutations in families with unusual and variable disorders of the skeletal muscle sodium channel. Nat Genet 2:148–152

Mount LA, Reback S (1940) Familial paroxysmal choreoathetosis: preliminary report on a hitherto undescribed clinical syndrome. Arch Neurol Psychiatry 44:841–847

Nakano T, Kondo K, Oguchi K, Yanagisawa N (1982) A late onset case of paroxysmal dystonic choreoathetosis induced by caffeine and aminophylline. Clin Neurol 23:199–202

Nygaard T, Wilhelmsen K, Risch N, Brown D, Trugman J, Gilliam T, Fahn S, et al (1993) Linkage mapping of dopa-responsive dystonia (DRD) to chromosome 14q. Nat Genet 5:386–391

Ptacek LJ, Tawil R, Griggs RC, Engel AG, Layzer RB, Kwiecinski H, McManis PG, et al (1994*a*) Dihydropyridine receptor mutations cause hypokalemic periodic paralysis. Cell 77: 836–838

Ptacek LJ, Tawil R, Griggs RC, Meola G, McManis P, Barohn RJ, Mendell JR, et al (1994*b*) Sodium channel mutations in acetazolamide-responsive myotonia congenita, paramyotonia congenita, and hyperkalemic periodic paralysis. Neurology 44:1500–1503

Richards RN, Barnett HJM (1968) Paroxysmal dystonic choreoathetosis. Neurology 18:461–469

Rojas CV, Wang JZ, Schwartz LS, Hoffman EP, Powell BR, Brown RHJ (1991) A Met-to-Val mutation in the skeletal muscle Na+ channel alpha-subunit in hyperkalaemic periodic paralysis. Nature 354:387–389

Spurr NK, Barton H, Bashir R, Bryson GM, Bushby K, Cox S, Gringrich JC, et al (1994) Report of the Third International Workshop on Human Chromosome 2 Mapping 1994. Cytogenet Cell Genet 67:216–237

Stoffel M, Espinosa R III, Powell K, Philipson L, Le Beau M, Bell G (1994) Human G-protein-coupled inwardly rectifying potassium channel (GIRK1) gene (*KCNJ3*): localization to chromosome 2 and identification of a simple tandem repeat polymorphism. Genomics 21:254–256

Teh BT, Silburn P, Lindblad K, Betz R, Boyle R, Schalling M, Larsson C (1995) Familial periodic cerebellar ataxia without myokymia maps to a 19-cM region on 19p13. Am J Hum Genet 56:1443–1449

Vahedi K, Joutel A, Van Bogaert P, Ducros A, Maciazeck J, Bach JF, Bousser MG, et al (1995) A gene for hereditary paroxysmal cerebellar ataxia maps to chromosome 19p. Ann Neurol 37:289–293

von Brederlow B, Hahn AF, Koopman WJ, Ebers GC, Bulman DE (1995) Mapping the gene for acetazolamide responsive hereditary paroxysmal cerebellar ataxia to chromosome 19p. Hum Mol Genet 4:279–284