Paroxysmal dystonic choreoathetosis
Genetic linkage studies in a British family

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Summary
Paroxysmal dystonic choreoathetosis (PDC) is characterized by attacks of involuntary dystonic and choreoathetoid movements, typically several hours in duration with no sign of abnormality between attacks. Inheritance is autosomal dominant and the PDC locus has recently been assigned to the distal long arm of chromosome 2 in two families. We describe a six-generation British family with PDC and describe the results of fine genetic mapping and candidate gene linkage analysis. As part of a genome-wide search, linkage to chromosome 2q was confirmed in this family. Positive LOD scores were obtained for six markers on 2q. A LOD score of 5.08 at a recombination fraction of 0.0 was obtained for the marker D2S163. Construction of haplotypes allowed definition of a disease interval of 4 cM between the flanking markers D2S295 and D2S377. Polymorphic tandem repeats within the candidate genes CHRND (delta polypeptide of the nicotinic acetylcholine receptor) and SLC4A3 were examined yielding LOD scores of −7.68 and 6.08, respectively, at a recombination fraction of 0.0. This excludes CHRND as a candidate. Our data confirm the assignment of the locus for PDC to chromosome 2q and provide evidence for locus homogeneity in PDC. We have narrowed the disease interval to 4 cM and our findings provide support for the involvement of the gene for the chloride/bicarbonate exchanger as a candidate gene for PDC.

Keywords: dystonia; paroxysmal; paroxysmal dystonic choreoathetosis; genetics, linkage

Abbreviations: CHRND = delta polypeptide of the nicotinic acetylcholine receptor; PCR = polymerase chain reaction; PDC = paroxysmal dystonic choreoathetosis; SLC4A3 = chloride/bicarbonate anion exchanger gene

Introduction
Paroxysmal dystonic choreoathetosis (PDC) (MIM # 11880) is an autosomal dominant disorder characterized by attacks of involuntary dystonic and choreoathetoid movements. Attacks typically start as hemi-dystonia and often progress to become generalized, affecting all limbs, trunk and neck muscles as well as causing dysarthria or anarthria in most patients. Clear consciousness is preserved throughout. Attacks last anything from several minutes to several hours and can be precipitated by a number of factors, including stress, excitement, alcohol, caffeine and tiredness. Onset is usually during early childhood (Fahn, 1994). Patients are entirely normal between attacks with no abnormality on examination. PDC is frequently misdiagnosed as epilepsy or hysteria, and does not respond to anticonvulsant drugs, unlike paroxysmal kinesigenic choreoathetosis, a condition in which short-lived involuntary movements are precipitated by sudden movement.

The first clear description of a patient with PDC was by Mount and Reback in 1940 (Mount and Reback, 1940). They reported an index case with typical PDC with onset in infancy. Twenty-seven other family members over five generations appeared to be similarly affected with an autosomal dominant pattern of inheritance. They named the condition ‘familial paroxysmal choreoathetosis’. Several other families with PDC have subsequently been reported including those of Richards and Barnett (1968), who coined the term ‘paroxysmal dystonic choreoathetosis’, and Lance (1977) who distinguished PDC from other paroxysmal dyskinesias (Forssman, 1961; Lance, 1963; Weber, 1967; Richards and Barnett, 1968; Lance, 1977; Tibbles and Barnes, 1980; Kurlan et al., 1987; Byrne et al., 1991; Schloesser et al., 1996).

PDC has recently been mapped to the long arm of chromosome 2 in two families originating from Poland and Italy. Fink et al. (1996) assigned the locus to a 15 cM interval between the flanking markers D2S164 and D2S159 and Fouad et al. (1996) defined a candidate interval of ~10 cM between D2S128 and D2S126.
We report here the results of refinement of the genetic map of the PDC locus using markers on chromosome 2 in a large British family. The results of linkage analysis using polymorphisms within candidate genes mapping to the region are also described.

Methods

Subjects

We traced the family members of two apparently unrelated index cases with typical PDC. With the help of the oldest surviving relative (III-13) of one index case we were able to trace the origins of both families to an ancestor living in London in the 19th century, who was said to suffer from involuntary movements typical of PDC.

The pedigree (Fig. 1) contains 27 affected individuals (20 still living) over six generations. Thirty-nine family members were interviewed by an author (P.R.J.) and a detailed history of attacks obtained. Physical and neurological examination was performed in all but five individuals (three affected and two unaffected). Individuals <8 years old were not included in the study and do not appear in Fig. 1. Attacks were witnessed in five individuals and recorded on videotape in four. Venous blood samples were obtained from 45 family members and spouses including all but one of those interviewed. The medical records of most affected family members were obtained. Patients were designated as phenotypically affected or unaffected at the time of interview on the basis of presence or absence of a history of typical attacks. Informed consent was obtained from all subjects, and the study was approved by the Joint Medical Ethics Committee of the National Hospital for Neurology and Neurosurgery.

DNA analysis

DNA was extracted from peripheral blood leucocytes using standard techniques. Microsatellite markers were amplified from genomic DNA using the polymerase chain reaction (PCR) as previously described (Reed et al., 1994). PCR reactions were performed in a 96-well microtitre plate in a reaction volume of 20 ml using 50 ng of DNA. PCR primers
were fluorescently tagged. PCR products were analysed by electrophoresis on a 4% acrylamide gel using an automatic DNA fragment analyser (Applied Biosystems: Prism 377) and Genescan software. Genotypes were assigned using the Genotyper (Version 1.0) programme (Applied Biosystems).

**Linkage analysis**

Sixty-seven microsatellite markers distributed over 16 chromosomes were analysed. Most of these were derived from a panel of markers suitable for genome-wide linkage mapping (Reed et al., 1994). The seven microsatellite markers on chromosome 2q were from the Généthon map (Gyapay et al., 1994). Polymorphic repeat sequences at the gene loci of the chloride/bicarbonate anion exchanger gene (SLC4A3) and the delta polypeptide of the nicotinic acetylcholine receptor (CHRN) were also analysed (Landa et al., 1994; Su et al., 1994). Pairwise LOD scores between the disease locus and D2S157 (2q35), D2S164 (2q35), D2S295 (2pter-qter), D2S163 (2q33-q37), D2S377 (2pter-qter), D2S126 (2q33-q37), D2S130 (2pter-qter), SLC4A3 (2q36) and CHRN (2q36-q37) were generated using the MLINK program of the FASTLINK version 3.0P linkage package. An autosomal dominant model of inheritance was used with a disease penetrance of 80% by the age of 8 years and a disease allele frequency of 0.0001.

**Haplotype analysis**

Haplotypes were assigned manually.

**Results**

**Patients**

Attacks typically started as a sensation of tightness in one limb followed by the onset of hemidystonia and chorea. Patients reported that if they were able to fall asleep at the onset of an attack, even for as short a period as 5 min, this would reliably abort the attack. If an attack could not be aborted it would usually progress to become generalized, resulting in dystonia or chorea and dystonia with choreoathetoid movements involving all limbs. Onset of symptoms was in infancy or early childhood (at <=5 years of age) in all cases. The duration of attacks varied between 30 min and 24 h, but most episodes lasted 2–3 h. Patients were fully aware throughout. Precipitants included alcohol, caffeine, excitement, stress, tiredness and hunger. Sudden movements did not trigger attacks. The frequency of attacks varied considerably between affected family members: from three to four episodes per week to only one episode per year. All adult patients reported a marked tendency for attacks to become less frequent as they became older, the highest frequency of attacks being during childhood and adolescence. Affected individuals were entirely normal between attacks.

General physical and neurological examination was normal in unaffected individuals and in affected individuals between attacks. There was no myokymia or spasticity. No family members gave a history of exertional cramping. Investigations performed in several patients, including brain imaging and EEG, were normal.

Twenty-six out of 66 at risk family members were themselves affected suggesting a penetrance of 79% in this pedigree. This figure depends on accurate recall by family members of affection status of individuals in generation II, many of whom are no longer alive. However, this figure is broadly in line with the 84% penetrance calculated by combining data from all the PDC kindreds with an unequivocal phenotype previously reported (Mount and Reback, 1940; Forssman, 1961; Weber, 1967; Richards and Barnett, 1968; Lance, 1977; Tibbles and Barnes, 1980; Byrne et al., 1991). There are 15 male-to-male transmissions in this pedigree.

**Linkage analysis**

Sixty polymorphic microsatellite markers on 16 chromosomes were analysed prior to the reported linkage on chromosome 2; there was no evidence for linkage to PDC for any of these markers. Following the assignment of the PDC locus to chromosome 2 in two other families, this region was examined in detail using seven closely spaced microsatellite markers mapping to this region. The Généthon map places these in the order: cen-D2S157-(9cM)-D2S164-(1cM)-D2S295-(2cM)-D2S163-(2cM)-D2S377-(2cM)-D2S126-(2cM)-D2S130-qter (Gyapay et al., 1994).

Pairwise LOD scores between microsatellite markers and PDC are given in Table 1. Maximum LOD scores for each marker were as follows: D2S164 (Z = 3.22 at θ = 0.1), D2S295 (Z = 5.22 at θ = 0.05), D2S163 (Z = 5.08 at q = 0.0), D2S377 (Z = 2.05 at θ = 0.1), D2S126 (Z = 4.13 at θ = 0.05) and D2S130 (Z = 5.87 at θ = 0.05). D2S377 was relatively uninformative in this family. These data confirm the assignment of the PDC locus to chromosome 2q in this family.

**Haplotype analysis**

Haplotypes for chromosome 2q markers are shown in Fig. 1. Construction of haplotypes allowed definition of a disease interval of 4 cM between the flanking markers D2S295 and D2S377. Informative recombinations occurred in individuals IV-19, V-5, V-9 and VI-14. Recombinations in individuals V-5 and V-9 indicate that the disease gene lies distal to D2S295. Phase could not be determined at D2S163 for individual VI-14 as DNA was not available from the unaffected father, so the exact position of the crossover could not be determined. Therefore the recombination in this individual places the disease gene proximal to D2S377. Two adult family members (V-17 and VI-9) had inherited the disease haplotype but were unaffected. Neither of these individuals abstained from alcohol or caffeine.
Candidate gene linkage analysis

Two genes which may be considered candidates for PDC are located on chromosome 2q36-q37. The chloride/bicarbonate anion exchanger (SLC4A3), a member of the family of solute transporters has been mapped to the interval between D2S126 and D2S164 at 2q36 (Su et al., 1994). CHRND is assigned to chromosome 2q36-q37. Both genes contain polymorphic tandem repeat sequences suitable for linkage studies. Two point LOD scores between these markers and PDC are given in Table 2. The CHRND marker gives a LOD score of 7.68 at a recombination fraction of 0.0 with at least two recombinations between CHRND and PDC in this family. This excludes CHRND as a candidate for PDC. There were no recombinations between PDC and SLC4A3. The SLC4A3 polymorphism gave a LOD score of 6.08 at a recombination fraction of 0.0.

Discussion

We have described a large British kindred with PDC and confirmed the assignment of the locus to the distal long arm of chromosome 2. Our data narrow the critical region for the PDC locus to a 4-cM region between the flanking markers D2S164 and D2S377. The 4-cM candidate interval for the PDC locus in our family lies within the overlapping region defined by the families described by Fink et al. (1996) and Fouad et al. (1996). Our data are therefore consistent with previously published genetic mapping data and provide further refinement of the position of the PDC locus on chromosome 2, so allowing a smaller genetic interval to be considered in future physical mapping of the disease gene.

The assignment of a British family with PDC to this region adds to the evidence for genetic homogeneity of PDC. Many members of our family exhibited marked sleep responsiveness, with rapid alleviation of episodes by very short periods of sleep, a phenomenon which has been described previously in PDC families (Forssman, 1961; Lance, 1963; Weber, 1967; Byrne et al., 1991), but in all other respects were phenotypically similar to the Italian and Polish-American kindreds mapping to 2q. The diverse geographical origins of these families suggest that in all three families the disease may have arisen as new mutations. A dominantly inherited paroxysmal movement disorder with similarities to PDC, named ‘paroxysmal choreoathetosis/spasticity’ has been assigned to a locus on chromosome 1p in a German kindred (Auburger et al., 1996). However, a constant spastic paraplegia was a prominent feature of the phenotype in this family and the age of onset was relatively late in childhood in some individuals. The attacks also differed from those in our family, and in others with classical PDC, in that diplopia and headache were features, and physical exercise was a precipitant similar to the ‘intermediate’ form of paroxysmal dystonia described by Lance (1977). Therefore, classical PDC and the form of paroxysmal choreoathetosis described by Auburger et al. (1996) appear to be distinct both clinically and genetically.

Autosomal dominant nocturnal frontal lobe epilepsy shows some similarities to PDC and has been mistaken for PDC in

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### Table 1 Pairwise LOD scores between PDC and chromosome 2q microsatellite markers

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<th>Marker</th>
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### Table 2 Pairwise LOD scores between PDC and chromosome 2q candidate gene polymorphisms

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<td>CHRND</td>
<td>−7.68</td>
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Refining the PDC locus on chromosome 2q

Fig. 2 Schematic representation of the genetic map of part of chromosome 2q showing the PDC candidate interval as determined by Fouad et al. (1996) (flanking markers D2S128 and D2S126), Fink et al. (1996) (flanking markers D2S164 and D2S159) and in the British PDC family (flanking markers D2S295 and D2S377). The position of the candidate gene SLC4A3 (mapped between flanking markers D2S128 and D2S126) (Su et al., 1994) is also shown. Sex averaged genetic distances are given in centimorgans.

clinical practice (Scheffer et al., 1995). It may be caused by mutations in the α4 subunit of the neuronal nicotinic acetylcholine receptor (Steinlein et al., 1995). Although there are no consistent EEG abnormalities associated with PDC, it has been proposed that it may represent a form of epilepsy. The delta subunit gene of the nicotinic acetylcholine receptor which maps to 2q36-q37 may therefore be considered a possible candidate for PDC. However, we were able to exclude the CHRN10 locus which does not segregate with the disease in this family.

Our data provide support for a role of the SLC4A3 gene in PDC. The SLC4A3 polymorphism yielded a LOD score of 6.08 at a recombination fraction of 0.0 and there were no recombinations between PDC and the SLC4A3 polymorphism in this family. SLC4A3 is the third member of the band-3-related family of anion exchangers. The brain isoform of the protein is expressed on neurons throughout the brain, particularly in the deep pontine grey matter, the caudal medulla and, interestingly when considering a disorder causing an abnormality of movement, a high level of expression is also seen in the substantia nigra (Alper, 1991). SLC4A3 is a membrane-bound protein which functions as a chloride/bicarbonate exchanger and an alkali extruder. It plays a role in the regulation of intracellular pH, cell volume and in regulation of intracellular chloride concentration (Kopito et al., 1989). The absence of recombination between PDC and SLC4A3, its expression pattern in the central nervous system, and physiological importance in the regulation of intra-neuronal homeostasis, all provide support for a role of this gene in PDC. Reports of patients with PDC responding to the carbonic anhydrase inhibitor acetazolamide are of interest in this context (Mayeux and Fahn, 1982; Bressman et al., 1988; Auburger et al., 1996). Acetazolamide produces a mild metabolic acidosis by inhibiting excretion of hydrogen ions in the renal tubule and could therefore be acting in these patients to correct an abnormal intracellular pH. However, not all patients with PDC respond to acetazolamide (Kurlan and Shoulson, 1983) and, in contrast to the kindreds linked to chromosome 2q, none of the patients showing an apparent response to acetazolamide were affected by pure PDC with a supporting family history.

PDC is a disabling condition which is refractory to most forms of treatment. As there are no pathological or biochemical clues to its pathogenesis, a molecular genetic approach is likely to represent the best strategy for understanding the pathophysiological basis of this condition, and for developing a rational form of treatment and means of molecular diagnosis. The next stage in such a strategy should include mutation analysis of the SLC4A3 gene.

Acknowledgements
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References


