A second paroxysmal kinesigenic choreoathetosis locus (EKD2) mapping on 16q13-q22.1 indicates a family of genes which give rise to paroxysmal disorders on human chromosome 16

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Summary
Paroxysmal kinesigenic choreoathetosis (PKC) is a rare paroxysmal movement disorder characterized by recurrent and brief attacks of choreiform or dystonic movements triggered or exacerbated by sudden voluntary movements. Some patients with PKC also have a history of infantile afebrile convulsions. PKC can be sporadic, or familial with autosomal dominant inheritance. PKC has been mapped to the pericentromeric region of human chromosome 16 in several Japanese families and in an African-American family, to regions which overlap by 9.8 cM (centiMorgan). Both regions overlap by 3.4 cM with a region containing a gene responsible for ‘infantile convulsions and paroxysmal choreoathetosis’ (ICCA). We have identified a second PKC locus (EKD2) on the long arm of chromosome 16 in a large Indian family with PKC. A maximum two-point LOD score of 3.66 (recombination fraction = 0.00, penetrance = 0.80) was obtained between PKC and D16S419. Haplotype and recombinant analysis localized EKD2 to a 15.8 cM region between D16S685 and D16S503. This region does not overlap with that identified in Japanese families, or with the ICCA locus. These results exclude one locus on chromosome 16 which causes both the ICCA and PKC syndromes; this suggests that there may be a cluster of genes on human chromosome 16 which lead to paroxysmal disorders.

Keywords: paroxysmal kinesigenic choreoathetosis; paroxysmal dyskinesia; linkage studies; haplotypes; chromosome 16

Abbreviations: ICCA = infantile convulsions and paroxysmal choreoathetosis; PKC = paroxysmal kinesigenic choreoathetosis; RE-PED-WC = rolandic epilepsy with paroxysmal exercise-induced dystonia and writer’s cramp

Introduction
Paroxysmal kinesigenic choreoathetosis (PKC; MIM 128200) is a relatively rare form of paroxysmal dyskinesia which has been recognized increasingly since its first description in the early 1960s (Kertesz, 1967). It is characterized by sudden brief attacks of unilateral or bilateral involuntary movements, including dystonic postures, chorea, athetosis or ballism, precipitated by sudden movement (Kertesz, 1967). The attacks occur most commonly when a patient stands up quickly from a sitting position; however, startle, hyperventilation and continuous exercise can also trigger them. The attacks usually last from seconds to 1–2 min, and occasionally up to 5 min. There can be up to 100 attacks per day, but the frequency of the attacks commonly decreases with age. Age at onset is usually during childhood or early adulthood, but can range from 6 months to 40 years. Consciousness is preserved. Males are affected more often than females, with an estimated ratio of 3 : 1 to 4 : 1 (Fahn, 1994; Bhatia, 1999). Some PKC patients or their relatives have a history of afebrile infantile convulsions, usually with a favourable outcome (Hudgins and Corbin, 1966; Sadamatsu et al., 1999). The disorder responds to antiepileptic drugs such as carbamazepine or phenytoin.

PKC can occur in a sporadic form, but most cases are familial with autosomal dominant inheritance and reduced
A second PKC locus on chromosome 16 penetrance (Fahn, 1994; Marsden, 1996). A PKC locus recently has been mapped to the pericentromeric region of chromosome 16 in eight Japanese families (Tomita et al., 1999) and in an African-American kindred (Bennett et al., 2000). The PKC region in Japanese families spans 12.4 cM (centiMorgan) and overlaps by 6.0 cM with a region responsible for ‘infantile convulsions and paroxysmal choreoathetosis’ (ICCA syndrome; MIM 602066), a recently identified neurological disorder with benign infantile convulsions and paroxysmal dyskinesias (Szepetowski et al., 1997; Lee et al., 1998). There is an increased prevalence of afebrile infantile convulsions in the Japanese families with PKC and it has been suggested that one gene may be responsible for both PKC and ICCA (Tomita et al., 1999). The PKC interval identified in the African-American family spans 16.7 cM and overlaps by 3.4 cM with the ICCA region and by 9.8 cM with the PKC region identified in Japanese families. The three loci overlap by 3.4 cM between markers D16S3100 and D16S517, suggesting that there may be one gene in this interval which gives rise to both ICCA and PKC (Fig. 1).

Here we report the results of a genome-wide linkage search on a large Indian PKC family, which maps a second PKC locus to the long arm of chromosome 16. This locus is distinct from both the ICCA locus and the PKC locus described in the Japanese families. These findings demonstrate the presence of at least two genes for PKC, and provide evidence for a cluster of genes on chromosome 16 which lead to paroxysmal disorders.

Subjects and methods

Subjects

Thirty-two individuals (26 family members and six spouses) from a large Indian family comprising 60 members were interviewed and personally examined by one of the authors (G.M.W.). The study was approved by the Joint Medical Ethics Committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology. All examined subjects gave informed consent and venous blood samples were obtained from all but individuals II:4 and IV:13. Thirteen people, who were deceased prior to the time of examination, have been reported as affected from their history. The male:female ratio was 1.8:1. The clinical presentation of PKC was typical in all affected family members, with brief attacks (of up to 2 min) of dystonic/choreic movements precipitated by sudden movements, with a frequency of 1–20 episodes per day. Age of onset was in early childhood (range: 7–13 years). None of the affected individuals was known to have a history of benign infantile convulsions. However, five members of the family (one definitely affected by PKC, one found to be affected from history and three unaffected subjects) had sporadic episodes of tonic–clonic generalized seizures in their teenage years, with spontaneous

Fig. 1 Schematic representation of the genetic map of part of human chromosome 16 showing: the ICCA region as determined by Szepetowski and colleagues (Szepetowski et al., 1997) (flanking markers: D16S401 and D16S517); the RE-PED-WC region as determined by Guerrini and colleagues (Guerrini et al., 1999) (flanking markers: D16S3133 and D16S3131); the PKC region as determined by Tomita and colleagues (Tomita et al., 1999) (flanking markers: D16S3093 and D16S416); the PKC region as determined by Bennett and colleagues (Bennett et al., 2000) (flanking markers: D16S3100 and D16S517); and the novel PKC locus as determined in our family (flanking markers: D16S685 and D16S503). The arrow indicates the position of the centromere, the dotted lines indicate the region of overlap between the ICCA locus and the previously published PKC regions. Note that the novel PKC locus described here overlaps only with the PKC region identified in the African-American family, but does not overlap with the PKC locus mapped in the Japanese families or with the ICCA locus. Asterisks denote markers whose location has been assigned on the basis of recombinations in previously reported linkage studies; the location and intermarker distances of other markers are taken from the Genetic Location Database consensus map.
remission. The clinical presentation and age of onset of seizures in these patients are different from those of benign infantile convulsions frequently reported in association with PKC; for this reason, only individuals with PKC were considered as affected in this study.

The inheritance of PKC was clearly autosomal dominant, with affected individuals spanning three consecutive generations. Penetration appeared to be high but not complete, as there was at least one unaffected obligate gene carrier (individual III:12). The family pedigree is shown in Fig. 2.

**DNA and linkage analysis**

DNA was extracted from leucocytes using standard techniques. A simulation study performed with the program SLINK (Ott, 1989) confirmed that the family was suitable for a genome-wide search (maximum expected LOD score: 3.98 at \( \theta = 0.00 \)). Prior to the reported linkage on chromosome 16, we analysed 350 highly polymorphic fluorescent microsatellite markers spanning the 22 autosomes with an average distance of 10 cM (Linkage Mapping Set version 2, PE Applied Biosystems, Foster City, Calif., USA). Microsatellite markers were amplified from genomic DNA using the PCR (polymerase chain reaction) technique as specified by the manufacturers, and electrophoresed on a denaturing acrylamide gel using a 377 DNA Sequencer (PE Applied Biosystems). DNA fragment size analysis was performed semi-automatically using the Genescan and Genotyper software (PE Applied Biosystems) to determine genotypes.

Two-point LOD scores were generated using the FASTLINK version of MLINK (Lathrop and Lalouel, 1984; Cottingham et al., 1993) using an assumption of autosomal dominant inheritance, age-related reduced penetrance (0.8 for individuals older than 14 years and 0.5 for individuals 14 years old or younger), a gene frequency of 0.0001 and equal allele frequencies for each marker. The results of our genome search mapped the PKC locus to the pericentromeric region of chromosome 16. Additional informative microsatellite markers were analysed to refine this localization. The position of these markers with respect to PKC and ICCA is shown in Fig. 1. Haplotypes were constructed to detect recombination events, assigning phase based on the minimum number of recombinants. Marker order and genetic distances were based on framework markers of the latest Genetic Location Database chromosome 16 consensus map (G-map, updated November 1999) and published recombinants (Szpetowski et al., 1997; Tomita et al., 1999; Bennett et al., 2000) (Fig. 1).

**Results**

Pairwise LOD scores between PKC and marker loci are given in Table 1. Positive results were obtained over the pericentromeric region of chromosome 16, with LOD score values >3 at \( \theta = 0.00 \) for markers D16S419, D16S3137 and D16S415, with a maximum LOD score at D16S419 (\( Z = 3.66, \theta = 0.00 \)). These markers are located telomeric to the PKC region identified in the Japanese families (Tomita et al., 1999).

All affected individuals in the family shared a common haplotype between D16S685 and D16S503 (Fig. 2). The lower extent of the region is determined by recombinations detected in subjects III:15 and IV:11 between markers D16S415 and D16S503. The upper extent of the region is defined in individual III:3 between D16S685 and D16S419. This recombination was more difficult to map, as both parents of III:3 are dead and the only unaffected brother (III:1) was unavailable for examination. The full parental haplotype segregating with the disease (black bar) was shared by most affected family members. Construction of the unaffected chromosome of the affected mother (II:2) was made possible by considering the haplotypes of individuals III:18 and III:22. These two individuals are the unaffected sisters of III:21, who is affected and carries the same haplotype as the other affected individuals in the family. III:18 and III:22 carry the same haplotype as III:3 for markers D16S3133–D16S685, but a different haplotype for markers D16S419–D16S503. It is likely that they inherited the haplotype not segregating with the disease (Fig. 2, white bar) from their affected father (II:6), brother of II:2 and presumably haploidentical for this region. It is therefore likely that individual III:3 is recombinant between D16S685 and D16S419. Clinical assessment, blood sampling and marker analysis have been carried out twice for this individual in order to avoid possible errors.

The disease haplotype did not segregate with epilepsy in the family, suggesting that generalized juvenile-onset epilepsy is not part of the PKC phenotype in this family.

These results assign a second PKC locus to a 15.8 cM region between markers D16S685 and D16S503, which is distinct from both the ICCA locus and the PKC locus described in Japanese families (Szpetowski et al., 1997; Tomita et al., 1999). This locus has been assigned the gene symbol EKD2 (episodic kinesigenic dyskinesia).

**Discussion**

One PKC locus has been mapped to a 12.4 cM interval on the pericentromeric region of chromosome 16, between D16S3093 and D16S416 (Tomita et al., 1999). A subsequent study confirmed linkage of PKC to a partially overlapping 16.7 cM interval between markers D16S3100 and D16S771 in an African-American family (Bennett et al., 2000). There is a common overlap of 3.4 cM between these two regions and the ICCA locus (Szpetowski et al., 1997). The present study maps a PKC locus to chromosome 16q13–q22.1, to a region which is close to, but distinct from the PKC locus identified in Japanese families (Tomita et al., 1999). The localization of PKC in the African-American family (Bennett et al., 2000) overlaps with both these regions. The African-American PKC locus may be allelic with either the Japanese or Indian PKC locus or represent a third gene (Fig. 1).
A second PKC locus on chromosome 16

Fig. 2 Pedigree of the family and haplotypes of marker loci. Black symbols denote individuals affected by PKC; individuals found to be affected from their history are denoted by a thick vertical bar. The plus symbol denotes individuals with a history of seizures. Deceased members are marked with a diagonal bar. A thin horizontal bar above the symbols indicates members of the family who were examined clinically. The black bar denotes the haplotype segregating with the disease in the family.
These results demonstrate genetic heterogeneity of PKC, and exclude the possibility that one gene in the pericentromeric region of human chromosome 16 gives rise to both PKC and ICCA. Variation exists between published genetic maps and between genetic and physical maps. This study has used framework markers of the Genetic Location Database consensus map for intermarker distances and relative position. Markers D16S401, D16S3133, D16S3131, D16S3093 and D16S411 were localized according to previously observed recombinants (Szepetowski et al., 1997; 1993), two loci for familial benign hypercalcaemia on the pericentromeric region of chromosome 19 (Heath et al., 1993; Lloyd et al., 1999) and several epilepsy genes causing phenotype different epilepsy syndromes on the long arm of human chromosome 8 (Steinlein et al., 1995; Zara et al., 1995; Wallace et al., 1996; Fong et al., 1998; Mikami et al., 1999).

The haplotype data are consistent with the value of penetrance (0.8) assigned for linkage analysis: of 16 individuals carrying the haplotype segregating with the disease, 12 (75%) were affected by PKC. However, two unaffected individuals carrying the disease haplotype completely or partially (IV:9 and IV:22) were 7 years old at the time of examination, so it remains possible that they might develop the disease later in their life. Individual IV:22 is particularly interesting, as she is recombinant between D16S419 and D16S3137. If she develops PKC, this will refine the localization to a 12.8 cM interval.

Another paroxysmal disorder, characterized by autosomal recessive rolandic epilepsy with paroxysmal exercise-induced dystonia and writer’s cramp (RE-PED-WC), has been mapped to the same region as the ICCA locus (flanking markers: D16S3133 and D16S3131) (Guerrini et al., 1999). In both syndromes, epilepsy is the most striking feature, suggesting that the underlying gene(s) for these two conditions are different from those which give rise to PKC.

The most significant observation in this study is the identification of a second PKC locus in close proximity to, but distinct from, that previously reported (Tomita et al., 1999). This raises the possibility that a family of genes in the pericentromeric region of chromosome 16 are responsible for multiple paroxysmal disorders (PKC, ICCA and RE-PED-WC). The identification of several duplicated regions and frequent chromosomal rearrangements in the pericentromeric region of human chromosome 16 supports this hypothesis (Loftus et al., 1999). Clusters of genes causing similar but distinct phenotypes, which map in close proximity, have been reported previously, e.g. two hereditary non-chromaffin paraganglioma loci on the long arm of chromosome 11 (Heutink et al., 1992; Mariman et al., 1993), two loci for familial benign hypercalcaemia on the pericentromeric region of chromosome 19 (Heath et al., 1993; Lloyd et al., 1999) and several epilepsy genes causing phenotype different epilepsy syndromes on the long arm of human chromosome 8 (Steinlein et al., 1995; Zara et al., 1995; Wallace et al., 1996; Fong et al., 1998; Mikami et al., 1999).

An investigation of the GeneMap ‘99 Database revealed a cluster of genes encoding solute carriers mapping to chromosome 16, comprising sodium/chloride and potassium/chloride co-transporters, neurotransmitter transporters and sodium/hydrogen exchangers. Most members of this cluster lie in the pericentromeric region of chromosome 16, within the ICCA and PKC intervals. Also, several members of small inducible cytokine subfamilies and a cluster of 14 metallothionein genes map to the region.

It has been postulated that ion channel genes cause a large number of paroxysmal conditions (Hanna et al., 1998). It is therefore likely that paroxysmal movement disorders mapping to chromosome 16 are also due to mutations in genes encoding or controlling ion channel function. To date, no ion channel genes have been identified within these candidate regions.

The identification of a second PKC locus in the pericentromeric region of chromosome 16 has important

### Table 1 Pairwise LOD scores between PKC and markers on chromosome 16

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<th>Markers</th>
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implications for the positional cloning of genes which give rise to paroxysmal movement disorders.

Electronic database information

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