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ORIGINAL INVESTIGATION

Wei-Ling Lee · Agnes Tay · Hian-Tat Ong
Li-Meng Goh · Anthony P. Monaco
Pierre Szepetowski

Association of infantile convulsions with paroxysmal dyskinesias (ICCA syndrome): confirmation of linkage to human chromosome 16p12-q12 in a Chinese family

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Abstract We have studied one family of Chinese origin, in which benign infantile convulsions and paroxysmal choreoathetosis (of the dystonic form) were co-inherited as a single autosomal dominant trait. This association is specific to ICCA syndrome, which we have recently described in four French families. Some patients in the new family also exhibit recurrence of epileptic seizures at a much later age, making the ICCA syndrome in this family atypical. DNA samples isolated from this family of 22 members (9 affected) have been tested with genetic markers at chromosome 16p12-q12, in which region the ICCA syndrome has previously been linked. Confirmation of linkage to this pericentromeric region of human chromosome 16 has been obtained and no critical meiotic recombination event has been detected in the ICCA region. This result suggests that, in contrast to marked clinical heterogeneity, the association of infantile convulsions with paroxysmal dyskinetic movements could be genetically homogeneous.

Introduction

The epilepsies are a heterogeneous group of disorders characterized by predisposition to seizures that represent an abnormal electrical activity of cerebral neurons, leading to paroxysmal clinical manifestations of various types. It is usual to distinguish between symptomatic epilepsies, arising from a variety of brain disorders or lesions, and idiopathic epilepsies, which are presumed to be of genetic origin. A genetic contribution to the etiology has been estimated to be present in about 40% of patients with epilepsy.

Most forms of human epilepsy show complex non-Mendelian modes of inheritance. However, progress in the mapping and cloning of epilepsy genes has mainly been obtained by studying rare forms of epileptic disorders inherited as Mendelian traits (Delgado-Escueta et al. 1994; Szepetowski and Monaco 1998). In addition to genes responsible for progressive epileptic syndromes, in which epileptic seizures are only part of a more general degenerative process, three genes have been identified that are involved in pure epileptic disorders. Mutations in the gene encoding the neuronal nicotinic acetylcholine receptor α4 subunit (CHRNA4) lead to autosomal dominant frontal lobe epilepsy (Steinlein et al. 1995, 1997). More recently, two homologous potassium channel genes, KCNQ2 and KCNQ3, have been identified and shown to be responsible for the two genetic forms of benign familial neonatal convulsions (BFNC; Singh et al. 1998; Charlier et al. 1998; Biervert et al. 1998).

Benign familial infantile convulsions (BFIC) represent another type of epileptic syndrome of childhood inherited as an autosomal dominant trait (Vigevano et al. 1992; Lee et al. 1993; Echenne et al. 1994; Caraballo et al. 1997). This syndrome is characterised by seizures occurring first at the age of 3–12 months and a favourable outcome. Linkage of BFIC to chromosome 19q has been shown in five families of Italian origin (Guipponi et al. 1997). In addition, we have shown that BFIC can concur and co-segregate with variably expressed paroxysmal choreoathetosis in four French families. This has led to the definition of a new neurological syndrome (ICCA: infantile convulsions and choreo-athetosis) that is inherited as an autosomal dominant trait and that we have linked to the pericentromeric region of chromosome 16 (Szepetowski et

W.-L. Lee
Department of Neurology, Tan Tock Seng Hospital, Singapore

A. Tay (✉) · L.-M. Goh
Department of Paediatrics, National University Hospital,
5 Lower Kent Ridge Road, Singapore 119074
e-mail: paetaya@nus.edu.sg, Tel.: +65 772 4420, Fax: +65 779 7486

A. Tay
Institute of Molecular and Cell Biology, Singapore

H.-T. Ong
Departmet of Paediatrics, National University Hospital, Singapore

A. P. Monaco · P. Szepetowski
The Wellcome Trust Centre for Human Genetics,
University of Oxford, Oxford, UK

al. 1997). This represents the first genetic evidence of a common basis for these two clinically distinct paroxysmal cerebral disorders.

Paroxysmal dyskinesias are also highly heterogeneous. Several classifications have been proposed (for a review, see Demirkiran and Jankovic 1995). Lance (1977) has distinguished between choreoathetosis of the dystonic, kinesigenic and mixed forms. Fahn (1994) has proposed a broader classification for "paroxysmal dystonia/choreoathetosis". The new classification of paroxysmal dyskinesias (Demirkiran and Jankovic 1995) is even more precise. Whatever classification is used, clinical and genetic heterogeneity have been observed. Genetic analyses could thus help in the classification of the various and often overlapping forms of dyskinesias and in the understanding of the unknown pathophysiology of these movement disorders. Linkage of paroxysmal dystonic choreoathetosis (paroxysmal dyskinesia of the dystonic form, according to Demirkiran and Jankovic 1995) to chromosome 2q has been shown (Fink et al. 1996; Fouad et al. 1996), whereas the more complex syndrome of paroxysmal choreoathetosis and episodic ataxia has been linked to chromosome 1p (Auburger et al. 1996).

We have identified an additional family of Chinese origin, in which infantile convulsions and paroxysmal dystonic choreoathetosis co-segregate as a unique autosomal dominant trait (Fig. 1). In addition to typical ICCA syndrome, recurrence of epileptic seizures in childhood is a feature in some of the patients (Table 1). In the present study, we have confirmed genetic linkage to 16p12-q12 in this additional family; this suggests that the disease, although clinically heterogeneous, could be genetically homogeneous.

Materials and methods

Clinical investigation

Individuals were considered as being affected if they had afebrile convulsions at the age of 3–12 months, attacks of involuntary movements and dystonic postures or a history of both convulsions and dyskinesia. Clinical investigation of the index patients included physical examination, blood electrolyte values, electroencephalography (EEG) and computerized tomography (CT). The family pedigree was ascertained and the clinical history of relatives was obtained. Some of these were also examined and interictal EEGs performed. All members of the family were asked to cooperate and informed consent was obtained from all subjects. Blood samples were collected in EDTA tubes prior to DNA extraction.

Genotyping

Genomic DNA was extracted by standard procedures. Highly polymorphic microsatellites markers (Callen et al. 1995; Dib et al. 1996) were analysed by polymerase chain reaction (PCR) amplification as

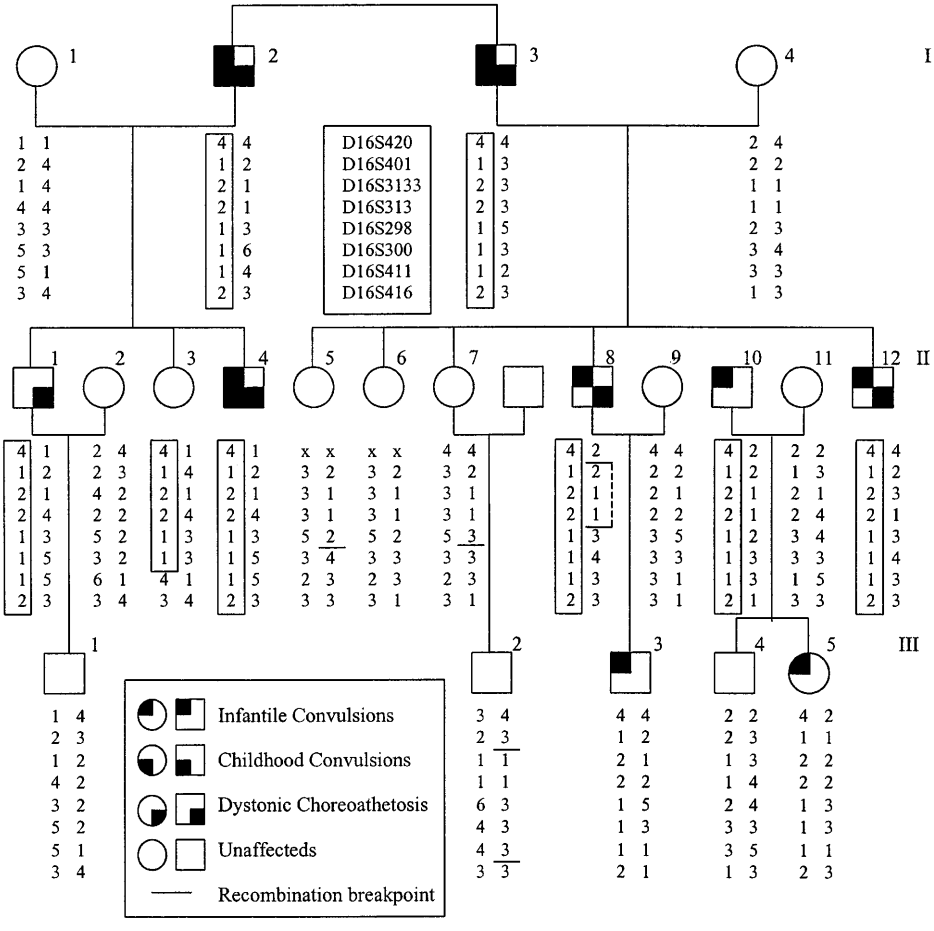

Fig. 1 Pedigree with infantile convulsions, childhood epileptic seizures and paroxysmal dystonic choreoathetosis

Table 1 Summary of clinical information (*GTC* generalised tonic-clonic convulsions, *DC* dystonic choreoathetosis)

Individual	Infantile seizures	Later events	Persistence of seizures into adulthood
	Age at onset/type	Age at onset/type	
I-3	Not known	6 years/DC and GTC	Yes
I-2	Not known	10 years/DC and GTC	Yes
II-12	4 months/GTC	15 years/DC	No
II-10	5 months/GTC	No	No
II-1	No	10 years/DC	No
II-4	5 months/GTC	12 years/DC and GTC	Yes
II-8	4 months/GTC	10 years/DC	No
III-5	4 months/GTC	None so far (aged 2 years)	–
III-3	5 months/GTC	None so far (aged 2 years)	–

previously described (Szepetowski et al. 1997), with primers labelled at the 5' terminus with a fluorescent dye. Fluorescent PCR products were analysed in two independent sets of experiments on Applied Biosystem 373 A (in Oxford) and 310 (in Singapore) sequencers, with GENESCAN and GENOTYPER software. Linkage analysis was performed with the parameters previously used to study ICCA syndrome (Szepetowski et al. 1997), by assuming an autosomal dominant mode of inheritance with penetrance at 0.8 and with a frequency of the disease allele at 0.0001, by means of the MLINK modification of the LINKAGE computer package (Lathrop and Lalouel 1984).

Results

Clinical information

The Chinese family first presented to one of the authors (W.-L.L.) with two cousins (III.3 and III.5) both having infantile seizures. Patient III.5 was 4 months old when she presented with three afebrile seizures within 1 day. The seizures lasted 2 min or less and varied from generalised tonic-clonic convulsions to episodes of uprolling of the eyes and twitching of the mouth. Serum electrolytes, glucose and electroencephalogram (EEG) were all normal. The patient had one further seizure at the age of 6 months but remained seizure-free up to the last outpatient visit at 2 years of age. Neurological and developmental assessment was normal throughout. Her cousin, III.3, presented at the age of 5 months with five afebrile seizures within 1 day. The seizures were either generalised tonic-clonic convulsions or only deviation of the eyes upwards or laterally. Serum electrolytes, cerebrospinal fluid, EEG and CT scan were all normal. He had no further seizures up to the last outpatient visit at the age of 2 years.

Their family history revealed the occurrence of infantile seizures in several other relatives (Fig. 1, Table 1). Infantile seizures started around the age of 4 months, with generalised convulsions; these ceased around the age of 1 year. In later childhood or early adolescence, paroxysmal dystonic choreoathetosis (dyskinesia of the dystonic form) and/or epileptic seizures developed. Individuals with paroxysmal dystonic choreoathetosis experienced the sudden onset of involuntary movements, such as twisted posture of the limbs or curling of the tongue, uprolling of the eyes and cramping of toes and/or hands. Consciousness was not impaired but gait was abnormal during the attack. Each attack lasted only a few seconds and was often subtle enough to be missed by casual bystanders. The frequency of attacks varied from 2–3 times a day to once in many months. Paroxysmal dystonic movements appeared to be triggered by excitement, stress and sudden movements.

In those patients with seizures beyond infancy, these were infrequent generalised tonic-clonic convulsions, although status epilepticus occurred rarely. Both paroxysmal dystonic movements and seizures responded to low doses of phenytoin and stopped by the age of 20–30 years, even on cessation of phenytoin treatment.

Of the interictal EEGs obtained from three adults, two were normal. Both these patients had only dystonic choreoathetosis with no seizures. The third patient, who had both paroxysmal dystonic choreoathetosis and seizures, had an abnormal EEG that showed focal slowing. None of the patients had neurological deficits or intellectual impairment.

Linkage analysis

DNA samples isolated from the family were tested with highly polymorphic markers situated at chromosome 16p12-q12, where linkage of the ICCA syndrome had previously been shown (Szepetowski et al. 1997). In addition to some of the Genethon markers (Dib et al. 1996) analysed in our former study, we used markers situated in the critical area (Callen et al. 1995). Confirmation of linkage was obtained with several informative markers, with a maximum two-point LOD score of 3.36 (θ=0.0) for D16S416 (Table 2).

As in the case of the ICCA families previously studied (Szepetowski et al. 1997), penetrance was not complete. One individual (II.3), although not affected, carried the disease haplotype from marker D16S420 to marker D16S300. This consequently lowered the LOD scores for markers situated in that particular subregion. A recombination event between markers D16S300 and D16S411 on the disease chromosome inherited by this individual led to

Table 2 Pairwise LOD scores with microsatellite markers at 16p12-q12

Markers	Theta						
	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D16S420	1.15	1.13	1.05	0.95	0.74	0.52	0.27
D16S401	2.57	2.54	2.37	2.15	1.66	1.09	0.48
D16S3133	2.54	2.50	2.34	2.13	1.64	1.08	0.48
D16S313	2.50	2.46	2.31	2.10	1.62	1.07	0.47
D16S298	2.60	2.56	2.40	2.18	1.68	1.11	0.49
D16S300	2.60	2.56	2.40	2.18	1.68	1.10	0.48
D16S411	3.21	3.15	2.92	2.62	1.98	1.29	0.56
D16S416	3.36	3.30	3.06	2.75	2.09	1.37	0.60

increased LOD scores for more distal markers (D16S411 and D16S416).

Meiotic recombination events could not be found in affected individuals with any of the markers used in this study. Since two of these, D16S401 and D16S517, respectively, defined the actual boundaries of the ICCA region, we could not narrow the critical region for the ICCA gene. Nevertheless, these results confirmed the existence, on chromosome 16p12-q12, of a gene responsible for variably associated epileptic seizures and paroxysmal dyskinesias.

Discussion

Despite the clinical discrepancies observed between the Chinese family studied here and the four French ICCA families, in which no recurrence of epileptic seizure occurred in late childhood or adolescence, we have confirmed linkage for this new family to the critical ICCA region. This indicates that the association of infantile convulsions with paroxysmal dyskinesias is specific to mutations in a single gene situated on chromosome 16p12-q12. The fact that the family studied here is of Chinese origin also argues in favour of genetic homogeneity. However, this does not rule out the possibility that other families with a similar phenotype will be excluded for linkage to chromosome 16 in future studies.

In the Chinese family, the ICCA phenotype is variably expressed, with patients displaying infantile convulsions, paroxysmal dystonic movements or both, whereas one individual, although carrying the disease haplotype in the critical ICCA region, remains unaffected. This has also been seen in the four French families (Szepetowski et al. 1997). When considering the dyskinetic component only, variable expressivity has been found in the first four families, with some patients displaying non-dystonic choreoathetotic movements and other dystonic choreoathetotic movements. This is apparently not the case in the Chinese family, since only choreoathetosis of the dystonic form has been found. The infantile convulsions are of the partial type in the French families, although generalisation might occur. In the Chinese family, patients seem to have generalised infantile convulsions only. One likely explanation is that these seizures are initially partial and generalized secondarily. Moreover, as stated above, epileptic seizures have continued in three patients of the Chinese family, even after the age of 10 years, which is not the case in the four typical ICCA families that we initially studied. This inter- and intra-familial variability that is exhibited in the ICCA families is not surprising and could be attributable to environmental factors, to genotype-phenotype correlation, with different mutations in the putative ICCA gene causing distinct, although similar, clinical disorders and/or to the existence of modifier gene(s).

Ion channel genes could be involved in several paroxysmal cerebral disorders. The common features of ion channel diseases, including paroxysmal symptoms and their high level of clinical variability, could be inherent to the close relationship existing between all these genes (Ptácek 1996; Bulman 1997; Doyle and Stubbs 1998). Factors influencing the expression of proteins involved with ion metabolism could play significant modifying roles in the phenotypic expression of these disorders, including the ICCA syndrome itself. It is interesting to note that, in one out of the BFNC pedigrees with KCNQ2 mutations (Singh et al. 1998), the age of onset shows greater variation than in typical BFNC families, with three patients having onset of seizures at 3, 4 and 5 months of age. This age of onset is the same as that in typical BFIC and ICCA syndromes. Moreover, 10% of BFNC patients go on to develop epilepsy in adulthood. In the new Chinese family that we have studied, epileptic seizures have persisted into adulthood in three patients. Similarities and overlaps between both types of convulsions could be sustained by the involvment of different, albeit homologous, ion channel genes.

Mutations in a calcium channel gene, CACNL1A4, have also been identified in families with hemiplegic migraine or episodic ataxia (Ophoff et al. 1996). Interestingly, one single large pedigree has been described in which typical infantile convulsions and hemiplegic migraine cosegregate (Terwindt et al. 1997) and epileptic seizures may occur during severe hemiplegic migraine attacks in other families (Ducros et al. 1997), whereas paroxysmal choreoathetosis and episodic ataxia can be inherited as a single autosomal dominant trait in some families (Auburger et al. 1996). In another large pedigree where paroxysmal dystonic choreoathetosis segregates as an autosomal dominant trait, classic migraine occurs in one third of the patients

(Hofele et al. 1997). Moreover, a new recessively inherited syndrome (REWCA) associating Rolandic epilepsy, paroxysmal kinesogenic dystonia (writers' cramp) and ataxia has recently been described in a single consanguineous family (Guerrini et al. 1997). Mutations in ion channel genes obviously could account for the variable association and co-segregation of the various paroxysmal cerebral disorders seen in some families. An as-yet undiscovered ion channel gene could thus be responsible for the ICCA phenotype.

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