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Mapping of a Locus for a Familial Autosomal Recessive Idiopathic Myoclonic Epilepsy of Infancy to Chromosome 16p13

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Myoclonic epilepsies with onset in infancy and childhood are clinically and etiologically heterogeneous. Although genetic factors are thought to play an important role, to date very little is known about the etiology of these disorders. We ascertained a large Italian pedigree segregating a recessive idiopathic myoclonic epilepsy that starts in early infancy as myoclonic seizures, febrile convulsions, and tonic-clonic seizures. We typed 304 microsatellite markers spanning the 22 autosomes and mapped the locus on chromosome 16p13 by linkage analysis. A maximum LOD score of 4.48 was obtained for marker D16S3027 at recombination fraction 0. Haplotype analysis placed the critical region within a 3.4-cM interval between D16S3024 and D16S423. The present report constitutes the first example of an idiopathic epilepsy that is inherited as an autosomal recessive trait.

Introduction

Myoclonic seizures are common features of many epilepsies at all ages and often represent nonspecific epileptic manifestations (Fejermann 1991). In the specific subgroup of myoclonic epilepsies, however, myoclonic seizures are invariably present and are the most prominent symptom. Among these syndromes, the classification of myoclonic epilepsies with onset in infancy and childhood has been a source of controversy because other types of seizures frequently accompany myoclonic attacks and the cerebral origin and etiology of epileptic discharges are uncertain. The International Classification of Epilepsies and Epileptic Syndromes (ICE) (Commission on Classification and Terminology of the International League against Epilepsy 1989) recognizes different myoclonic epilepsies with onset in the first year of life. They include idiopathic benign myoclonic epilepsy in infancy (BIME), the symptomatic early myoclonic encephalopathy, and severe myoclonic epilepsy of infancy (SIME).

Familial myoclonic epilepsies of infancy have rarely been described (Fujiwara et al. 1990; Dravet et al. 1992*a*), although an increased incidence of idiopathic epilepsy or febrile convulsions in relatives of probands has been frequently described (Dalla Bernardina et al. 1982; Dravet et al. 1992*b;* Lin 1998). The sporadic nature of these myoclonic disorders has further complicated their classification into syndromic entities, and, therefore, many cases of infantile myoclonic epilepsy remain unclassified (Jeavons 1977; Aicardi 1986).

To date, very little is known about the etiology of idiopathic myoclonic epilepsies, although genetic factors are thought to play an important role (Minassian et al. 1995). Two loci predisposing to juvenile myoclonic epilepsy (MIM 254770) have been mapped to chromosome 6p and 15q (Liu et al. 1995; Elmslie et al. 1997), and a locus for an adult form of benign familial myoclonic epilepsy (MIM 601068) has been mapped to chromosome 8q24 in Japanese families (Mikami et al. 1999; Plaster et al. 1999).

To date, no data are available on genetic factors involved in myoclonic epilepsy of infancy. We ascertained a large Italian pedigree segregating an autosomal recessive idiopathic myoclonic epilepsy that starts in early infancy, characterized by very frequent and often long-lasting myoclonic seizures, febrile convulsions, and generalized tonic-clonic seizures (GTCS). In this article, we report the genetic mapping of an autosomal recessive locus for idiopathic myoclonic epilepsy of infancy to chromosome 16p13.

Subjects and Methods

Case Report

Two related branches (fig. 1) make up the Italian family. The founders of generation I were not consanguineous, despite origin from the same geographic area (Naples), and were not reported to be affected. The four individuals (two sib pairs) of generation II did not show

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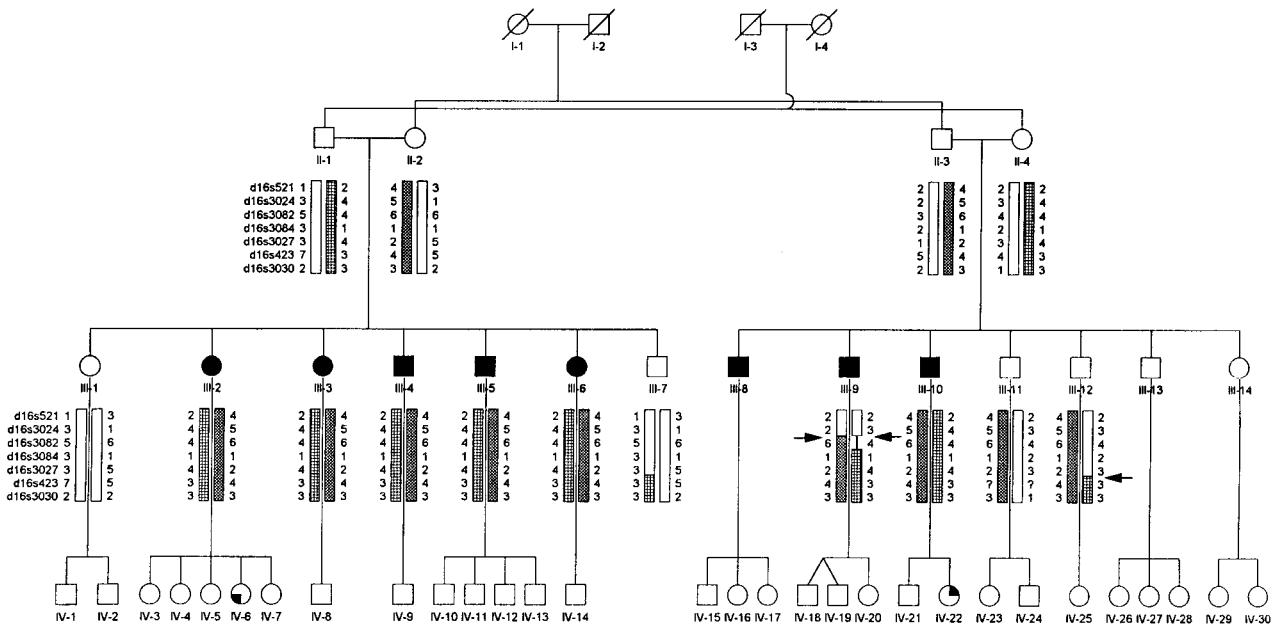

Figure 1 Pedigree and chromosome 16p13 haplotypes of the Italian family segregating idiopathic myoclonic epilepsy of infancy. *Filled symbols,* affected subjects; *unfilled symbols*, individuals with no history of seizures. Individuals IV-6 and IV-22 had GTCSs and febrile convulsion, respectively. Individuals III-8, III-13 and III-14 did not consented to the study. *Arrows,* key recombinations that define the critical region.

any clinical symptoms of the disease or any epilepsy-related episodes. Electroencephalograms (EEGs; recorded at mean age 60 years) were normal for individuals II-1, II-3, and II-4 but not for individual II-2, who showed mild localized paroxysmal abnormalities over the temporal regions. Of 14 individuals of generation III, 11 consented to the study, and 7 of them are affected. Clinical features of myoclonic epilepsy may be summarized as follows: onset at 4–8 mo of age with GTCS in subjects III-2, III-3, III-4, and III-5; appearance of myoclonic seizures at age 5–36 mo and persistence to adulthood in all patients; febrile convulsions at age 5 mo to 3 years in individuals III-2, III-3, III-4, III-5, and III-6; very frequent myoclonic seizures (several times a day) in childhood, lasting for many hours, sometimes preceding a convulsive seizure, spontaneous or induced by fatigue or drowsiness, acoustic stimuli or variations in light intensity, the intense "green" color, repetitive movements of the hands; GTCS in all affected individuals, more frequent in childhood and adolescence, sporadic or absent in adult age; interictal EEGs normal or with mild abnormalities; ictal EEGs showing bisynchronous spike waves; cranial computed topography scan and magnetic resonance imaging (MRI) negative for any detectable brain lesions; and good control of both myoclonic and GTCSs with Valproate.

The fourth generation accounts for 30 individuals. Twenty are the offspring of affected individuals, and none shows any symptom of the disease. Individual IV-4, however, showed an idiopathic generalized epilepsy that started at age 19 years (two GTCSs, with normal MRI), and individual IV-16 had febrile convulsions at age 5 years.

DNA Samples and Genotyping

Ten milliliters of peripheral blood were obtained from consenting individuals and were used for DNA extraction. Microsatellite markers were selected from the Généthon map (Dib et al. 1996). Markers were typed by following two different procedures: (a) PCR amplifications were performed by use of γ-[^{32}P]-dATP end-labeled forward primers and then separated onto 6% denaturating gel that was subsequently vacuumed and exposed to autoradiography; and (b) sets of 10–12 PCR amplifications were electrophoresed on 6% denaturating gels that were subsequently blotted on nylon membranes. Filters were hybridized with multiple sets of two to three radiolabeled primers that specifically recognize nonoverlapping amplifications and that were then exposed to autoradiography (Tournier-Lasserve et al. 1993).

PCR amplifications were carried out as described by Gennaro et al. (1999) with 35 cycles of 94°C for 40 s and 55°C for 30 s. In a few selected cases, a specific annealing temperature was used.

Linkage Analysis

Two-point and multipoint linkage analysis was performed by respective use of the MLINK and LINKMAP

programs of the LINKAGE package version 5.1 (Lathrop et al. 1985) in the FASTLINK implementation (Cottingham et al. 1993). A variable number of equifrequent alleles were used for LOD score calculations according to the actual heterozygosity of the marker within the white population. Marker order and distances were obtained from the Généthon map (Dib et al. 1996). A fully penetrant autosomal recessive disease allele with a frequency of .001 was assumed for linkage analysis. The phenocopy rate was set at 0.

Results

We first typed a set of 181 microsatellite markers covering the 22 autosomes at an average distance of ~20 cM. Because no suggestive evidence of linkage was found with the initial screen, we typed a second set of 123 markers in order to reduce the intermarker distance to ~10 cM. We observed a maximum LOD score of 1.74 at recombination fraction (θ) .2 with marker D16S521 on chromosome 16p13. Additional markers were typed to obtain a detailed genotypic map of the region in our family. Two-point LOD scores and haplotypes are shown in table 1 and figure 1, respectively.

The maximum LOD score of 4.48 was observed for D16S3027 at $\theta = 0$. Furthermore, no obligate recombinants were observed for D16S3082 and D16S3084 (maximum LOD scores of 1.81 and 2.94, respectively, at $\theta = 0$). Four-point LOD scores of the regions peaked at 4.69 (data not shown). Key recombinations were observed in individual III-9 for D16S3024 and in individual III-12 (age 42 years) for D16S423, which allowed the localization of the critical region to a 3.4-cM interval of chromosome 16p13 between markers D16S3024 and D16S423 (fig. 2).

Discussion

The idiopathic epilepsies are a very heterogeneous group of neurologic disorders: among them are very common disorders with a complex mode of inheritance and rare syndromes inherited as Mendelian traits. Although very little is known about the genetic factors implicated in genetically complex idiopathic epilepsies, substantial progress has been made in the rare Mendelian forms of epilepsy. Mutations in genes encoding potassium channels (Biervert et al. 1998; Charlier et al. 1998; Singh et al. 1998), the neuronal acetylcholine receptor α4 subunit (Steinlein et al. 1995), and the sodium channel β1 subunit (Wallace et al. 1998) have been, respectively, associated with familial benign neonatal convulsions (BFNC [MIM 121200, 121201]), autosomal dominant nocturnal frontal lobe epilepsy (MIM 600513), and a syndrome characterized by generalized epilepsy and febrile seizures (GEFS+) (MIM 604236).

Furthermore, autosomal loci responsible for benign familial infantile convulsions (MIM 601764), benign adult familial myoclonic epilepsy (MIM 601068), and several Mendelian subsets of genetically complex epilepsies such as childhood absence epilepsy (MIM 600131), juvenile myoclonic epilepsy (MIM 254770), and partial epilepsy (MIM 604364) have been identified on chromosomes 8q24, 6p21.2-p11, 10q22-q24, and 22q11-q12 by means of linkage analysis (Liu et al. 1995; Ottman et al. 1995; Guipponi et al. 1997; Fong et al. 1998; Mikami et al. 1999; Plaster et al. 1999; Xiong et al. 1999).

Although an increasing number of Mendelian idio-

Table 1

Two-Point LOD Scores for Chromosome 16p13 Markers

	LOD AT θ					
LOCUS	0	.05	.2	.3	.4	.1
D16S521	$-\infty$	1.70	1.74	1.45	.94	.34
D16S3024	$-\infty$	1.50	1.72	1.52	1.01	.37
D16S3082	1.81	1.61	1.41	.99	.58	.20
D16S3084	2.94	2.61	2.27	1.59	.93	.32
D16S3027	4.48	4.05	3.61	2.68	1.68	.64
D16S423	$-\infty$	2.89	2.77	2.17	1.39	.52
D16S3030	$-\infty$	2.99	2.85	2.22	1.41	.52

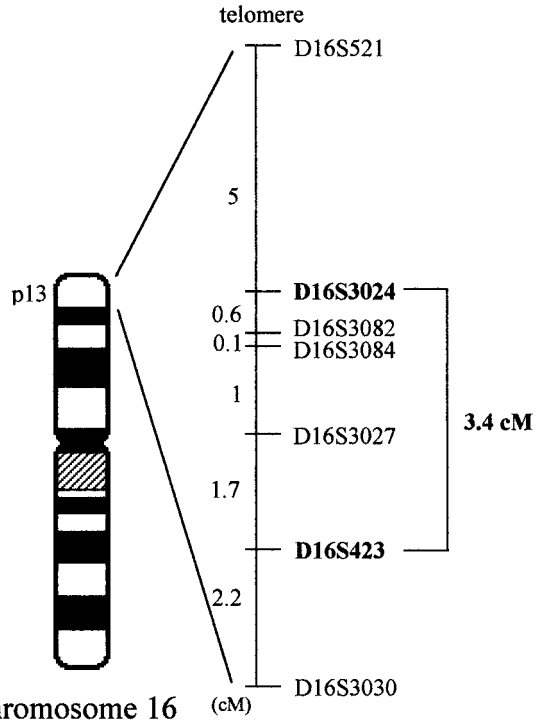

Figure 2 Genetic map of chromosome 16p13 markers. Order of markers and distances are taken from the Généthon map. Markers harboring the critical region are indicated in bold.

pathic epilepsies have been described and their underlying genes localized or cloned, their mode of inheritance is autosomal dominant. We report here the mapping of a locus predisposing to a familial myoclonic epilepsy of infancy to chromosome 16p13.

To the best of our knowledge, this is the first example of an idiopathic epilepsy that is inherited as autosomal recessive trait. It is possible that recessive alleles causing idiopathic epilepsy are rare. Alternatively, their frequency may be underestimated because of difficulty in mapping recessive traits in clinically and genetically heterogeneous disorders.

In the family described here, we identified two different haplotypes segregating with the disease, which suggests that two different mutations might likely be present and therefore that the affected family members should be compound heterozygotes. This finding is consistent with available data indicating that founders of generation I are not consanguineous. In this hypothesis, at least two mutant alleles should be present within the Italian population, and hence this gene could play a significant role. We cannot, however, exclude that a single mutation, arisen from a common ancient founder, is segregating in the family we describe here and that different haplotypes have been originated by ancestral crossovers that occurred close to the disease gene.

The myoclonic epilepsy segregating in the reported kindred is not identifiable with any epileptic syndrome described in ICE. It does, however, resemble SIME in several features, such as constant presence of GTCS, high frequency of febrile convulsions, long-lasting and frequently provoked myoclonic seizures, and persistence of clinical manifestations in adult life (Guerrini and Dravet 1997), and it shares with BIME the normal psychomotor development, the absence of neurologic deficit, and the good response to therapy (Vigevano et al. 1997). Intermediate forms between BIME and SIME have already been described, and the hypothesis that myoclonic epilepsy of infancy might represent a clinical continuum with variable expression and severity has been postulated (Lombroso 1990). By eventually isolating the mutant gene, we could verify whether the myoclonic epilepsy segregating in the reported kindred represents a novel clinical entity or a specific expression of a broader clinical picture.

We have mapped a gene for a familial idiopathic myoclonic epilepsy of infancy within a 3.4-cM region of chromosome 16p13. Within this region, three genes potentially involved in epileptogenesis have been mapped: the voltage-dependent chloride channel 7 gene (CLCN7), the synaptogyrin III gene (SYNGR3), and the solute carrier family 9 isoform 3 regulatory factor 2 gene (SLC9A3R2).

CLCN7 is broadly expressed in different tissues and, together with CLCN6, represents a specific branch within the chloride-channel gene family (Brandt and Jentsch 1995). Although very little is known about CLCN7-specific functions, other members of the chloride-channel gene family have been implicated in repolarizing the cell membrane when expressed in Xenopus oocytes (Pusch et al. 1995). Because in BFNC (MIM 121200, 121201) the moderate reduction of K^+ repolarizing current has been demonstrated to be epileptogenic (Schroeder et al. 1998), CLCN7 may represent an appealing candidate.

SYNGR3 encodes a synaptic vesicle membrane protein (Kedra et al. 1998). To date, synaptogyrins have not been implicated in the etiology of epilepsy; however, knockout mice of synapsin I and II (which are parts of synaptic vesicle membranes) show spontaneous seizures in the absence of any detectable structural brain abnormalities (Rosahl et al. 1995).

SLC9A3R2 was implicated in the regulation of the sodium/hydrogen-exchanger isoform 3 (Brant et al. 1995; Yun et al. 1997). Because a mutation in the sodium/hydrogen-exchanger isoform 1 (SLC9A1) was found to cause generalized seizures in the slow-wave epilepsy mouse (Cox et al. 1997), the abnormal activity of the related isoform 3 caused by altered regulation might also be epileptogenic.

Our work discloses important new information regarding the potential mode of transmission of myoclonic epilepsies with pediatric onset and provides evidence for a new locus implicated in idiopathic epilepsy on chromosome 16p. Identification of genes involved in the myoclonic epilepsies of infancy could provide a more precise diagnosis of the various syndromes and improve genetic counseling to patients and families. In the future, new therapies may be developed to treat myoclonic epilepsies resistant to currently available drugs.

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Electronic-Database Information

Accession numbers and the URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for juvenile myoclonic epilepsy [MIM 254770], benign adult familial myoclonic epilepsy [MIM 601068], BFNC [MIM 121200, 121201], autosomal dominant nocturnal frontal lobe epilepsy [MIM 600513], GEFS+ [MIM 604236], benign familial infantile

convulsions [MIM 601764], childhood absence epilepsy [MIM 600131], and partial epilepsy [MIM 604364])

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