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Clinical Research

Benign Familial Infantile Convulsions: Linkage to Chromosome 16p12-q12 in 14 Families

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Summary: *Purpose:* Benign familial infantile convulsions (BFIC) is a form of idiopathic epilepsy. It is characterized by clusters of afebrile seizures occurring around the sixth month of life. The disease has a benign course with a normal development and rare seizures in adulthood. Previous linkage analyses defined three susceptibility loci on chromosomes 19q12-q13.11, 16p12-q12, and 2q23-31. However, a responsible gene has not been identified. We studied linkage in 16 further BFIC families.

Methods: We collected 16 BFIC families, without an additional paroxysmal movement disorder, of German, Turkish, or Japanese origin with two to eight affected individuals. Standard two-point linkage analysis was performed.

Results: The clinical picture included a large variety of seizure semiologies ranging from paleness and cyanosis with altered consciousness to generalized tonic–clonic seizures. Interictal EEGs showed focal epileptiform discharges in six patients, and three ictal EEGs in three distinct patients revealed a focal seizure onset in different brain regions. In all analyzed families, we found no evidence for linkage to the BFIC loci on chromosomes 19q and 2q, as well as to the known loci for benign familial neonatal convulsions on chromosomes 8q and 20q. In 14 of the families, the chromosome 16 locus could be confirmed with a cumulative maximum two-point lod score of 6.1 at marker D16S411, and the known region for BFIC could be narrowed to 22.5 Mbp between markers D16S690 and D16S3136.

Conclusions: Our data confirm the importance of the chromosome 16 locus for BFIC and may narrow the relevant interval. **Key Words:** Idiopathic epilepsy—Genetics—Linkage—Benign familial infantile convulsions—Benign familial neonatal convulsions—Benign partial epilepsy of infancy.

Epilepsy is one of the most common neurologic disorders, with a cumulative lifetime incidence of 3% (1). Approximately 25 to 50% of these epilepsies are idiopathic (2–4) (i.e., with a predominant genetic origin and without an underlying neurologic disorder or a structural brain lesion). In some of the rare idiopathic epilepsies with a mendelian inheritance, the causative mutations could be identified. With one exception, all these mutations were found in ligand- or voltage-gated ion channel genes (5–11). Such findings augment our knowledge about the pathophysiologic concepts of epilepsy and may help to identify new targets for pharmacotherapy, as was recently shown for the novel anticonvulsant drug retigabine (RGB), which activates neuronal KCNQ potassium channels mutated in benign familial neonatal convulsions (12). In several linkage studies, further susceptibility regions could be identified in other idiopathic epilepsy syndromes [idiopathic generalized epilepsy (13,14); rolandic epilepsy (15); febrile seizures (16–19)], but the mutated genes are not known.

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Benign familial infantile convulsions (BFIC, OMIM 601764, 605751, 606052) is another idiopathic epileptic syndrome for which the underlying gene has not been identified. It is characterized by afebrile seizures with a focal onset [mainly complex partial (CPSs) and/or secondarily generalized tonic–clonic seizures (GTCSs)] occurring between ages 3 and 12 months. The disease has a benign course with a usually normal development, an excellent response to anticonvulsant drugs (AEDs), and rare seizures in adulthood (20–24). BFIC shows an autosomal-dominant mode of inheritance with a high penetrance. Linkage to chromosomes 19q12-q13.11 (25), 2q23-31 (26), and 16p12-q12 (27) has been described.

Several other hereditary neurologic syndromes with epileptic seizures and/or paroxysmal symptoms such as dyskinesias have been linked to the same region on chromosome 16p12-q12: infantile convulsions with paroxysmal choreoathetosis [ICCA; OMIM 602066 (28–30)], paroxysmal kinesiogenic choreoathetosis [PKC; OMIM 128200 (31–33)], and autosomal-recessive rolandic epilepsy with paroxysmal dystonia and writer's cramp (34). As for pure BFIC, for none of these syndromes has a causative mutation been identified. PKC is characterized by brief attacks of involuntary movements including dystonic postures, choreoathetosis, and ballism, which are induced by sudden movements. Up to 100 attacks per day last seconds to minutes (35). The onset of the disease is usually in early childhood or adolescence. ICCA was defined for families in which BFIC and PKC occurred within the same pedigree or even in the same individuals (28). Rolandic epilepsy, also called benign epilepsy of childhood with centrotemporal spikes (BECTS), is characterized by clusters of simple partial seizures starting between ages 3 and 13 years and typical interictal epileptiform discharges in the centrotemporal region. Seizures usually respond well to the first AED (36).

In some families, BFIC also can occur in combination with familial hemiplegic migraine (FHM), a severe and rare autosomal dominant form of migraine associated with transient hemiparesis ((37), OMIM 602481). In two families with FHM, in which BFIC partially cosegregates, two different mutations in the gene *ATP1A2* encoding the $\alpha 2$ subunit of the Na^+/K^+-ATPase were recently identified ((38), OMIM 182340).

Benign familial neonatal convulsions (BFNC; OMIM 121200, 121201) is clinically similar to BFIC, but seizures appear as a cluster in the first days and weeks of life. In a few cases, however, the onset was described to occur as late as at age 3 months (39–41). Mutations in the genes *KCNQ2* (20q13.32-13.33) and *KCNQ3* (8q24.22) encoding two voltage-gated potassium channels have been identified to be responsible for BFNC (42–44). Mutations in these two genes lead to a reduction of the resulting potassium current and an impairment of membrane repolarization, explaining the neuronal hyperexcitability.

Recently Heron et al. (10) identified mutations in the sodium channel gene *SCN2A* in a new syndrome, which they named benign familial neonatal/infantile convulsions (BFNIC). *SCN2A* is located on chromosome 2q23-31, close to one of the BFIC loci (26,45). Within the two families described, seizures occurred in clusters nearly exclusively in the first weeks of life, thus between the typical periods observed for BFNC and BFIC.

Here we report clinical investigations and linkage studies in 16 BFIC families without a clearly cosegregating accompanying neurologic disorder of German (8), Turkish (1) or Japanese (7) origin with two to eight affected family members.

PATIENTS AND METHODS

Clinical data

All included patients and unaffected family members, or their parents, gave informed consent to the clinical and genetic investigations. All procedures were in accordance with the Helsinki Convention and approved by the Ethical Committees of the University of Ulm and the Tokyo Women's Medical University. Information about the seizure frequency and semiology, the neurologic state and development, electroencephalogram (EEG), and brain imaging was obtained from medical history and examination of the patients, their parents, or other relatives during visits in the various clinics involved in the study, at home or by telephone calls, and from medical records. Classification of seizures and epilepsy syndromes was performed according to the Commission on Classification and Terminology of the International League Against Epilepsy (46,47).

Genetic investigations

Ethylenediaminetetraacetic acid (EDTA) blood samples were obtained from available affected and unaffected family members. Genomic DNA was extracted from leukocytes by standard procedures. Markers were used from the Human Screening Set/Version 8.8a and additional markers from the Genethon human linkage map (48–50). Microsatellite DNA polymorphisms were amplified by polymerase chain reaction (PCR) with the following conditions in a final volume of 50 μl: 50 ng DNA, 30 pmol of each primer (one fluorescent labeled with Cy5), 15 m*M* dNTP, 5 μl buffer (500 m*M* KCl, 200 mM Tris HCl, 25 m*M* $MgCl_2$, 0.01% gelatin, pH 8.4), 0.2 μl Taq polymerase (Pharmacia Biotech, Freiburg, Germany). Samples were amplified in a thermocycler (Whatman Biometra, Goettingen, Germany) in the following conditions: 95°C for 3 min; 35 cycles of 30 s, 95°C; 45 s for annealing and 30 s at 72°C; followed by a final extension period of 2 min at 72°C. PCR products were loaded on a 6%-denaturating polyacrylamide gel for electrophoresis with an Alf Express automated sequencer (Pharmacia Biotech). Genotypes were scored relative to 95-, 300-, and 400-bp

standards. Two-point linkage analysis was performed by the MLINK subroutine of the Linkage package program (51) using an autosomal dominant mode of inheritance with a disease allele frequency of 0.0001 and a penetrance of 70% for lod score calculations. Obligate disease allele carriers were defined as nonaffected.

RESULTS

Clinical data

A summary of the clinical data is provided in Table 1. In 16 BFIC families of German (8), Turkish (1) and Japanese (7) origin, data were obtained from a total of 200 family members. Sixty-seven affected individuals were diagnosed as having BFIC (39 female, 28 male subjects). Three patients had other epilepsy syndromes and were therefore treated as nonaffected in the genetic analysis: patients II.2/BFIC8 and III.3/BFIC12 were classified as having epilepsy with generalized tonic–clonic seizures (GTCSs), which occurred after age 5 years. EEGs of these patients were not available. Patient III.1/BFIC12 had only febrile seizures around age 4 years. The neurologic examination was normal in all affected family members. In most BFIC patients, GTCSs with or without a witnessed focal onset were described, but isolated CPSs occurred (Table 1). The semiology, as described by relatives, included loss of consciousness without any prominent behavioral abnormalities, paleness, cyanosis, generalized hypotonia, gaze deviation, focal cloni of the face or the extremities, and generalized clonic seizures. Seizure onset was between months 2 and 7 of life. Seizures disappeared latest after the age of 18 months. Most of the patients were seizure free after starting the medication. Phenobarbital (PB) was the most common drug used, but carbamazepine (CBZ), phenytoin (PHT), valproate (VPA), or sulthiam (STM) also were administered. The medication was stopped between ages 3 months and 4 years. No relapse of seizures in childhood or adulthood was reported in any of the patients.

Most of the interictal EEGs did not show epileptic discharges. In a few patients, interictal epileptic discharges appeared in the frontal (IV.1/BFIC15, II.3/BFIC5, II.2/BFIC5, II.1/BFIC5) or the occipital lobe (III.1/BFIC3, III.1/BFIC6). In three patients, ictal EEGs could be recorded. In patient IV.2/BFIC15, the ictal pattern started with a right frontal rhythmic activity spreading first over the ipsilateral and then to the contralateral hemisphere (Fig. 1). In patient III.2/BFIC11, ictal epileptic discharges occurred in the left hemisphere during a CPS. The third ictal EEG (patient III.2/BFIC8) showed epileptic discharges bilaterally in the occipital lobe. In 18 patients, brain imaging was available [magnetic resonance imaging (MRI) or cranial computed tomography (CCT)] and classified as normal in 17. In patient IV.2/BFIC15, morphologic abnormalities in form of a few unspecific periventricular white matter lesions were identified.

Most of the patients did not have additional neurologic symptoms apart from epileptic seizures; in particular, paroxysmal dyskinesias or dystonia were not reported for any of the patients. However, in family BFIC19, migraine with aura occurred in patients I.1, I.2, II.5, and II.6, and without aura in patient II.3. The auras were reported only with visual symptoms; no evidence for sensory or motor symptoms was found, as described in FHM. Furthermore, the migraine did not clearly cosegregate with BFIC, because both parents (I.1/I.2) were affected by migraine with aura.

Genetic investigations

The results of the linkage analysis are summarized in Tables 2 and 3. One of the collected families of Japanese origin was too small for linkage studies (BFIC6). Linkage analysis over all families yielded highly negative cumulative 2-point lod scores for the known BFIC loci on chromosomes 2q and 19q, as well as for the BFNC loci on chromosomes 8q and 20q (Table 2). To exclude those loci definitely in as many families as possible, we also performed haplotype analyses (results not shown). The chromosome 19q12-13.11 locus could be excluded in 11 families, whereas in four families, the markers were not informative enough. The 2q23-31 locus was clearly excluded in all families. Furthermore, haplotype analysis excluded the known loci for BFNC on chromosomes 20q13.32-13.33 and 8q24.22 in 14 and 13 families, respectively.

In contrast, 14 of the collected families were linked to the chromosome 16p12-q12 locus, with a maximum cumulative 2-point lod score of 6.1 at marker D16S411. Because of recombinations detected in individuals III.2 and III.4 of family BFIC13 as well as in III.1 and III.2 of BFIC19 between markers D16S690 and D16S753, and individual III.7 of BFIC14 between D16S411 and D16S3136 (Fig. 2), the known region for BFIC (27) could be narrowed to a 22.5-Mbp interval (D16S690–D16S3136) (Fig. 3). In one family of Japanese origin (BFIC5), linkage was excluded for chromosome 16 and for all other loci investigated in this study.

Furthermore, we excluded the locus for FHM and BFIC on chromosome 1q21-23 (38) in family BFIC19 by linkage analysis (results not shown).

DISCUSSION

The clinical analysis of our patients of three different ethnic origins (German, Japanese, and Turkish) revealed a homogeneous picture of complex partial or (secondary) GTCSs with onset at age 2 to 7 months and a benign course of the disease. The three ictal EEG recordings confirmed a focal onset of the seizures in those patients (22). We also detected interictal epileptiform discharges, which are only rarely seen in BFIC patients (22,52–54).

Three patients had clinical syndromes not classifiable as BFIC. One patient showed pure febrile seizures, and two

TABLE 1. *Clinical data of family members affected with epilepsy*

Family	Individual (sex, age/yr)	Onset/end of seizures (age/mo)	Type of seizure	Number/frequency of seizures (effect of treatment)	Treatment (period (age/mo))
BFIC1(D)	II.2 (f, 65)	6/7	GTCS	Cluster	None
	II.4 (f, 68)	6/7	GTCS	Cluster	None
	III.3(m, 47)	6/8	GTCS	Cluster	None
	III.6 (f, 37)	6/7	GTCS	Cluster	None
	III.7 (f, 47)	6/7	GTCS	Cluster	PB (6–24)
	IV.2 (m, 12)	6/9	GTCS	2/d in 4 d (sf)	PB (6–24)
	IV.4 (f, 15)	6/10?	GTCS	Cluster	None
BFIC2(J)	II.2 (f, 25)	4/8	GTCS	?	None
	III.1 (f, 0.3)	3/14	GTCS	3 × 4/d (sf)	PB (6–18)
	III.2 (f, 2)	4/10	GTCS	2 × 2/d (sf)	VPA (10–18)
BFIC3(J)	II.1 (f, 28)	5/8	GTCS	?	None
	II.4 (f, 30)	5/8	GTCS	?	None
	III.2 (m, 0.9)	4/12	GTCS	2	None
	III.3 (m, 4)	4/10	GTCS	3 (sf)	CBZ (8–18)
BFIC5(J)	I.1 (m, 46)	6/?	GTCS	2–3	None
	II.1 (m, 5)	5/6	CPS	Cluster (sf)	CBZ (6–24)
	II.2 (m, 13)	4/4	CPS	Cluster (sf)	PB (6–40)
	II.3 (m, 15)	6/6	GTCS	Cluster (sf)	PB (6–36)
BFIC6(J)	II.2 (f, 22)	6/?	GTC	2–3	None
	III.1 (m, 8)	7/8	CPS	2 (sf)	CBZ (7–24)
BFIC7(J)	I.2 (f, 50)	5/6	GTCS	2	None
	II.2 (f, 27)	5/6	GTCS	2	None
	I.2 (m, 0.5)	5/5	GTCS	4 × 4/d (sf)	PB (6–18)
BFIC8(J)	I.1 (m, 58)	Infancy	GTCS	?	None
	II.2 (m, 32)	5 yr/25 yr	GTCS	?	CBZ
	II.3 (m, 28)	5/10	GTCS	Cluster	None
	III.1 (m, 2)	6/14	CPS	Cluster	None
	III.2 (m, 0.6)	6/10	CPS	10 × /d (sf)	VPA (6–12)
BFIC9(J)	II.2 (f, 26)	3/10	GTCS	Clusters	None
	III.1 (f, 3)	5/13	CPS, GTCS	10/d (sf)	CBZ (6–18)
	III.2 (f, 0.5)	5/15	CPS	10–15/d (sf)	VPA (6–12), CBZ (13–34)
BFIC10(D)	II.1 (m, 70)	6/?	GTCS	?	None
	II.3 (f, 63)	6/∼18	GTCS	1/wk–2/d	None
	II.5 (f, 58)	6/∼18	GTCS	?	None
	III.1 (f, 34)	4/5	GTCS	5 (sf)	PH (4–24)
	III.3 (m, 33)	4/5	GTCS	7–8 (sf)	?
	III.5 (f, 24)	4/4	GTCS	5 (sf)	PB (4–24)
	IV.1 (f, 3)	5/5	GTCS	10 in 2 d (sf)	PB (5–now)
BFIC11(T)	II.2 (f, 27)	?	?	?	?
	II.4 (f, 30)	?	?	?	?
	III.1 (m, 9)	5/8	?	?	PB (5–24)
	III.2 (m, 5)	6/6	GTCS/CPS	5 in 1 d (sf)	PB (6 mo–?)
	III.3 (m, 3)	6/6	GTCS/FS	2 in 2 d (sf)	PB (6 mo–?)
BFIC12(D)	II.2 (f, 60)	?/?	CPS	?	None
	III.6 (f, 27)	3/18	CPS	1–3/d (sf)	None
	III.5 (f, 36)	6/12	CPS	4–10/d (sf)	None
	III.3 (m, 33)	16y/?	GTCS	?	VPA (16 yr–?)
	III.1 (m, 21)	4y/5y	FS	?	None
	IV.1 (f, 8)	2/9	CPS/GTCS	5/d (sf)	CBZ
BFIC13(D)	II.3 (m, 79)	?/?	?	?	None
	III.2 (f, 34)	8/8	CPS	1 in 1 d (sf)	PB (8–48)
	III.3 (f, 39)	8/8	CPS	3 in 3 wk (sf)	CBZ (8–48)
	IV.2 (f, 17)	8/9	CPS	2 in 1 d (sf)	PB (8–48)
BFIC14(D)	II.8 (f, 65)	?/?	?	?	Yes (?)
	III.2 (m, 40)	5/5	CPS	3 in 1 d (sf)	PB (5–48)
	III.7 (m, 6)	?	?	?	?
	III.10 (f, 35)	6/6	CPS	2–3/d (sf)	PHT (6–36)
	IV.1 (f, 4)	6/6	CPS	3–4/d (sf)	CBZ (6–now)
BFIC15(D)	II.1 (m, 65)	?	?	?	?
	IV.1 (f, 3)	6/6	AtS	2 in 1 d (sf)	STM (6–24)
	IV.2 (m, 5)	4/12	CPS/GTCS	9/2 d, 12 mo 3/2 d	PB (4–22)
BFIC18(D)	II.2 (f, 39)	3/3	GTCS	5–6 in a few d (sf)	PHT (3–54)
	II.4 (f, 35)	6/?	CPS/GTCS	?	?
	III.2 (m, 9)	7/14	FS/CPS	5–6 in 7 mo (sf)	PHT (14–now)
BFIC19(D)	II.3 (m, 42)	6/6	GTCS	2 in 14 d	None
	III.1 (m, 4)	6/6	GTCS	4 over 3 wk (sf)	PB (6–36)
	III.2 (f, 2)	5/5	GTCS	6 in 2 d (sf)	PB (5–now)
	III.4 (f, 8)	4/4	GTCS/CPS	3/10 d, ∼20/1 d (sf)	PB (4–7)
	III.5 (f, 15)	6/12	GTCS	3 in 6 mo	None
	III.7 (m, 12)	4/6	GPS/GTCS	8/3 in 8 wk (sf)	PB (6–15)

Individuals are denoted according to the pedigrees in Fig. 2. The age is given in the decimal system.

f, female; m, male; sf, seizure free; (D), family of German origin; (J), family of Japanese origin; (T), family of Turkish origin; GTCS, generalized tonic–clonic seizure; CPS, complex partial seizure; FS, febrile seizure; CBZ, carbamazepine; PB, phenobarbital; PHT, phenytoin; VPA, valproate; STM, sulthiam; d, days; wk, weeks; mo, months; yr, years.

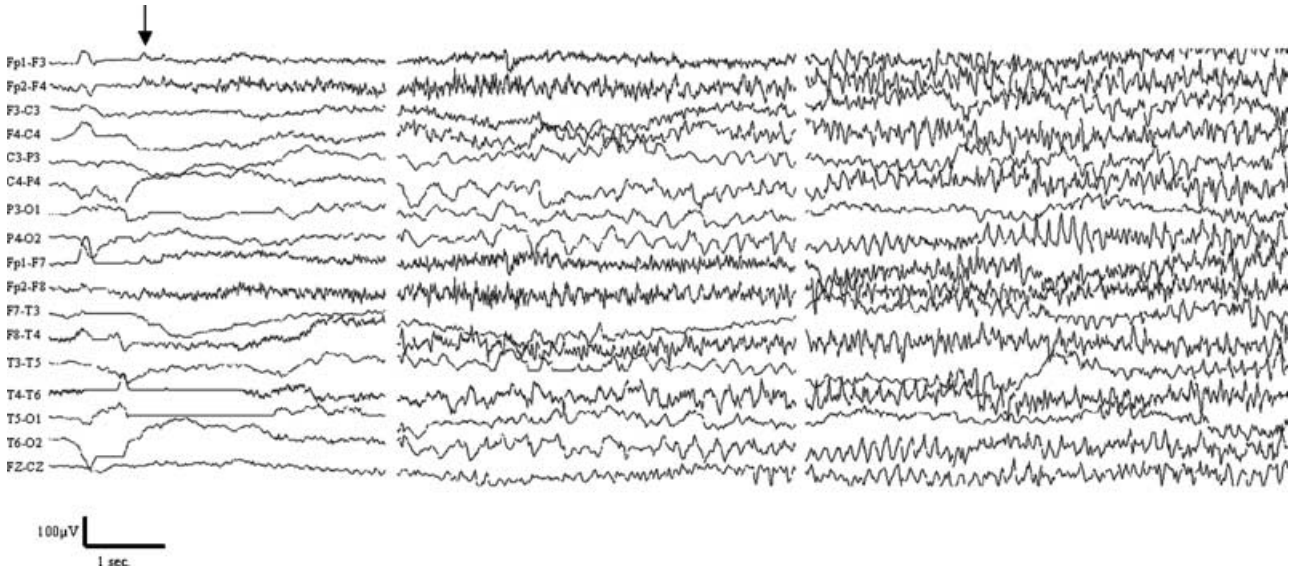

FIG. 1. Ictal EEG of patient IV.2/BFIC15. Routine EEG recording of individual IV.2/BFIC15 during a seizure by using surface electrodes placed according the international 10–20 system. *Arrow,* Onset of ictal discharges in form of a rhythmic activity starting in the right frontal lobe with subsequent spreading over the ipsilateral and later to the contralateral hemisphere.

patients experienced GTCSs with an onset at age 5 or 16 years. For the linkage analysis, these patients were treated as unaffected. However, because two of these individuals (III.1/BFIC12 and III.3/BFIC12) carried the linked haplotype on chromosome 16, this finding may suggest an overlap of BFIC with other epilepsy syndromes, which has not been described in other families with BFIC (25–27). In rare cases of ICCA, a persistence of GTCS until adult age was described (29).

TABLE 2. *Two-point linkage analysis: excluded loci*

Chromosomal localization	Mbp	Lod score at different recombination fractions θ				
		0.0	0.1	0.2	0.3	0.4
2q23-31						
D2s1353	15.7	−57.8	−9.1	−4.1	−1.7	−0.6
D2s382	16.4	−17.4	−1.9	−0.6	−0.2	−0.1
D2s2330	16.4	−55.1	−7.7	−3.3	−1.4	−0.6
19q12-13.11						
D19s433	30.9	−37.7	−4.0	−1.3	−0.4	−0.2
D19s868	33.9	−15.7	−1.6	−0.3	0.1	0.1
D19s245	34.6	−11.5	−1.8	−0.8	−0.4	−0.1
20q13.32-13.33						
D20s171	57.6	−31.9	−4.1	−1.4	−0.3	−0.1
D20s173	58.6	−25.9	−2.2	−0.4	0.2	0.2
8q24.22						
D8s284	130.2	−38.9	−5.3	−2.0	−0.6	−0.1
D8s529	132	−34.2	−4.9	−2.0	−0.8	−0.2
D8s256	133.3	−18.4	−0.3	0.8	0.9	0.5

Cumulative two-point linkage analysis was performed by using an autosomal dominant mode of inheritance with a disease allele frequency of 0.0001 and a penetrance of 70% for lod score calculations. Shown is the exclusion of the known benign familial infantile convulsion (BFIC) loci on chromosomes 19q12-13.11 and 2q23-31 and the known loci for benign familial neonatal convulsions (BFNC) on chromosomes 20q13.32-13.33 and 8q24.22.

In family BFIC19, migraine with or without aura was associated with BFIC in some of the affected individuals but did not clearly cosegregate with the BFIC phenotype. The locus for FHM with BFIC (38) was excluded by linkage analysis. However, because migraine is a very common disease and because, conversely, migraine might not have occurred in the third generation of this family because of a later age at onset, we cannot exclude cosegregation of BFIC and migraine with aura in this family. Therefore the syndrome in family BFIC19 either might be different from the "pure BFIC" syndrome in the other

TABLE 3. *Two-point linkage analysis: linkage to chromosome 16p12.2-q12.2*

Chromosomal localization	Mbp	Lod score at different recombination fractions θ				
		0.0	0.1	0.2	0.3	0.4
16p12.2-q12.2						
D16s403	22.8	−19.4	−0.8	0.2	0.2	0.1
D16s3131	25.8	−11.3	2.5	2.2	1.3	0.5
D16s769	26.0	−14.1	0.7	1.2	0.9	0.3
D16s3093	26.6	−10.4	−0.2	0.6	0.6	0.4
D16s690	27.8	−8.3	2.0	2.2	1.6	0.8
D16s753	31.2	4.6	4.0	2.9	1.6	0.5
D16s3044	47.0	0.6	1.2	1.3	1.1	0.6
D16s3080	49.3	2.5	3.0	2.5	1.6	0.7
D16s411	49.4	6.1	5.2	3.8	2.3	1.0
D16s3136	50.3	−0.7	1.7	1.5	1.0	0.5
ata55A11	50.8	−2.6	2.8	2.2	1.4	0.6
D16s2623	51.7	−3.2	2.5	2.0	1.2	0.5
D16s419	52.6	−7.7	2.4	2.6	2.0	1.1
D16s3137	53.3	−11.0	1.0	1.7	1.4	0.7

Positive linkage in 14 of the collected benign familial infantile convulsion (BFIC) families to chromosome 16p12.2-q12.2, with a maximum cumulative lod score of 6.1 at marker D16S411. The same linkage parameters as indicated in Table 2 were used.

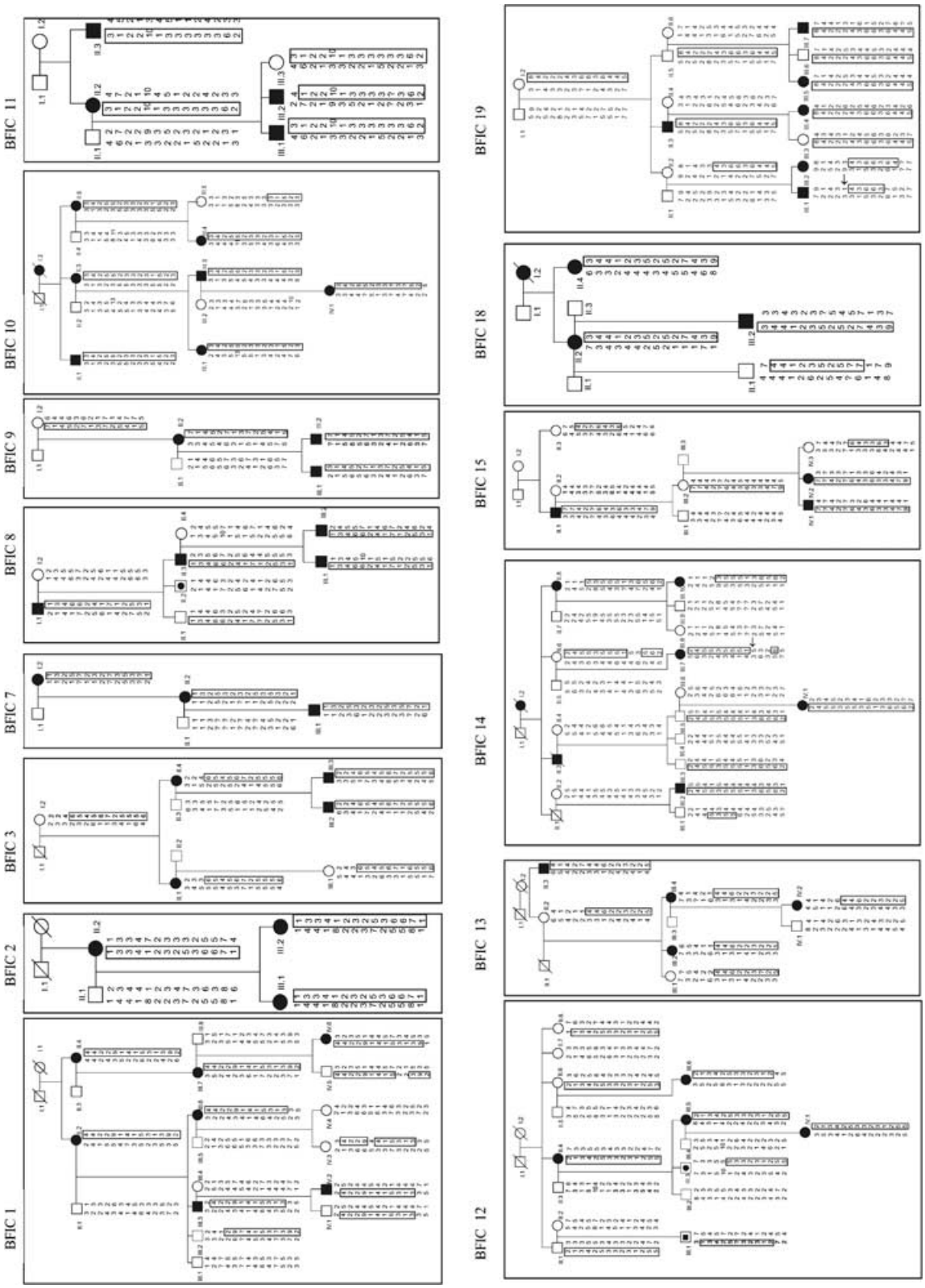

FIG. 2. Haplotype analysis of benign familial infantile convulsion (BFIC) families on chromosome 16p12-q12.■●, males (*square*) and females (*circle*) affected with BFIC; □○, nonaffected males and females; ⊡, males affected with epilepsy with generalized tonic–clonic seizures; ▣, male affected with febrile seizures. Markers used in the linkage analysis (from top to bottom): D16S403, D16S3131, D16S769, D16S3093, D16S690, D16S753, D16S3044, D16S3080, D16S411, D16S3136, ATA55A11, D16S2623, D16S419, D16S3137. Recombinations in individuals III.1/BFIC19 between markers D16S690 and D16S753, and individual III.7/BFIC14 between D16S411 and D16S3136 defining the critical interval between markers D16S690 and D16S3136 (*arrows*).

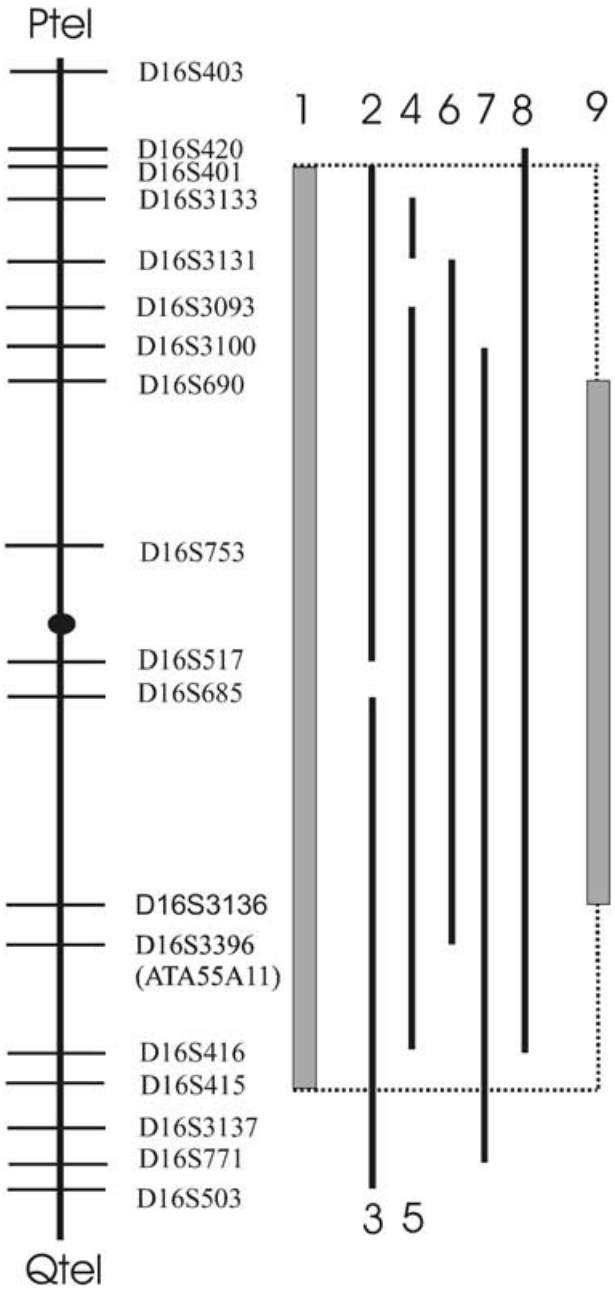

FIG. 3. Comparison of the susceptibility regions on chromosome 16 defined in different linkage studies as indicated. 1, BFIC (27); 2, ICCA (28); 3, PKC (32); 4, RE-PED-WC (34); 5, PKC (31); 6, ICCA (30); 7, PKC (33); 8, ICCA ; and 9, present study.

families, or migraine with aura could be part of the chromosome 16–linked BFIC syndrome (see discussion later).

We did not find evidence for phenocopies, as the linked allele between markers D16S690 and D16S3136 was found in all family members classified as having BFIC. Nevertheless, some nonaffected family members also shared this haplotype, indicating a reduced penetrance of 66%, as assumed by other studies (27).

During the haplotype analysis, double recombinations were found in individuals IV.3/BFIC1 (markers D16S690 to D16S3044, 9.2 cM), IV.5/BFIC1, II.6/BFIC14 (markers D16S411 to D16S2623, 2.3 cM), and III.8/BFIC14 (markers D16S2623 and D16S3137, 1.6 cM). True recombinations, but also genotyping errors or physiologic inversions, could be the underlying cause for this finding. Because several genomic gaps are known in this region, the number of double recombinations might be increased (UCSC: www.genome.ucsc.edu).

In one family with a typical BFIC phenotype (BFIC5, Table 1), we could exclude all loci known for BFNC and BFICs, including the chromosome 19q12-13.11, 2q23-31, 20q13.32-13.33, 8q24.22, and the chromosome 16 locus. This finding suggests further genetic heterogeneity in BFIC.

Our study emphasizes the importance of the chromosome 16p12.2-q12.2 locus for BFIC, as first described by Caraballo et al. (27), with a significant cumulative lod score of 6.1 at marker D16S411. The susceptibility region could be narrowed to 22.5 Mbp between the markers D16S690 and D16S3136. Individual III.7/BFIC14 defines the q-telomeric boundary due to a recombination between microsatellites D16S411 at D16S3136, although she shares one more marker (D16S419) of the disease haplotype (Fig. 2). This most q-telomeric part of the haplotype, including marker D16S419, is definitely excluded by patients III.3/BFIC1 and III.1/BFIC19, in whom a second recombination also defines the p-telomeric boundary of the critical interval. If family BFIC19 is disregarded because of a potentially different phenotype (see discussion earlier), the cumulative lod score would be 5.2 at marker D16S411, but narrowing of the critical interval would be unchanged because of the recombinations found at the same sites in other families (Fig. 2).

The same region was found to be linked to other syndromes with different combinations of epilepsy and paroxysmal movement disorders (27–34), suggesting one responsible gene in this region. Because the detected regions, however, did not overlap entirely, several similar genes associated with these diseases might be located close together in the critical area. Furthermore, the complexity of the interpretation of the results in this region might arise from genotyping errors or inconsistencies of the available genetic maps (27). A common pathophysiologic mechanism of these epileptic and dyskinesic syndromes is nevertheless suggested, not only by the same identified locus on chromosome 16, but also because all of these symptoms could be well explained by an enhanced membrane excitability and because both PKC and BFIC respond usually well to AEDs (35).

According to a databank gene scan performed in this region (UCSC: www.genome.ucsc.edu, NCBI: www.ncbi.nlm.nih.gov, GDB: www.gdb.org), we could not identify any sequence susceptible to an ion channel

gene, as found previously in other forms of idiopathic epilepsies (5–11). Several sodium glucose cotransporters and hypothetical genes with similarity to a carboanhydrase were identified, and might be interesting candidates. Recently the gene *KST1,* coding for a human sodium/glucose cotransporter in the 16p12.1 region, was excluded for BFIC (55).

In conclusion, our study confirms linkage in 14 of 15 BFIC families to chromosome 16p12-q12, emphasizes the importance of this locus for BFIC, and may narrow the critical region to a 22.5-Mbp interval.

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