Familial Occurrence of Febrile Seizures and Epilepsy in Severe Myoclonic Epilepsy of Infancy (SMEI) Patients with *SCN1A* Mutations

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Summary: *Purpose:* The role of the familial background in severe myoclonic epilepsy of infancy (SMEI) has been traditionally emphasized in literature, with 25–70% of the patients having a family history of febrile seizures (FS) or epilepsy. We explored the genetic background of SMEI patients carrying *SCN1A* mutations to further shed light on the genetics of this disorder.

Methods: We analyzed the occurrence of FS and epilepsy among first- and second-degree relatives (N = 867) of 74 SMEI probands with *SCN1A* mutations (70 de novo, four inherited) and compared data with age-matched and ethnically matched control families. Familial clustering and syndromic concordance within the affected relatives in both groups were investigated.

Results: The frequency of FS or epilepsy in relatives of SMEI patients did not significantly differ from that in controls (FS: 13 of 867 vs. 12 of 674, p = 0.66; epilepsy: 15 of 867 vs. six of 674,

p = 0.16). Different forms of epilepsy were identified in both relatives of SMEI probands and controls. Twenty-eight relatives with FS and epilepsy were distributed in 20 (27%) of 74 SMEI families; among the controls, 18 affected relatives were clustered in 13 (18.5%) of 70 families. No pedigree showed several affected members, including the four with inherited mutations.

Conclusions: A substantial epileptic family background is not present in our SMEI patients with *SCN1A* mutations. These data do not confirm previous observations and would not support polygenic inheritance in SMEI. The investigation of the family background in additional series of SMEI patients will further shed light on the genetics of this syndrome. **Key Words:** Severe myoclonic epilepsy of infancy—Voltage-gated sodium channel αsubunit type A—Genetics.

Severe myoclonic epilepsy of infancy (SMEI) is an intractable epileptic encephalopathy typically beginning during the first year of life with febrile or afebrile hemiclonic or generalized prolonged seizures. Subsequently, other seizure types occur, including myoclonic, partial, and absence seizures. Photosensitivity is common.

Psychomotor-development stagnation begins around the second year of life (Dravet et al., 2005). Ataxia, pyramidal signs, and interictal myoclonus can also appear. According to the International League Against Epilepsy (ILAE) classification, SMEI is defined as "syndrome undetermined as to whether it is focal or generalized" (Commission on Classification and Terminology of the International League Against Epilepsy, 1989) because both generalized and focal seizure types occur. SMEI is usually a sporadic condition and familial cases are rarely reported (Singh et al., 2001; Dravet et al., 2005). SMEI has often been associated with a family history of seizure disorders (Dravet et al., 2005), but only one study specifically addressed this issue and found an increased frequency of febrile seizures (FS) and epilepsy in relatives of SMEI patients (Benlounis et al., 2001).

In recent years, heterozygous de novo mutations in the α-subunit type A of the voltage-gated sodium channel gene (*SCN1A*) have been reported in patients with SMEI (Claes et al., 2001), suggesting a monogenic nature for this disorder. However, the recurrence of epilepsy and FSs in SMEI families and the identification of *SCN1A* mutations in parents with mild epileptic phenotypes (Gennaro et al., 2003; Nabbout et al., 2003; Fukuma et al., 2004; Kimura et al., 2005) led to the hypothesis that additional inherited genetic factors could influence the familial epileptic background and modulate the expression of *SCN1A* mutations (Fujiwara et al., 2003). In this scenario, SMEI would be an oligogenic disorder caused by the association of a major player—*SCN1A*—with one or more additional genetic partners.

In this study, we investigated familial antecedents of FSs and epilepsy in 74 SMEI patients with *SCN1A* mutations to explore the genetic background of a homogeneous group of SMEI patients and to shed light on the genetics of this disorder.

METHODS

Inclusion criteria

Patients

Diagnosis of SMEI was made according to the following criteria: (a) no history of acquired brain injury, (b) normal cognitive and motor development before seizure onset, (c) onset of febrile or afebrile seizures, generalized or unilateral, before age 1 year, (d) myoclonic jerks, (e) intractable epilepsy, and (f) psychomotor delay within 2 years from seizure onset. For the purposes of this study, we selected SMEI patients referred from 15 Italian Epilepsy Centers and showing SCN1A mutation. Probands were selected blind to familial antecedents.

Controls

Among subjects admitted to Gaslini Institute, Genova, for acute traumatic or infectious events, we selected 70 subjects (43 boys, 27 girls) with no evidence of neurologic disease and matched for sex and age with the SMEI group.

Genetic analysis

Genomic DNA was extracted from peripheral blood according to standard methods. *SCN1A* exons were amplified by polymerase chain reaction (PCR) in 29 fragments from genomic DNA of each patient. PCR fragments were then analyzed by denaturing high-performance liquid chromatography on the WAVE automated instrument (Transgenomic Inc., Omaha, NE, U.S.A.). Fragments showing an abnormal chromatogram compared with control were subsequently sequenced (Nabbout et al., 2003). For each SMEI proband, possible hereditary transmission of mutations was excluded by testing parents' DNA.

Genealogic documentation and clinical evaluation of relatives

Detailed pedigrees extending to second-degree relatives were constructed for SMEI probands and controls. Information was collected from parents or relatives through semistructured interviews. Clinical and instrumental data of the affected family members were analyzed to determine the type and etiology of seizures. Epileptic seizures and syndromes were classified according the ILAE criteria (Commission on Classification and Terminology of the International League Against Epilepsy, 1981, 1989). "Febrile seizure plus" (FS$^+$) indicated recurrent FS extending beyond 6 years, or with afebrile generalized tonic–clonic seizures (GTCS) (Singh et al., 1999; Singh et al., 2001). The term "unclassified seizures" was used when data were insufficient to diagnose a specific seizure type or epilepsy syndrome.

Statistical analysis

Descriptive statistics of qualitative data were performed and reported in terms of absolute frequencies. The comparison of qualitative data between groups of subjects was made by means of the χ^2 test, or the Fisher's exact test in case of expected frequencies less than five. Subgroup analyses have been performed for first- and second-degree relatives in both SMEI patients and controls. The Bonferroni correction was applied for all the subgroups analyses (p_B). All the tests were two-sided, and a p value < 0.05 was considered statistically significant.

Sample power estimates were calculated according to the method described by Fleiss et al (1980), assuming an α error of 0.05, a β error of 0.2 (i.e., a power of 80%). Considering an estimated relative frequency of FS in the control group of 0.02, the minimum number of subjects needed to detect a difference ≥ 0.03 is equal to 653, for each group. Considering an estimated relative frequency of epilepsy in the control group of 0.01, the minimum number of subjects needed to detect a difference ≥ 0.02 is equal to 866, for each group.

The statistical package Stata release 7 (Stata Corporation, college Station, TX, U.S.A.) was used for all the analyses, and the package NQuery Advisor release 5.0, for the sample-size estimation.

RESULTS

Epileptology of probands

The study included 74 consecutive SMEI patients (38 boys, 36 girls) with SCN1A mutation. Age at first seizure was 3–12 months (mean, 5.4 months). Initial seizures were FSs in 49 cases, afebrile tonic or clonic or tonic–clonic in 25. All probands showed an intractable epilepsy and different type of seizures. During follow-up, all the probands experienced at least one generalized seizure; 42 had absence seizures, 58 had unilateral motor seizures, and 56 had other types of partial seizures, mainly complex partial. Between 1 and 4 years of life, all subjects manifested segmental or massive myoclonic jerks. Status epilepticus occurred in 48 cases, whereas almost half of the patients showed ataxia.

Genetic findings

The list of SCN1A mutations identified in SMEI patients is reported in Table 1. Seventy mutations occurred de novo, whereas four were inherited from the mother. Twenty-four mutations, including the four inherited, have been previously published (Nabbout et al., 2003; Gennaro et al., 2006). Twenty-seven (36.4%) SMEI probands showed SCN1A missense mutations. Truncating mutations (nonsense and frameshift) occurred in 38 subjects (51.3%), whereas mutations affecting the splice sites were found in nine (12.2%).

Familial occurrence of febrile seizures and epilepsy

A total of 214 first-degree (108 male, 106 female) and 653 second-degree (308 male, 345 female) relatives (total, 867) of SMEI probands were ascertained. Control group included 184 first-degree (97 male, 87 female) and 490 second-degree (241 male, 249 female) relatives (n = 674). None of the pedigrees showed evidence of consanguinity. Affected relatives of SMEI probands and controls are reported in Table 2.

Febrile seizures in relatives

Among relatives of SMEI probands, 13 (eight female, five male) out of 867 (1.5%) individuals had single (nine cases) or multiple (four cases) of FS. No subject experienced isolated FS[+]. Within the control group, 12 (four females, eight males) relatives of 674 (1.8%) had single (10 cases) or multiple (two cases) of FS.

No significant difference (p = 0.74) was found between first-degree (four of 184) and second-degree relatives (eight of 490) of controls, indicating that the age of relatives did not bias the ascertainment of FS. Overall, the frequency of FS in relatives of SMEI patients did not significantly differ from that in controls (13 of 867 vs. 12

TABLE 1. *SCN1A mutations in 74 patients with SMEI*

Family ID	Mutation DNA	Mutation Protein	Inheritance	Type
1	c.969 T>A	Y323X	De novo	Premature truncation
2	c.1165C>T	Q389X	De novo	Premature truncation
3	c.3697G>C	G1233R	De novo	Missense
4	c.5008-5011delTGTT	F1671fsX1679	De novo	Premature truncation
5	c.5674C>T	R1892X	De novo	Premature truncation
6	c.4388T>C	F1463S	De novo	Missense
7	c.680T>G	I227S	De novo	Missense
8	c.5285G>A	G1762E	De novo	Missense
9	IVS2+3T>G	N/A	De novo	Splicing
10	c.5141T>G	M1714R	De novo	Missense
11	c865G>C	E289X	Maternal	Premature truncation
12	c.5656C>T	R1886X	De novo	Premature truncation
13	c.249C>G	Y83X	De novo	Premature truncation
14	c.853-856delCTTC	A285fsX290	De novo	Premature truncation
15	c.234G>T	E78D	De novo	Missense
16	c.664C>T	R222X	De novo	Premature truncation
17	iIVS24+2A>G	N/A	De novo	Splicing
18	c.4486delG	Q1496fsX1500	De novo	Premature truncation
19	c.680T>G	I227S	De novo	Missense
20	c.2435C>G	T808R	De novo	Missense
21	c.1624C>T	R542X	De novo	Premature truncation
22	iIVS4+1C>T	N/A	De novo	Splicing
23	c.4542C>A	S1516X	De novo	Premature truncation
24	c.3295 G>T	E1099X	De novo	Premature truncation
25	c890C>T	c890C>T	Maternal	Missense
26	del3867-3869CTT	delF1298	De novo	Premature truncation
27	IVS1+5 G>A	N/A	De novo	Splicing
28	c.2678T>A	L893X	De novo	Premature truncation
29	c.838T>C	W280R	De novo	Missense
30	c.4277T>G	L1426R	De novo	Missense
31	c.1129C>T	R377X	De novo	Premature truncation
32	c.664C>T	R222X	De novo	Premature truncation
33	c.2536G>A	E853K	De novo	Missense
34	c.992insT	L331fsX339	De novo	Premature truncation
35	del c.3997-4002	M1333X	De novo	Premature truncation
36	c.1177T>C	R393C	De novo	Missense
37	c.484A>C	T162P	De novo	Missense
38	c.5339T>C	M1780T	De novo	Missense
39	c5002C>G	P1668A	Maternal	Missense
40	c.3173delAAGA	K1058fs1079X	De novo	Premature truncation
41	c.4352C>T	P1451L	De novo	Missense
42	c.277-278delTT	N94X	De novo	Stop
43	c.3733C>T	R1245X	De novo	Stop
44	c.1177C>A	R393S	De novo	Missense
45	c5240insAA	1747Nfs1779X	Maternal	Premature truncation

TABLE 1. *Continued.*

Family ID	Mutation DNA	Mutation Protein	Inheritance	Type
46	c.5414-5415delTT	F1805X	De novo	Stop
47	c.5040insAA	M1681fsX1715	De novo	Stop
48	del c.2916-2919	T970fsX972	De novo	Stop
49	IVS10+1G>T	N/A	De novo	Splicing
50	c.1276T>A	Y426N	De novo	Missense
51	c.1121delC	S374fsX378	De novo	Stop
52	c.5674C>T	R1892X	De novo	Stop
53	c.3733C>T	R1245X	De novo	Stop
54	IVS18+1G>T	N/A	De novo	Splicing
55	del c.3696G ins22	L1235fsX1243	De novo	Stop
56	c.5318C>T	S1773F	De novo	Missense
57	c.664C>T	R222X	De novo	Stop
58	c.5531delCAAA	N1845fsX1856	De novo	Stop
59	c.650C>A	T217K	De novo	Missense
60	c.2825T/C	L942P	De novo	Missense
61	c.4424T>C	L1475S	De novo	Missense
62	del c.387-389 ATT	delL129	De novo	Stop
63	c.1837C>T	R613X	De novo	Stop
64	c.1149C>G	F383D	De novo	Missense
65	c.5668delG	E1890fsX1910	De novo	Stop
66	c.383C>A	S128X	De novo	Stop
67	c.5657delG	R1886fsX1910	De novo	Stop
68	c.3734G>A	R1245Q	De novo	Missense
69	IVS18-1G>A	N/A	De novo	Splicing
70	c.580G>A	D194N	De novo	Missense
71	c.4265A>G	Y1422C	De novo	Missense
72	IVS25-1G>C	N/A	De novo	Splicing
73	IVS5+1G>C	N/A	De novo	Splicing
74	c.1042G>T	G348X	De novo	Stop

of 674; p = 0.66). Stratification analysis indicate that the frequency of FS was not significantly increased in either first-degree (three of 214 vs. four of 184; $p_B = 0.98$) or second-degree relatives (10 of 653 vs. eight of 490; $p_B = 1.00$) compared with controls.
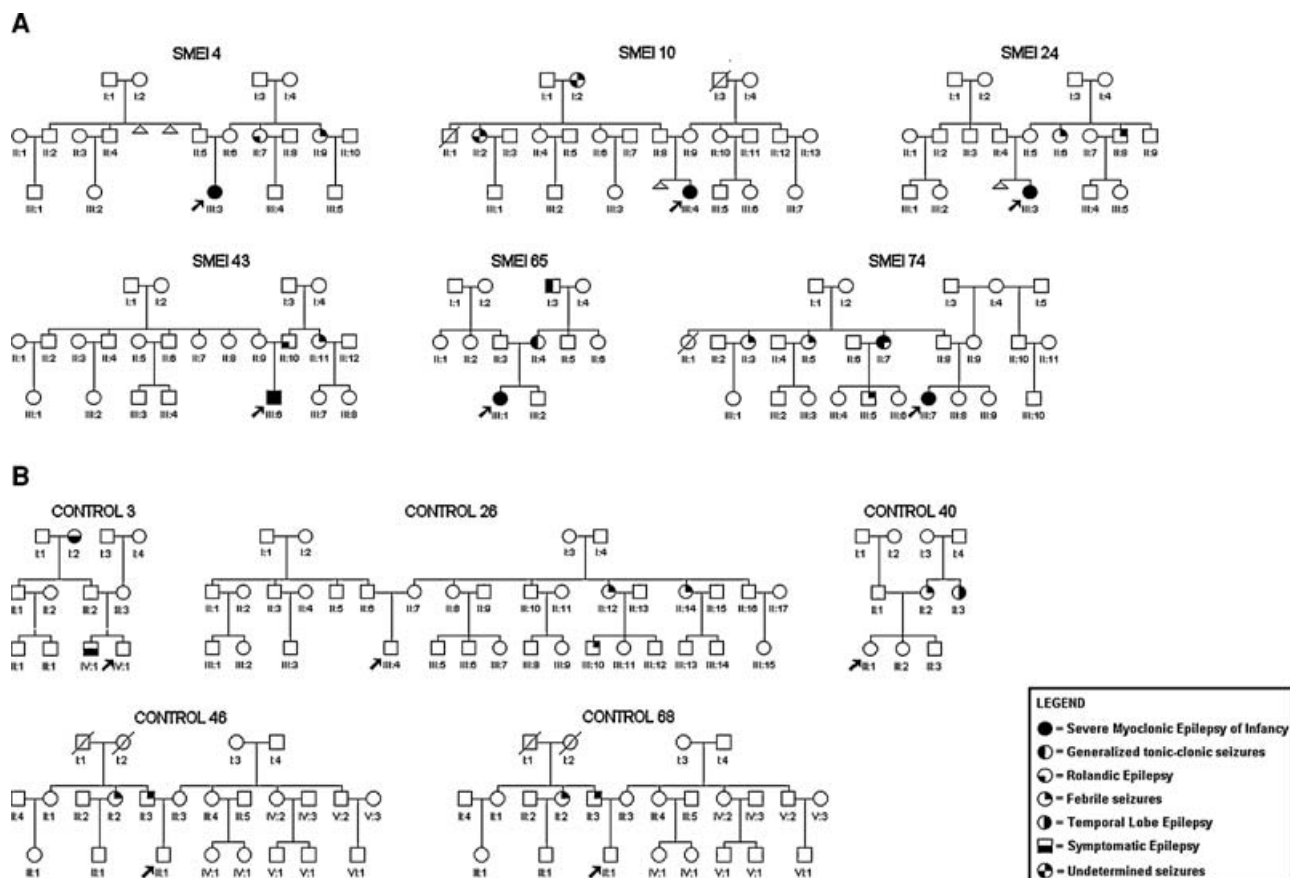
Epilepsy in relatives

Among SMEI relatives,15 (10 female, five male) of 867 (1.7%) individuals had a history of epilepsy (Table 2). Within the control group, epilepsy was found in six of 674 (0.9%) relatives without a significant difference between first- and second-degree relatives (two of 184 vs. four of 490; p = 0.67).

The frequency of epilepsy in relatives of SMEI was not significantly increased compared with controls (15 of 867 vs. six of 674; p = 0.16).

Epilepsy phenotypes in relatives of SMEI patients and controls were the following:

- Idiopathic generalized epilepsy: four SMEI relatives were affected by epilepsy with GTCSs. One had FS$^+$. Childhood absence epilepsy (CAE) occurred in one SMEI relative. One subject from the control group had epilepsy with GTCS;
- Idiopathic partial epilepsy: three SMEI relatives had typical benign epilepsy with centrotemporal spikes (BECTS);
- SMEI: two siblings carrying inherited mutations were affected by SMEI;
- Cryptogenic epilepsy: one individual belonging to the control group was affected by temporal lobe epilepsy (TLE) with onset at age 52 years;
- Symptomatic epilepsy: one SMEI relative had seizures due to perinatal hypoxic–ischemic encephalopathy. In the control group, one subject had mesial temporal sclerosis with TLE; three individuals were affected by epilepsy due to perinatal hypoxic ischemic damage (n = 2) and stroke sequelae (n = 1);
- Undetermined seizures: two SMEI relatives, belonging to the same family, had rare, drug-responsive

TABLE 2. *Occurrence of febrile seizures and epilepsy among relatives of SMEI probands and controls*

Seizure type	Familial antecedents									
	SMEI					Controls				
	First degree (n = 214)			Second degree (n = 653)	Total (n = 867)	First degree (n = 184)			Second degree (n = 490)	Total (n = 674)
	Mother	Father	Sibs			Mother	Father	Sibs		
Febrile seizures	1^a	1	1	10	13	1	2	1	8	12
Idiopathic generalized epilepsy					6					1
CAE	-	-	-	1	1	-	-	-	-	0
GTCS	2	1	-	1	4	1	-	-	-	1
FS$^+$	-	-	-	1	1	-	-	-	-	0
Idiopathic partial epilepsy					3					0
BECTS	-	1	-	2	3	-	-	-	-	0
SMEI	-	-	2^a	-	2	-	-	-	-	0
Cryptogenic epilepsy	-	-	-	-	-	-	-	-	1	1
Symptomatic epilepsy	-	-	-	1	1	-	-	1	3	4
Undetermined seizures	1^a	-	-	2	3	-	-	-	-	0

CAE, childhood absence epilepsy; GTCS, generalized tonic–clonic seizure; FS$^+$, febrile seizures plus; BECTS, benign epilepsy with centrotemporal spikes.
^{a}Relatives carrying *SCN1A* mutations.

FIG. 1. Pedigrees of SMEI probands with de novo *SCN1A* mutations (**A**) and control subjects (**B**), showing two or more affected relatives.

seizures diagnosed as absences, during the menopausal age. A mother bearing a truncating mutation experienced focal seizures during early adolescence (Nabbout et al., 2003). However, as no EEG was available, we considered these cases affected by undetermined seizures.

Idiopathic generalized epilepsy occurred more frequently in relatives of SMEI probands (six of 867) than in controls (one of 674), but this difference is not statistically significant ($p_B = 0.47$). The frequency of epilepsy was not increased in either first-degree relatives of SMEI probands (seven of 214 vs. two of 184; $p_B = 0.99$) or second-degree relatives (eight of 653 vs. four of 490; $p_B = 0.94$) compared with controls.

Familial clustering

Twenty-eight relatives with FSs and epilepsy were distributed in 20 (27%) of 74 SMEI families: 13 (17.6%) showed a single additional affected relative (six with FSs, seven with epilepsy), six (8.1%) had two additional affected relatives, and one (1.3%) showed three additional affected members. Among SMEI families with multiple affected members, four showed clinical heterogeneity (Fig. 1A): families 4 and 65 had two relatives with FSs and BECTS; family 43 had two subjects with FSs and one

with IGE and FS$^+$. In family 24 and family 74, relatives were concordant for FS and IGE, respectively (Fig. 1A). Family 10 showed two relatives affected by epilepsy of undetermined type. In family 45, the mother had a single FS during infancy, and the proband's brother was affected by SMEI. Recently we demonstrated that the mutation was inherited by the affected siblings from the mother who showed a somatic mosaicism (Gennaro et al., 2006).

Within the control group, 18 affected relatives were clustered in 13 (18.6%) of 70 families: eight (11.4%) families showed one affected subject, and five (7.1%) showed two affected subjects (Fig. 1B). None of the SMEI or control families showed a history of seizures in both the paternal and maternal branches.

DISCUSSION

The role of the family background in SMEI has been traditionally emphasized in the literature. It was reported by different studies that 25–70% of SMEI patients have a positive family history of FSs or epilepsy (Singh et al., 2001; Nabbout et al., 2003; Scheffer et al., 2003; Dravet et al., 2005). However, only Benlounis et al. (2001) approached this issue methodically and found that the frequency of FSs and epilepsy was significantly increased in relatives of
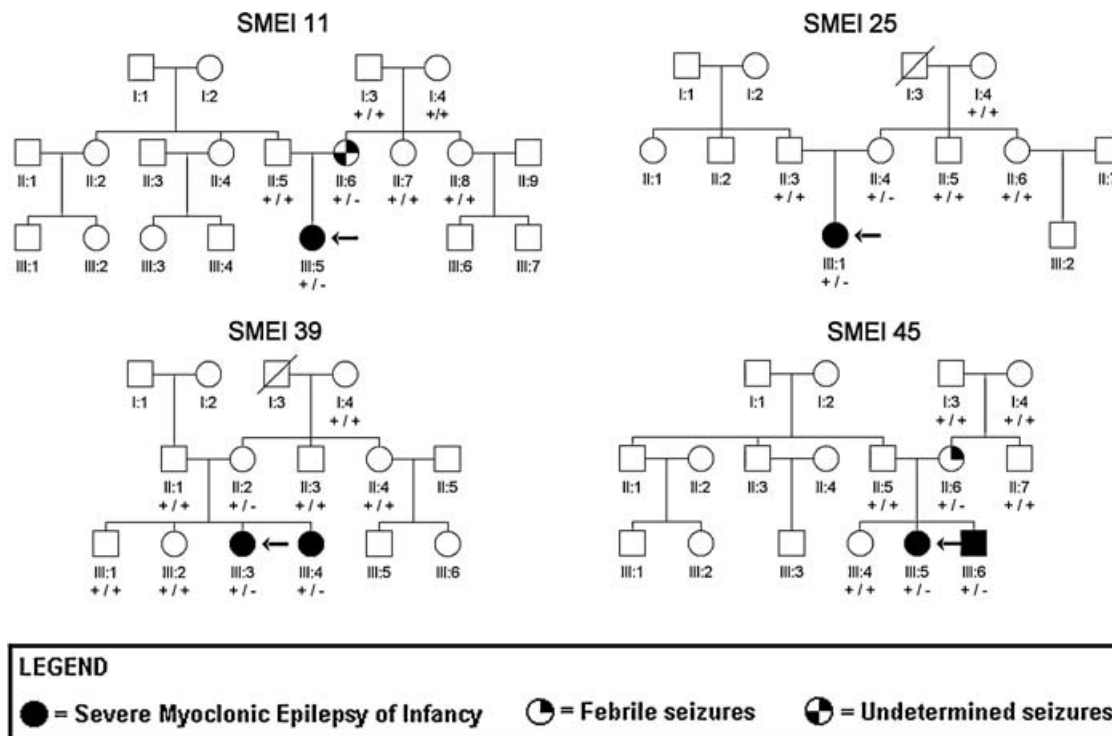
FIG. 2. Pedigrees of SMEI probands with inherited *SCN1A* mutations. +, wild-type allele; −, *SCN1A* mutation.

SMEI patients compared with a control group. As epilepsy in relatives had the characteristics of idiopathic generalized epilepsy (IGE), the authors concluded that SMEI is genetically linked to FS and to other phenotypes of IGE. The identification of de novo *SCN1A* mutations in SMEI probands and the lack of *SCN1A* mutations in families with common forms of IGE (Escayg et al., 2001) or FS (Malacarne et al., 2002) challenged this view and suggested our investigation of the familial antecedents of FS and epilepsy in a homogeneous group of SMEI patients showing *SCN1A* mutations.

In our series, 27% of SMEI probands characterized by *SCN1A* mutations have a family history of seizures. However, because of the high frequency of FS (2–5%) and epilepsy (∼0.5–1%) in the general population (Hauser et al., 1991), this finding is not indicative by itself of an increased risk for relatives of SMEI probands. We found that FS and epilepsy occur in 18.5% of age-matched and ethnically matched control families. Moreover, systematic analysis of first- and second-degree relatives of SMEI probands and controls demonstrated that the frequency of FS and epilepsy was not significantly increased in our series of SMEI families carrying *SCN1A* mutations.

Power estimates, calculated on the actual frequency of FS in the control population, indicate that the available sample can detect a ≥2.5-fold increase frequency of FS among SMEI relatives. Because of the lower frequency of epilepsy with respect to FS, the available sample can detect a ≥3-fold increased frequency of epilepsy among

SMEI relatives. Minor differences (e.g., twofold) in FS and epilepsy frequencies between relatives of SMEI patients and the general population cannot be detected by our study and would require a sample size on the order of thousands.

Our findings are in contrast with the study of Benlounis et al. (2001). The criteria of selection of SMEI population could be responsible of conflicting findings. In Benlounis et al. (2001), the SMEI sample was selected blind to molecular analysis, and it is likely to be genetically heterogeneous, as a proportion of SMEI patients do not carry *SCN1A* mutations (Mulley et al., 2005). The family background of SMEI patients without *SCN1A* mutations—who were not included in our study—may differ from that of patients bearing *SCN1A* mutations. The characteristics of the control population could also have affected statistical outcomes. In Benlounis et al. (2001) control families showed a very low frequency of FS (1 of 222; < 0.5%) and no family members with epilepsy. Conversely, in the present study, the frequency of FS and epilepsy among relatives of controls was similar to that observed in general population (Hauser et al., 1991), with a homogeneous distribution among first- and second-degree relatives of controls.

It has been reported that FS[+] and IGE are the more frequent epileptic phenotypes among relatives of SMEI probands (Benlounis et al., 2001; Singh et al., 2001). In the present study, different forms of epilepsy were identified in both relatives of SMEI probands and controls.

IGEs occurred more frequently in the SMEI group, but the difference is not statistically significant.

The analysis of familial clustering failed to identify pedigrees with several affected members. The majority (13 of 20, 65%) of SMEI pedigrees with a family history showed a single relative affected by FS or epilepsy. Moreover, families with multiple affected relatives were equally frequent in SMEI and control groups (9.5% vs. 7.1%).

Interestingly, the four families segregating *SCN1A* mutations have no second-degree members affected by epilepsy or FS and do not show a significant familial clustering. Accordingly, in these families, none of the second-degree relatives carries a *SCN1A* mutation. Furthermore, we showed that the mutation occurred de novo in the proband's mother in two instances (SMEI 11, SMEI 45; Fig. 2).

The family history for FS and epilepsy previously reported in different clinical series as well as the variable expressivity of *SCN1A* mutations in parents of SMEI probands suggest that mutations in *SCN1A* could not be the only genetic player in the etiology of SMEI (Scheffer et al., 2003; Mulley et al., 2005).

However, in this study, we showed that a substantial epileptic family background is not present in 74 SMEI patients with *SCN1A* mutations. In addition, the four families with inherited mutations do not show several affected members, probably because of the de novo origin of the mutations in the parents. These data and our recent observation that somatic mosaicism may account for variable expressivity of *SCN1A* mutations in SMEI families would suggest that SMEI is a monogenic rather than a multifactorial condition (Gennaro et al., 2006). The investigation of the family background and the detection of somatic mosaicism in additional series of SMEI patients will further shed light on the genetics of this syndrome.

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