Idiopathic epilepsies with seizures precipitated by fever: clinical and genetic study of 132 patients

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Abstract

Purpose: Mutations in the genes coding for subunits of ion channels have been associated with epilepsy. Of these known epilepsy genes *SCN1A,* coding for the α subunit of the sodium channel, is currently the most clinically relevant gene. The majority of *SCN1A* mutations lead to severe myoclonic epilepsy of infancy (SMEI) including borderline SMEI (SMEB) and to generalized epilepsy with febrile seizures plus (GEFS+). Both syndromes have febrile seizures (FS) as a common clinical feature. The aim of this study was to achieve a better definition of the spectrum of phenotypes that might be associated with *SCN1A* mutations. We aimed to performing phenotype-genotype correlations of *SCN1A* mutations with specific epilepsy syndromes.

Methods: We selected 132 patients in whom most seizures occurred during febrile episodes. A clinical and genetic study focussing on *SCN1A* screening was performed.

Results: Patients were classified as follow: SMEI/ SMEB = 55; GEFS+ spectrum= 25; sporadic myoclonic astatic epilepsy= 3; classical FS= 10; other phenotypes= 25. We identified 40 *SCN1A* mutations. Of the 40 mutations 37 were found in patients with SMEI in whom mutations were missense in 16 probands (2 familial) and truncating in 21 (2 familial). The remaining 3 missense mutations were associated with GEFS+. Missense mutations in the pore forming parts (S5-S6) of the Na+ channel occurred in 10 out of the 16 SMEI (62,5%) and only in one of the three GEFS+ patients. Mutations in the pore forming region seem to correlate in 70% with the classical SMEI type and only with 30% of the SMEB phenotype. Analysis of the age of seizures onset between SMEI patients with: a) *SCN1A* truncating mutations, b) *SCN1A* missense mutations and c) no *SCN1A* mutations showed that the differences of the age of FS onset was extremely significant between the three groups (p=0,0007, ANOVA test). Patients with truncating mutations had the earlier

onset of FS, patients with missense mutations had an intermediate onset, and individuals without *SCN1A* mutations had the latest age of FS onset.

Conclusion: We obtained a prevalence of about 70% of *SCN1A* mutations in SMEI and SMEB patients and of 12% in GEFS+ probands confirming the predominant and important role of *SCN1A* in patients with SMEI. None of the other patients with fever-provoked seizures carried mutations in *SCN1A* gene. The high correlation between SMEI and *SCN1A* mutations suggests a phenotypical specificity of *SCN1A* rather than a dysfunction of neurons exacerbated by high body temperature

Introduction

Voltage-gated sodium channels are essential for the generation and propagation of action potentials in the brain as well as in other excitable tissues. Mutations in the genes coding for the α1 and β1subunits of the neuronal sodium channel (*SCN1A* and *SCN1B* respectively) have been associated with epilepsy. The overwhelming majority of *SCN1A* known mutations lead to severe myoclonic epilepsy of infancy (SMEI) and to generalized epilepsy with febrile seizures plus (GEFS+) (Mulley et al., 2005).

GEFS+ is a complex and heterogeneous familial syndrome with autosomal dominant inheritance in some families in which febrile seizures (FS) and febrile seizures plus (FS+) are the predominant phenotypes (Scheffer and Berkovic, 1997; Singh et al., 1999a). *SCN1A* and *SCN1B* mutations have been found in about 10% of GEFS+ families (Bonanni et al., 2004; Escayg et al., 2001; Escayg et al., 2000; Scheffer et al., 2000; Wallace et al., 2001; Wallace et al., 2002; Wallace et al., 1998).

Severe myoclonic epilepsy of infancy (SMEI) or Dravet's Syndrome is an epileptic encephalopathy presenting with prolonged febrile or afebrile generalized or unilateral clonic seizures in the first year of life in an otherwise normal infant (Dravet, 2002). Between 1 to 4 years other seizure types occur including myoclonic, absences and partial seizures, developmental delay becomes also apparent within the second year of life. Several authors described group of patients sharing most but not all the characteristic clinical features of SMEI patients and designated them as 'borderline SMEI (SMEB)' (Fukuma et al., 2004; Oguni et al., 2001). A family history of epilepsy is often found in SMEI patients and affected relatives have epilepsy phenotypes consistent with GEFS+ (Scheffer and Berkovic, 1997; Singh et al., 1999a; Singh et al., 1999b). Mutations of *SCN1A* have been found in 40 to 100% of SMEI patients and are *de novo* in the majority of individuals (Claes et al., 2001; Nabbout et al., 2003; Sugawara et al., 2002; Wallace et al., 2003).

Clinical analysis of the phenotypes shows clearly that the first clinical sing of mutations in *SCN1A* is recurrent, often prolonged, seizures provoked by fever in infancy. This evidence suggests that, in clinical practice, mutations in *SCN1A* could be suspected in every child with fever-provoked seizures. Febrile seizures are the most common convulsive event in humans and about 13% of patients with epilepsy have a history of FS (Frucht et al., 2000). The largest percentage of FS (25%) is observed in temporal lobe epilepsy, often characterized by the sequence of prolonged FS in childhood, hippocampal sclerosis (HS) and refractory temporal lobe seizures (Baulac et al., 2004). Sporadic patients with TLE and HS have been described in GEFS+ families with *SCN1A* and *SCN1B* mutations (Abou-Khalil et al., 2001; Wallace et al., 2002). These data, thus confirm the role of sodium channel genes in decreasing seizures threshold causing fever-provoked seizures in infancy and more specific seizure types later in the course of the syndrome. Modifier genes and environmental factors might be also involved to determine the phenotype specificity and the epilepsy outcome.

Following these considerations, we performed a clinical and genetic study focussing on *SCN1A* screening of a large series of patients with various epilepsy phenotypes whose common clinical feature was represented by the occurrence of fever-provoked seizures. The present study aimed to achieve a better definition of the spectrum of phenotypes that might be associated with *SCN1A* mutations. We also aimed to performing phenotype-genotype correlations of *SCN1A* mutations with specific epilepsy syndromes and possibly describe the clinical characteristics of fever-provoked seizures associated with *SCN1A* mutations.

Methods

Patients recruitment

We recruited subjects with either focal or generalized idiopathic epilepsies whose seizures were particularly increased during febrile episodes. Patients were collected from child neurologists and epileptologists around Italy and other European countries. Informed consent was obtained from parents or guardians.

Clinical classification

A detailed clinical history including pre and perinatal antecedents, seizures age of onset and semiology, occurrence of fever-provoked seizures, cognitive functions, EEG recordings and MRI were directly collected from the parents and/or other relatives for patients seen at the Epilepsy, Neurophysiology and Neurogenetic Unit of the Department of Child Neurology and Psychiatry of the Stella Maris Foundation, Pisa, Italy. For patients referred from other centres around Italy or Europe, medical records from child neurologists, paediatricians, hospitals and other treating doctors were collected and reviewed whenever possible. Genealogical information and family trees were also collected when available.

Epileptic seizures and epilepsy syndrome diagnoses were performed according to the International League Against Epilepsy classifications (Engel, 2001; ILAE, 1981; ILAE, 1989). Seizures lasting longer than 30 minutes were classified as status epilepticus, whereas seizures lasting at least several minutes but definitely less than 30 where considered as ‘prolonged seizures’. Classical SMEI was diagnosed when all of the following features were present: onset in the first year with hemiclonic or generalized febrile or afebrile seizures, evolution of myoclonic seizures, atypical absences and partial seizures, and progressive cognitive impairment (Dravet, 2002). The term “borderline” severe myoclonic epilepsy of infancy (SMEB) was used for cases without a number of the key features of

SMEI (e.g. lack of generalized spike wave discharges, lack of myoclonus, limited number or atypical seizure types) (Dravet, 2002; Fukuma et al., 2004; Oguni et al., 2001). Patients with febrile seizures beyond age 6 years and/or with afebrile seizures in early or mid childhood were classified as febrile seizure plus (FS^+) (Scheffer and Berkovic, 1997; Singh et al., 1999a).

When classical FS, FS+ with or without other seizure types, occurred in families we considered them as part of the GEFS+ spectrum (Scheffer and Berkovic, 1997; Singh et al., 1999a). Myoclonic astatic epilepsy (MAE) was considered as sporadic if no other family members had had seizures or it was included in the GEFS+ spectrum when it presented in families (Scheffer and Berkovic, 1997; Singh et al., 1999a). Patients in whom despite clinical information a diagnosis could not be made were designated as 'unclassified epilepsy'.

Molecular analysis

Peripheral blood samples were obtained from patients and from almost all of their parents, genomic DNA was extracted using standard protocol. The 26 coding exons of the *SCN1A* gene were amplified by PCR reaction with primers designed to amplify each exon and the flanking intron splice site. PCR products were then analyzed by denaturing high performance liquid chromatography (dHPLC) on a Wave automated instrument (Transgenomic Inc., Cheshire). Primers sequences and dHPLC condition are available upon request. Abnormal profiles observed on dHPLC screening were subsequently analyzed by direct sequencing. Sequencing analysis on both DNA strands was performed on a ABI3100 *avant* sequencer (Applied Biosystems, CA, U.S.A.) using the BigDyes v1.1 terminator Cycle Sequencing Kit following the manufacturer's protocol. Sequences were analyzed using as reference for the Seqscape program (Applied Biosystems, CA, U.S.A.) the genomic region of the Genbank sequence NC_000002 encompassing the *SCN1A* gene.

Available parents' DNA was checked for the mutation identified in their child by direct sequencing.

Sequence changes were interpreted as mutations when they resulted in: a) truncating mutation (frameshift, stop or splice site mutation; b) missense mutations when the change lead to an amino-acid substitution that was not previously reported as polymorphism, was not present in 200 control alleles and/or it arose *de novo*. The coding synonymous and intron nucleotide changes were investigated *in silico* using the Neural Network Splice Site Prediction software (http://www.fruitfly.org/seq_tools/splice.html) in order to identify alteration in the mRNA splicing process. The mutations found were coded using the DNA mutation checker (http://www.ebi.ac.uk/cgi-bin/mutations/check.cgi) and the protein P35498 of the Swiss-Prot Database reference.

Genotype-phenotype correlations

Based on epilepsy syndrome diagnosis, the 132 patients were divided in: 1) SMEI, 2) GEFS+, and 3) other phenotypes. A clinical analysis focussed on seizure types and age of seizures onset, with particular attention to seizures provoked by fever, was performed in the three groups. Based on previous studies of genotype-phenotype correlations of SMEI patients (Ceulemans et al., 2004; Fukuma et al., 2004; Ohmori et al., 2003) we further subdivided our SMEI probands in three groups according to the *SCN1A* analysis: a) truncating, b) missense and c) no mutations. ANOVA test was used for statistical analysis to evaluate differences of age of seizures onset between the three groups of SMEI patients.

To evaluate the existence of hot spots regions, where mutations of *SCN1A* are more likely to be found, we compared the number of the identified mutations in each exon (including 10 nucleotides up and down stream the exon) to the number of expected mutations using Fisher's exact test. We also performed a Fisher's exact test to evaluate the location of *SCN1A* mutations grouping the exons according to their coding protein domain:

N-terminal + DI; Loop 1; DII; Loop 2; DIII; Loop 3 and DIV + C-term.

Results

A total of 132 probands, 74 females and 59 males, with idiopathic epilepsies including febrile seizures (FS) were studied. Patients were collected from various European countries including: Italy (82), United Kingdom (24), Portugal (23), Spain (1), Denmark (1) and Israel (1). The mean age at the time of the study was 9 years (median 6,5 ranging from 1 to 38 years). Epilepsy phenotypes of the 132 probands are shown on Table 1.

Epilepsy syndromes

1. Severe myoclonic epilepsy of infancy (SMEI)

We studied 55 patients (27 females and 28 males) with a clinical diagnosis of SMEI (39) and SMEB (16 patients). Mean age at the time of the study was 9,5 years (median 8,6 ± 5,1, ranging from 2,5 to 20,8). Patients were divided in 3 groups according the *SCN1A* mutational screening result: 1) truncating, 2) missense and 3) no mutations. Detailed clinical information of patients with truncating and missense mutations are presented on Table 2 and 3 respectively.

a) Group 1: patients with SCN1A truncating mutations

Truncating mutations were identified in 21 patients with SMEI (18) and SMEB (3) (see Table 2 and Figure 1a). All patients had recurrent FS and in the majority of patients FS were the first clinical expression occurring between the 4^{th} to 5^{th} months of life (mean 4,6 months; median 4; ranging from 2 to 9). Status epilepticus, either febrile (14 patients; 66%), afebrile (13 patients; 62%) or both (7 patients; 33%), was reported in all patients. Febrile status epilepticus had a mean age at onset of 9,6 months (median 7, ranging from 3 to 40), afebrile status epilepticus had a mean age at onset of 12,5 months (median 9, ranging from 3 to 48). Afebrile tonic-clonic, tonic or hemiclonic seizures, often prolonged, occurred in 19 (90%) patients, mean age at onset was 13 months (median 10, ranging from

3 to 42). Symmetric or asymmetric bilateral myoclonic seizures occurred in 14 (67%) patients and in the majority of them began during the second year of life (mean age of onset 19 months; median 13; ranging from 4 to 38). Complex partial seizures were seen in 16 (76%) patients with a mean age of onset at 23 months (median 18; ranging from 3 to 72). Atypical absences occurred in 13 (62%) patients with mean age of onset at 34 months (median 36; ranging from 10 to 72). All patients had cognitive impairment ranging from mild to severe. A family history for seizures including FS was present in 11 (52%) patients. The sister of a SMEI patient (see Table 2, mutation IVS4+1 G>A splicing) had a clinical history also consistent with SMEI with prolonged febrile seizures beginning around 6 months of age and followed by the later appearance of myoclonic seizures. Their father had had FS from infancy to childhood. Detailed clinical information of each patient and further data regarding EEG, photosensitivity and therapy are reported on Table 2.

b) Group 2: patients with SCN1A missense mutations

Missense mutations were found in 16 patients, 8 males and 8 females with SMEI (11) and SMEB (5) (see Table 3 and Figure 1b). Recurrent, often prolonged, FS were seen in all patients with mean age at onset of 6,7 months (median 7; ranging from 3 to12). Status epileptics was present in 12 patients (75%) either febrile (9 patients; 56%), afebrile (8 patients; 50%) or both (5 patients; 35.5%). Febrile status epilepticus had a mean age at onset of 11 months (median 10; ranging from 4 to 28); afebrile status had a similar mean age at onset (median 5; ranging from 4 to 42). All but one patient had afebrile tonic-clonic, tonic or hemiclonic seizures, often prolonged, beginning during the first year of life (mean age of onset 10 months; median 7,5; ranging from 3 to 30). Clusters of symmetric or asymmetric myoclonic seizures occurred in 11 (69%) patients, age at onset was known in 10 of them, with a mean of 25 months (median 24; ranging from 7 to 60). Atypical absence seizures beginning around the third year of life (mean 38 months; median 40; ranging from

7 to 72) were seen in 7 (44%) of the 16 patients. Complex partial seizures were documented in 13 (81%) patients with a mean age at onset of 17 months (median 12; ranging from 3 to 42). All patients had cognitive impairment ranging from mild to severe. A family history of seizures including FS, was present in 9 of the 16 patients (56%) and in two of them relatives with seizures were found in both paternal and maternal branches of the family. Detailed clinical information of each single patient and further data regarding EEG, photosensitivity and therapy are reported on Table 3.

c) Group 3: patients without SCN1A mutations

Molecular analysis did not reveal *SCN1A* mutations in 18 patients (7 females and 11 males), 8 with SMEB and 10 with SMEI. FS beginning around age 10 months (median 8,5; ranging from 4 to 24 months) occurred in all patients. Status epileptics was documented in 12 patients (67%) either febrile (10 patients; 55.5%), afebrile (7 patients; 39%) or both (5 patients 28%). Febrile status epilepticus began around the age of 12 months in most patients with the exception of one in whom status appeared at the age of 7,5 years (median 12; ranging from 4 months to 7,5 years). Afebrile status had a mean age at onset of 10 months (median 9; ranging from 4,5 to 18). Afebrile seizures occurred in 13 (72%) patients with mean age of onset at 13 months (median 10; ranging from 2 to 14). Myoclonic seizures with mean age at onset of 26 months (median 22,5, ranging from 13 to 60) were present in 8 (44%) patients. Absence seizures were reported in 9 patients (50%), age of onset was known in 7 of them and had a mean of 24 months (median 24; ranging from 5 to 51). Partial seizures occurred in 11 (61%) patients with mean age of onset of 26 months (median 24; ranging from 8 to 60). Borderline cognitive functions were seen in 5 patients the remaining had from mild to severe cognitive impairment. A family history of seizures including febrile seizures was present in 10 probands (55,5%).

2. Generalized epilepsy with febrile seizure plus (GEFS+)

A diagnosis of generalized epilepsy with febrile seizures plus was made in 24 patients (13 females and 11 males) aged from 1 to 26 years (mean 8; median 6,5 years). Febrile seizures occurred in all patients and had a mean age at onset of 17 months (median 12, ranging from 5 to 60). Only two patients had, at 10 and 35 months respectively, a single episode of febrile and afebrile status epilepticus. Afebrile tonic-clonic seizures were seen in 19 patients with mean age at onset of 43 months (mean 19; ranging from 3 to 108). Other seizure types included: myoclonic (seven patients), absences (two patients), myoclonic-astatic (six patients) and partial seizures (nine patients).

Of the 24 patients with GEFS+ spectrum three patients (12,5%) were found to harbour *SCN1A* mutations (see Figure 1b). Clinical details of the 3 patients are reported on Table 4.

3. Other phenotypes

Classical febrile seizures (see Table 1) were diagnosed in 10 patients, mean age of FS onset was 15 months (median 12,5; ranging from 8 to 31). All 10 patients had had not other seizure types. One patient had right HS on MRI and a mild deficit of the cognitive functions. A positive family history of epilepsy was detected in 9 probands and in one of them there was a bilinear occurrence of seizures. None of them had *SCN1A* mutations.

Idiopathic generalized epilepsy of various subtypes (see Table 1) with antecedents of FS was documented in 14 patients and none of them carried *SCN1A* mutations. Three patients had *sporadic myoclonic astatic epilepsy* and *SCN1A* screening was also negative.

The remaining 25 patients had *various epilepsy phenotypes* (see Table 1); the majority of them (18), despite the analysis of EEG and clinical information, could not be classified. As described in the methods (selection criteria), all patients had, either focal or generalized seizures, or both, which increased in frequency during febrile episodes. Mean

age of seizures onset was 20 months (median 17, ranging from neonatal period to 84 months). None of them had *SCN1A* mutations.

SCN1A molecular analysis

dHPLC analysis of *SCN1A* in the 132 patients included in the study showed 64 different abnormal chromatograms. Direct sequencing revealed that 39 out of the 64 dHPLC abnormal profiles corresponded to 40 mutations: 19 missense, 5 nonsense, 10 frameshift, 4 splice site mutations and 2 silent nucleotide substitution that *in silico* seemed to modify the mRNA splicing process (see Figure 1a and b). Molecular analysis of the 34 available parent's showed that 28 mutations were *de novo* and 6 were inherited (2 truncating and 4 missense mutations) (see Tables 2, 3 and 5). Of the 40 *SCN1A* mutations identified in our study, 33 had not been previously reported. The position of the missense mutations within the *SCN1A* protein is shown in the graphic (see Figure 2). The direct sequencing of the remaining 25 dHPLC abnormal chromatograms showed that they were polymorphisms (12 not been previously reported, data not shown).

Genotype-phenotype correlations

The analysis of the age of seizures onset between the three groups of SMEI patients with: a) *SCN1A* truncating mutations, b) *SCN1A* missense mutations and c) no *SCN1A* mutations showed that the differences of the age of FS onset was extremely significant between the three groups (p=0,0007, ANOVA test). Patients with truncating mutations had an earlier seizures onset, around the 5th month of age, patients with missense mutations had a later FS onset, around the 7th month and finally individuals without *SCN1A* mutations had an age of onset around the 10th month of life. For the remaining seizure types – febrile and afebrile status afebrile tonic-clonic or hemiclonic, myoclonic, absence and partial seizures – there were not statistically significant differences in age of seizures onset amongst the 3 groups of patients.

The statistical analysis comparing the number of the identified mutations in each exon to the number of expected mutations did not find statistically significant differences between exons ($p>0,05$) (Fisher's exact test). The highest number of mutations (10 out 40) was found in exon 26. This data however, did not reach a statistically significant p-value because this is the biggest exon, therefore carrying a higher chance of being hit by a mutation. A similar analysis was performed grouping the exons according to their coding protein domain (N-terminal + DI; Loop 1; DII; Loop 2; DIII; Loop 3 and DIV + C-term) and similarly there were not statistically significant differences in the domains ($p> 0,05$).

Discussion

Mutation rates

In our cohort of 132 patients with idiopathic epilepsies with fever-provoked seizures in infancy and early childhood, classical SMEI was diagnosed in 39 (30%) and SMEB in 16 (12%) patients. Mutations of *SCN1A* were identified in 29 classical SMEI (74%) and 8 SMEB patients (22%). We obtained a prevalence of about 70% of *SCN1A* mutations in SMEI and SMEB patients, which is intermediate between the lowest percentage of 35% (Nabbout et al., 2003; Wallace et al., 2003) and the highest reported by the Belgium (100%) (Claes et al., 2001) and Japanese groups (78%) (Fujiwara et al., 2003; Ohmori et al., 2002; Sugawara et al., 2002). Ascertainment bias is likely to influence the proportion of patients carrying *SCN1A* mutations identified by the different authors. Although SMEI is a well-defined epilepsy syndrome clinicians are still debating whether myoclonic seizures are an essential feature of SMEI. Supported by the definition endorsed by the International League Against Epilepsy, in this study myoclonic seizures were not regarded as an essential diagnostic criteria for classical SMEI. We, instead, based our clinical classification predominantly on age of onset and on the occurrence of prolonged febrile hemiclonic or generalized seizures often evolving into status epilepticus. The results of *SCN1A* analysis supported our diagnostic criteria; the 29 SMEI patients carrying mutations manifested febrile seizures at onset, followed by febrile or afebrile seizures evolving into status epilepticus in most of them, whereas myoclonic seizures were noted only in 25 out of the 37 (67.5%) patients with mutations.

Clinical criteria for SMEB are even less defined, therefore easily over-or underestimated. Due to these nosological issues the correlation of SMEB with *SCN1A* mutations in our patients decreased to 50%. Furthermore, in our study, the milder SMEB phenotype correlated with a higher proportion of missense (70%) rather than truncating

mutations (30%). Electroclinical and genetic molecular findings suggest that SMEI and SMEB are closely related, representing a spectrum of phenotypes that could be categorized under the term of Dravet's syndrome. Clinical differences in some patients seem to be related to the type and location of *SCN1A* mutations whereas in other patients there might be some yet-undiscovered molecular genetic mechanisms.

Is SMEI an inherited disorder?

The majority of *SCN1A* mutations (89%) were *de novo,* whereas only 4 (11%) were inherited (2 truncating and 2 missense mutations). One of the two inherited truncating mutations was transmitted from a father who had a single seizure (Table 2, D1293delX1299 mutation). The second familial *SCN1A* truncating mutation (Table 2 IVS4+1 G>A splicing) was found in the probands' sister whose phenotype was also consistent with SMEI. The two siblings inherited the mutation from a mildly affected father with only FS from infancy to childhood. A literature review showed only one other family described in which *SCN1A* truncating mutation was inherited from a mildly affected mother (Nabbout et al., 2003).

In our cohort of SMEI probands, similarly to previous studies (Nabbout et al., 2003; Wallace et al., 2003), 56% had close relatives with seizures including febrile seizures, favouring the hypothesis that SMEI might be an inherited disorder. This hypothesis is confirmed by several observations including the family here reported in which both sisters had SMEI and *SCN1A* mutation, as well as the reports of additional families in which at least two affected members had SMEI, (Fujiwara et al., 1990; Gennaro et al., 2003; Kimura et al., 2005; Singh et al., 2001) including two families with *SCN1A* missense and truncating mutations (Gennaro et al., 2003; Kimura et al., 2005). High rates of family history of epilepsy and familial occurrence of SMEI are however, hard to reconcile with the finding that the majority of *SCN1A* mutations in SMEI patients are de novo.

Genotype–phenotype correlations

We observed a mild predominance of truncating (52,5%) over missense (47,5%) mutations. Patients with SMEB (8) where more likely to have missense (5/16; 31%) than truncating mutations (3/21; 14%), whereas patients with classical SMEI (29) had both truncating (18/21, 86%) and missense mutations (11/16, 69%).

Similarly to previous studies, we found that missense mutations in the pore forming parts S5-S6 of the channel occurred in 10 out of the 16 SMEI/SMEB patients (62,5%) and only in one of the three GEFS+ patients (see Figure 2). Mutations in the pore forming region seem to correlate in 70% with the classical SMEI type and only with 30% of the SMEB phenotype. Unlike previous studies, we did not find missense mutations in the voltage sensor part S4. The remaining six patients with SMEI/SMEB and two with GEFS+ carried missense mutations outside the important pore S4-S6 region (see Figure 2).

Statistical analysis of the mean age of onset of FS showed that patients with truncating mutations have the earliest onset followed by patients with missense mutations having an intermediate onset and individuals without *SCN1A* mutations showing the latest age of onset around the 10^{th} month of life. The difference between the three groups was statistically very significant (p=0,0007).

Several recurrent mutations are emerging as the number of published studies increases (Mulley et al., 2005). In our patients only six of the 39 mutations identified, had been previously described (see Tables 2 and 3). In four unrelated SMEI patients we found a truncating mutation (IVS4+1 G>A, Table 2 and Figure 1a) recurring twice and two missense mutations affecting the same aminoacid (Arg393Cys and Arg393His, see Table 3 and Figure 1b).

The *SCN1A* mutations so far identified seem to be scattered across SCN1A protein with some clustering occurring in the C-terminus, to some extent in the N-terminus and in

the loops between the segments 5 and 6 of the first three domains (Mulley et al., 2005). The statistical analysis (Fisher's exact test) of the position of the mutations did not uncover hot spot regions in our patients. The highest number of mutations (10 out 40) was found in exon 26. This data however, did not reach a statistically significant p-value because this is the biggest exon, therefore carrying a higher chance of being hit by a mutation.

GEFS+ and SCN1A mutations

Amongst GEFS$^+$ phenotypes the rate of *SCN1A* mutations was around 12%. The W1204R mutation identified in a child with FS+ and his father had previously been reported in a GEFS+ family (Escayg et al., 2001). The proband of this small GEFS+ family had a left hippocampal sclerosis (HS) on MRI scan. Spampanato et al. (2003) performed some functional studies of the W1204R mutation and showed it to cause an alteration of the voltage-dependent channel gating that could result in neuronal hyperexcitability (Spampanato et al., 2003). The remaining two *SCN1A* mutations have not previously been reported.

We identified two aminoacid substitutions: Arg604His and Ala1161Thr in a patient with myoclonic astatic epilepsy within a GEFS+ family. The same substitutions were previously described in three patients with JME (Escayg et al., 2001). These changes did not co segregate with seizure disorder in the JME families but were not identified in controls either (Escayg et al., 2001). In our patient the two changes, although not identified in 200 control alleles, were both inherited from the unaffected father thus suggesting that they are most likely to be rare polymorphisms.

How much genetic overlapping is there between SMEI and GEFS+?

The clinical similarities between SMEI and GEFS+, including the frequent occurrence of febrile seizures, the family history and the shared molecular genetic aetiology, have prompted the hypothesis that these disorders represent two extremes in

clinical presentation of the same condition (Claes et al., 2003; Singh et al., 2001). Molecular evidences of a shared aetiology include the finding that a mutation in the same aminoacid can give rise to both conditions: the mutation R1648C caused GEFS+ in a family (Escayg et al., 2000) whereas the mutation R1648H caused SMEI in another patient (Ohmori et al., 2002). More than one hundred *SCN1A* mutations have been defined to date and a mutational diversity between SMEI and GEFS+ has been observed. *SCN1A* truncating mutations occur only in SMEI patients whereas missense mutations are found in both SMEI and GEFS+. Furthermore, missense mutations causing SMEI are predominantly found in the pore-forming region of *SCN1A* whereas mutations associated with GEFS+ are spread throughout the gene. A SMEI patient within a GEFS+ family carrying a mutation of the $GABA_A$ receptor γ2 subunit gene is also on record (Harkin et al., 2002).

SCN1A and MRI abnormalities

Usually, neuroimaging studies in SMEI do not demonstrate brain lesions (Dravet, 2002; Nabbout et al., 2003) but studies designed to evaluate MRI abnormalities with special attention on the temporomesial structures, have not been performed. Only a retrospective study is on record, reporting that 10 of the 14 patients with SMEI had HS although none of the patients had temporal lobe epilepsy (Siegler et al., 2005). None of the 14 patients was tested for *SCN1A* mutations.

In our cohort of patients we identified three patients with *SCN1A* mutations and clearcut focal MRI brain abnormalities. In these patients MRI abnormalities did not seem to be an epiphenomenon but they contributed to the patient's epileptogenesis. The MRI study of one patient with SMEB and with complex partial seizures arising from the right temporal region, showed high signal intensity in the right temporal lobe with hippocampus volume reduction (Table 2, Figure 3A). The second patient with FS+ and focal seizures showed left HS on MRI (Table 3, patient 1 and Figure 3B) and the third patient with FS+ had left

temporal lobe hypoplasia on MRI (Table 3, patient 2). The true incidence of focal MRI brain abnormalities in SMEI patients needs to be further studied with properly designed studies. However, the apparently low rate of structural abnormalities raises the possibility that the occurrence of congenital or secondary MRI abnormalities requires further factors, either genetic or aquired, in addition to *SCN1A* mutations and prolonged FS.

Phenotypes other than SMEI and GEFS+

None of the patients with idiopathic epilepsies with fever-provoked seizures besides SMEI and GEFS+ carried mutations in the *SCN1A* gene. Recently, a large Italian family with simple FS cosegregating in affected family members was reported to carry a *SCN1A* missense mutation (Mantegazza et al., 2005). We did not identify *SCN1A* mutations in the 10 patients with simple FS and with a family history of FS (data not shown) included in this study, suggesting that *SCN1A* causes familial simple FS only in rare families.

In conclusion, *SCN1A* is the most relevant epilepsy gene with the largest number of epilepsy-related mutations so far identified. The high correlation between SMEI and *SCN1A* mutations suggests a phenotypical specificity of *SCN1A* rather than a dysfunction of neurons exacerbated by high body temperature. What causes SMEI when there is no detectable *SCN1A* mutation remains to be identified.

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Table 1: epilepsy phenotypes of the 132 patients screened for *SCN1A*

Syndromic phenotypes	**N° of patients**	***SCN1A* mutations**
1) Severe myoclonic epilepsy of infancy (SMEI / SMEB)	55	37 (67%)
2) GEFS+ spectrum	25	3 (12%)
3) Other phenotypes		
Sporadic MAE	3	0
FS	10	0
CAE	7	0
CAE>JME	2	0
JAE	2	0
JME	1	0
IGE-TCS	2	0
Uncl epilepsy with fever-sensitive sz	18	0
Infantile convulsions	2	0
Atypical BRE	1	0
Idiopathic fever-sensitive focal epilepsy	3	0
Total	132	40 (30%)

BRE= benign rolandic epilepsy; *CAE*= childhood absence epilepsy; FS= febrile seizures; *IGE-TCS*= idiopathic generalized epilepsy with tonic-clonic seizures; GEFS+= generalized epilepsy with febrile seizures plus; *JME*= juvenile myoclonic epilepsy; *JAE*= juvenile absence epilepsy; *MAE*= myoclonic astatic epilepsy; *SMEB*= borderline severe myoclonic epilepsy of infancy; *sz*= seizures; *Uncl*= unclassified

Table 2: clinical information of the 21 SMEI/SMEB patients with *SCN1A* truncating mutations

Gender	Age (yrs)	FS onset*	FS status onset*	Afeb status onset*	Afeb sz onset*	Myo sz onset*	Abs sz onset*	Partial sz onset*	EEG	IPS	AEDs	Efficacy	Family history	Syndrome Phenotype	SCN1A screening	
M	10.5	3	3	3	_	Unk	Unk	3	GSW	Y	VPA+ TPM +CLB+STP	Partial sz reduction	N	SMEI	c.1702 C>T Arg568X (Ohmori et al., 2002)	Parents not available
F	3.2	9	14	_	12	12	24	14	multiF SW	N	VPA+ PB+CLB	N	Y	SMEI	c.570 G>A Trp190X	De novo
F	12,4	3	_	9	7	12	_	_	bifrontal SW	N	VPA+ TPM	Partial sz reduction	Y	SMEB	c.1857_1858insGCAAC S620ins624X	De novo
F	17,7	5	_	_	11	20	36	37	GSW & multiF	N	VPA+TPM+LTG+CZP	N	Y	SMEI	IVS1 -3 C>A	De novo
F	14	3	_	24	10	38	36	12	GSW	N	VPA+STP	Partial sz reduction	N	SMEI	c.249 C>G Tyr83X (Nabbout et al., 2003)	De novo
F	9.5	6	40	_	18	30	42	38	GSW & multiF	Y	VPA+TPM	Partial sz reduction	bilineal	SMEI	c.2586 A>G Arg862Arg	De novo
F	16.3	4	4	Uncertain	5	Yes	Yes	_	GSW	Y	VPA+TPM	Y	N	SMEI	c.731_732delGT V244delX275	De novo
M	6.2	3	9	_	20	7	_	20	Right T S	Y	VPA +TPM+CLB	Partial sz reduction	N	SMEI	IVS16 -1 G>A acceptor splice site	De novo
F	6.4	5	_	12	12	14,5	24	12	GSW & Focal	Y	VPA +TPM+CZP	Partial sz reduction	N	SMEI	c.1624 C>T Arg542X	De novo
F	4.8	3	_	11	3	12	_	3	MultiF SW	Y	VPA+ TPM+ CZP	Y	N	SMEI	c.2608_2614delGCAAAAT A870delX874	De novo
M	6	2	_	48	36	36	72	72	GSW	Y	VPA+ TPM+ CZP	Partial sz reduction	N	SMEI	c.5367_5368delCA F1789delX1793	Parents not available
M	8	3	13	13,5	6	10	10	6	GSW	Y	VPA + PB+ ETS + GVG	N	Y	SMEI	c.3347delC P1116delX1119	De novo
M	12.1	9	9	_	9	24	48	48	Unkown	Y	VPA+TPM+CLB+STP	Partial sz reduction	Y	SMEI	c.3878delA D1293delX1299	Familial
M	4.8	6	6	6	4	_	_	6	T SW > R	N	VPA+CZP+CLB + STP	Partial sz reduction	N	SMEB	IVS4+1 G>A splicing (Fujiwara et al., 2003)	De novo
F	17	2.5	3	_	4	_	36	unk	MultiF SW	Y	VPA+PB+ CLN	N	Y	SMEI	c.3276insAT I1242ins1270X	De novo
M	14,4	4	5	8	6	32	30	24	Focal SW	N	CBZ+TPM	Partial sz reduction	Y	SMEI	c.5620delCGGGTTCT R1874delX1941	De novo

M	8,8	5	6	6	4	4	24	24	GSW	N	TPM+Acetaz	Partial sz reduction	Y	SMEI	c.5295delTTTT F1765delX1777	De novo
F	7	6	_	6	_	Y	Y	_	Slow backgr	N	VPA+ TPM+STP	Partial sz reduction	Y	SMEI	IVS4+1 G>A splicing (Fujiwara et al., 2003)	Familial
F	5	7	7	_	42	_	_	-	GSW	N	PB+VPA+ TPM+CLP	N	N	SMEB	c.4933 C>T Arg1645X	Parents not available
M	17,5	3	3	10	24	12	24	36	GSW & multiF SW	Y	VPA+TPM + CZP	N	Y	SMEI	c.4589insA K1517ins1536X	De novo
F	4,1	7	8	6	16	_	36	16	GSW	Y	VPA+CLB+STP	Partial sz reduction	N	SMEI	c.2415 G>A Leu805Leu	de novo

Legend:
Abs= absence seizures; *Acetaz*= acetazolamide; *Backgr*= background; *CLB*= clobazam; *CLN*= clonazepam; *F*= female; *FS*= febrile convulsions; *GWS*= generalized spike-wave; *IPS:* Photosensitivity; *M*= male; *MR*= mental retardation; *Myo*= myoclonic seizures; *MultiF*= multifocal; *N*= negative; *PB*= phenobarbital; *R*= right; *S*= spikes; *SMEB*= severe myoclonic epilepsy of infancy borderline; *SMEI*= severe myoclonic epilepsy of infancy; *SW*= spike-waves; *T*= temporal; *TPM*= topiramate, *STP*= stiripentol; *VPA*= valproic acid; *Y*= Yes; — absent; * age of seizures onset in months

Table 3: clinical information of the 16 SMEI/SMEB patients with *SCN1A* missense mutations

Gender	Age (yrs)	FS onset*	FS status onset*	Afeb status onset*	Afeb Sz onset*	Myo sz onset*	Abs sz onset*	Partial sz onset*	EEG	IPS	AEDs	Efficacy	Family history	Syndrome Phenotype	SCN1A screening	
F	15	7	10	13	9	36	48	9	GSW & multiF	N	VPA+LTG+CLB	N	Y	SMEI	c.4888 G>A Val1630Met	Familial
M	5.7	10	_	_	10	24	24	24	GSW & multiF	N	Unk	Unk	Y	SMEI	c.4822 G>T Asp1608Tyr	De novo
M	8.6	10	_	42	30	Unk	Unk	36	GSW & multiF	N	VPA+TPM+PB	N	Y	SMEI	c.5359 Glu1787Lys	Parents not available
M	9.4	7	7	_	24	40	40	40	GSW	N	VPA+CBZ+CLB	N	Bilineal	SMEI	c.2870 G>T Trp957Leu	Parents not available
M	5.6	7	_	5	4,5	_	_	42	GSW	Y	VPA + TPM + CLN	Partial sz reduction	Bilineal	SMEB	c302G>A Arg101Gln (Fukuma et al., 2004)	De novo
F	6.1	4	4	4	_	_	48	24	GSW	N	LTG+TMP+CLB+STP	N	Y	SMEI	c.4762 T>C Cys1588Arg	De novo
F	4.6	10	12	_	8	_	_	_	MultiF	N	TPM+PB	Partial sz reduction	Y	SMEB	c.1066 A>G Arg356Gly	Familial
F	3,9	4	_	4	4	_	_	14	GSW	N	TPM+PB	Partial sz reduction	N	SMEB	c.4973 C>G Thr1658Arg	Parents not available
M	20.8	4	4	5	5	_	_	_	GSW	N	Unk	Unk	N	SMEB	c.1072 C>A Pro358Thr	Parents not available
F	10.7	3	14	12	11	24	_	Unk	GSW & multiF	Y	VPA+ CLN + acetaloz	N	Y	SMEI	c.4408 G>T Gly1470Trp	De novo
F	9.7	3	_	_	3	7	7	3	multiF	Y	VPA+ TPM + CLN	N	N	SMEI	c.4240 A>T Asn1414Tyr	De novo
F	8.6	3	_	_	20	_	_	3	GSW	N	VPA+TPM	Unk	Y	SMEB	c.5146 T>C Cys1716Arg	De novo
F	11.9	4	28	_	5	60	28	5	GSW & multiF	N	VPA + CLN + CLB	N	N	SMEI	c.1178 G>A Arg393His (Claes et al., 2003)	De novo
M	4,8	12	_	_	14	20	_	12	GSW & multiF		LTG+CLB	N	N	SMEI	c.965 G>T Arg322Ile	Parents not available
M	13,1	12	12	_	7	7	72	7	GSW & MultiF	Y	PB+CZP+ CBZ	N	N	SMEI	c.1177 C>T Arg393Cys	De novo
M	4,7	8	8	4	4	12	_	4	GSW & Focal	N	PB+VPA	N	N	SMEI	c.5348 C>T Ala1783Val	De novo

Legend:
Abs= absence seizures; *Acetaz*= acetazolamide; *Afeb*= afebrile; *Backgr*= background; *CBZ*= carbamazepine; *CLB*= clobazam; *CLN*= clonazepam; *F*= female; *FS*= febrile convulsions; *GWS*= generalized spike-wave; *IPS*: Photosensitivity; *LTG*= lamotrigine; *M*= male; *MR*= mental retardation; *Myo*= myoclonic seizures; *MultiF*= multifocal; *N*= negative; *PB*= phenobarbital; *TPM*= topiramate*; S*= spikes; *SMEB*= severe myoclonic epilepsy of infancy borderline; *SMEI*= severe myoclonic epilepsy of infancy; *Sz*= seizures;, *STP*= stiripentol; *Unk*: unkown; *VPA*= valproic acid; *Y*= Yes; *Yrs*= years — absent; * age of seizures onset in months.

Table 4: clinical details of 3 patients with GEFS+ and *SCN1A* mutations

	Gender	Age (y)	FS onset*	Afeb sz onset*	Myo sz onset*	Abs onset*	Partial sz onset*	EEG	AEDs	Cognitive	Family	Syndrome Phenotype	MRI	*SCN1A* screening	
1	M	4.9	8	40	_	_	48	L T slowing	TPM	Language delay	Y	FS+	L HS	c3610 T>C Trp1204Arg (Escayg et al., 2000)	Familial
2	M	2,2	6	7	_	_	_	N	VPA	N	Neg	FS+	L T hypoplasia	c.220 T>C Ser74Pro	De novo
3	M	14	24	108	36	36	8	GSW	LEV +VPA	mild MR	Y	MAE/GEFS+	N	c.5060 T>C Phe1687Ser	Familial

Legend
Abs= absences; *AEDs*= antiepileptic drugs; *Afeb*= afebrile; *FS*= ferbile seizures; *GSW*= generalized spike-waves; *HS*= hippocampal sclerosis; *L*= left; *LEV*= levetiracetam; *M*=male; *Myocl*= myoclonic; *MR*= mental retardation; *N*= normal; *Neg*= negative; *T*= temporal; *TPM*= topiramate; *VPA*= valproic acid; *= months; *Y*=yes; *y*=years

Figures legend

Figure 1

a) Graphic representation of the SCN1A protein showing the location of the truncating mutations. In the upper part of the figure are reported mutations leading to truncated SCN1A protein and to mRNA splicing alteration (underlined) associated with classical severe myoclonic epilepsy of infancy; in the lower part are reported the truncating mutations associated with borderline severe myoclonic epilepsy of infancy (SMEB).

b) Graphic representation of the SCN1A protein showing the location of the missense mutations. In the upper part of the figure are reported missense mutations associated with classical severe myoclonic epilepsy of infancy (SMEI); in the lower part are reported the missense mutations associated with borderline severe myoclonic epilepsy of infancy (SMEB) and with generalized epilepsy with febrile seizures plus (GEFS+).

Figure 2

Graphic representation showing the location of the missense mutation according to the protein domains: N or C terminal; pore region; insulating region; voltage sensor; interdomain and other regions. Within the protein domains mutations have also been subdivided according to the phenotype: white for GEFS+, grey for SMEB and black for SMEI

Figure 3

A) Brain MRI scan of a patient with SMEI and *SCN1A* truncating mutation showing high signal intensity in the right temporal lobe with hippocampus volume reduction (arrows).

B) Brain MRI scan of a patient with GEFS+ spectrum and *SCN1A* missense mutation showing left hippocampal sclerosis (arrows)