The spectrum of *SCNIA*-related infantile epileptic encephalopathies

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The relationship between severe myoclonic epilepsy of infancy (SMEI or Dravet syndrome) and the related syndrome SMEI-borderland (SMEB) with mutations in the sodium channel alpha 1 subunit gene *SCNIA* is well established. To explore the phenotypic variability associated with *SCNIA* mutations, 188 patients with a range of epileptic encephalopathies were examined for *SCNIA* sequence variations by denaturing high performance liquid chromatography and sequencing. All patients had seizure onset within the first 2 years of life. A higher proportion of mutations were identified in patients with SMEI (52/66; 79%) compared to patients with SMEB (25/36; 69%). By studying a broader spectrum of infantile epileptic encephalopathies, we identified mutations in other syndromes including cryptogenic generalized epilepsy (24%) and cryptogenic focal epilepsy (22%). Within the latter group, a distinctive subgroup designated as severe infantile multifocal epilepsy had *SCNIA* mutations in three of five cases. This phenotype is characterized by early onset multifocal seizures and later cognitive decline. Knowledge of an expanded spectrum of epileptic encephalopathies associated with *SCNIA* mutations allows earlier diagnostic confirmation for children with these devastating disorders.

Keywords: *SCNIA*; SMEI; SMEB; epileptic encephalopathy; channelopathies

Abbreviations: dHPLC = denaturing high performance liquid chromatography; GEFS+ = generalized epilepsy with febrile seizures plus; ICEGTC = intractable childhood epilepsy with generalized tonic clonic seizures; LGS = LennoxGastaut syndrome; MAE = Myoclonicastatic epilepsy; PCR = polymerase chain reaction; SCNIA = sodium channel alpha 1 subunit gene; SMEB = SMEI-borderland; SMEB-M = SMEI-borderland-myoclonic seizures; SMEB-O = SMEI-borderland more than one feature; SMEB-SW = SMEI-borderland-spike wave; SMEI = severe myoclonic epilepsy of infancy

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Introduction

SCN1A, the gene encoding the sodium channel alpha 1 subunit, has emerged as the most important of the epilepsy genes currently known (Mulley *et al.*, 2005). *SCN1A* mutations underlie more than 70% of patients with the epileptic encephalopathy severe myoclonic epilepsy of infancy (SMEI or Dravet syndrome) (Dravet *et al.*, 1982; Claes *et al.*, 2001; Mulley *et al.*, 2005). More than 170 documented mutations are associated with SMEI and the related syndrome of borderland SMEI, known as SMEB. Truncation mutations account for nearly 50% of mutations found in SMEI, with the remainder comprising missense, splice site and deletion mutations (Mulley *et al.*, 2005). These mutations affect many domains of the gene with suggested clustering of missense mutations occurring in the N- and C-termini and the S5–S6 pore-forming regions of the protein (Kanai *et al.*, 2004). Recently intragenic and whole gene deletions have been identified in a few cases of SMEI without truncation, missense or splice-site mutations (Madia *et al.*, 2006; Mulley *et al.*, 2006; Suls *et al.*, 2006).

Approximately 95% of *SCN1A* mutations in SMEI patients arise *de novo*. The remaining cases have familial mutations with milder phenotypes in other family members often consistent with the generalized epilepsy with febrile seizures plus (GEFS+) spectrum (Scheffer and Berkovic, 1997; Escayg *et al.*, 2000; Singh *et al.*, 2001; Fujiwara *et al.*, 2003; Nabbout *et al.*, 2003a; Scheffer, 2003; Kimura *et al.*, 2005). Recently, germline and somatic *SCN1A* mutational mosaicism has been reported in unaffected parents (or parents mildly affected with febrile seizures) where their children have SMEI or SMEB (Depienne *et al.*, 2006; Gennaro *et al.*, 2006; Marini *et al.*, 2006; Morimoto *et al.*, 2006).

SMEI or Dravet syndrome is a distinctive syndrome with seizure onset in the first year of life, typically beginning with prolonged febrile hemiclonic seizures or generalized tonic–clonic seizures (Dravet, 1978; Dravet *et al.*, 1982, 2005). The disorder evolves with other seizure types such as myoclonic, focal, absence and atonic seizures developing between 1 and 4 years of age. Development is normal in the first year of life followed by developmental slowing and regression. Pyramidal signs and ataxia may evolve. Cognitive outcome is usually poor and seizures remain refractory for those who survive to adulthood (Jansen *et al.*, 2006).

The phenotypic spectrum of patients with *SCN1A* mutations has been extended beyond SMEI. The related syndrome SMEB (Ohmori *et al.*, 2003; Fukuma *et al.*, 2004) refers to children who lack several of the key features of SMEI such as myoclonic seizures or generalized spike-wave activity (Sugama *et al.*, 1987; Dravet *et al.*, 2005). In two studies, 26% (7/27) and 88% (15/17) of SMEB patients were found to have *SCN1A* mutations respectively (Ohmori *et al.*, 2003; Fukuma *et al.*, 2004). As with SMEI, these mutations are spread throughout the gene with a mixture of types of mutation including truncation, missense and splice-site changes (Mulley *et al.*, 2005). A subgroup of SMEB has been variously described as intractable childhood epilepsy with generalized tonic clonic seizures (ICEGTC, originally called high voltage slow waves grand mal by Japanese authors) or Severe idiopathic generalized epilepsy of infancy with generalized tonic–clonic seizures. These infants have generalized tonic–clonic seizures beginning in the first year of life without the evolution of other seizure types and they follow a similarly unfavourable developmental course to children with SMEI (Fujiwara *et al.*, 1992; Kanazawa, 1992, 2001; Sugama *et al.*, 1987; Doose *et al.*, 1998). In one series, 7/10 ICEGTC patients had missense mutations in *SCN1A* (Fujiwara *et al.*, 2003); truncation, missense and splice-site mutations were reported in 3/18 patients described as severe idiopathic generalized epilepsy of infancy (Ebach *et al.*, 2005). We reported the only case so far of West syndrome with an *SCN1A* mutation (Wallace *et al.*, 2003).

Given the overlapping yet heterogeneous clinical features of these epilepsy syndromes, we postulated that *SCN1A* mutations may be associated with other phenotypes. Here we studied unselected patients with severe epileptic encephalopathies (including SMEI) with onset primarily during the first year of life.

Material and methods
Clinical methods

Patients with epileptic encephalopathies of unknown cause were referred by paediatric neurologists and neurologists from Australia and around the world. Epileptic encephalopathies are defined as disorders in which there is a temporal relationship between deterioration in cognitive, sensory and motor function and epileptic activity comprising frequent seizures and/or extremely frequent 'interictal' paroxysmal activity (Nabbout and Dulac, 2003). Cases were only included where magnetic resonance imaging was normal or showed non-specific features without a definite aetiology. A subset of 14 patients, included in this study, with so-called 'vaccine encephalopathy' has been published previously (Berkovic *et al.*, 2006).

Electroclinical data were obtained on all patients with specific emphasis on early seizure history including age of onset, occurrence of status epilepticus, presence of fever sensitivity, clinical photic sensitivity and evolution of other seizure types. A detailed early developmental history was obtained with attention to acquisition of early milestones, timing of plateau or regression of development and current functioning. Other important details included general and neurological examination, family history of seizure disorders and results of EEG, video-EEG monitoring and neuroimaging studies. Results of other available investigations such as chromosomal analysis were also obtained.

SMEI was defined according to the following criteria: onset in the first year of life of convulsive seizures which were hemiclonic or generalized; myoclonic seizures; other seizure types which could include focal seizures, absence seizures, atonic seizures, tonic

seizures; normal development in the first year of life with subsequent slowing including plateauing or regression; generalized spike-wave activity and either normal MRI or non-specific findings.

SMEB was divided into subgroups based on the absence of specific features that are regarded as required for the diagnosis of SMEI. SMEB-M referred to patients who did not have myoclonic seizures but otherwise satisfied SMEI criteria. SMEB-SW defined patients who had all the SMEI criteria but had never had generalized spike-wave activity documented on EEG. SMEB-O referred to patients who had more than one feature that was not in keeping with SMEI; examples include absence of generalized spike-wave activity recorded on EEG, a normal developmental outcome and absence of myoclonic seizures. SMEB included cases with ICEGTC where they followed the same course but only had convulsive seizures.

Cryptogenic generalized epilepsy (CGE) denoted individuals who have multiple seizure types, generalized sharp and slow activity and intellectual disability with no known aetiology. Lennox–Gastaut syndrome (LGS) defined patients with tonic seizures and slow generalized spike-wave activity and abnormal development (Commission on Classification and Terminology of the International League Against Epilepsy, 1989; Beaumanoir and Blume, 2005). Myoclonic–astatic epilepsy (MAE) referred to individuals with myoclonic–astatic seizures and other generalized seizure types with generalized spike-wave activity and variable developmental outcome (Doose *et al.*, 1970; Guerrini *et al.*, 2005).

A further subgroup was called cryptogenic focal epilepsy where an individual had focal seizures and uni- or multifocal EEG epileptiform patterns. These individuals showed a variable degree of intellectual disability and usually had normal neuroimaging. Several individuals were included with abnormal neuroimaging that did not account for the clinical presentation such as hydrocephalus, bilateral periventricular leucomalacia, etc.

Other syndromes were defined according to the ILAE classification (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). Patients were called 'unclassified' if we had insufficient evidence to make a syndrome diagnosis or the patient did not fit into a recognized syndrome despite detailed evaluation.

The Austin Health Human Research Ethics Committee approved this study. Informed consent was obtained from the parents or guardians of minors and from adult subjects of normal intellect. In the case of adults with intellectual disability, legal consent was obtained from the appropriate government authority or legal guardian.

Molecular analysis

Molecular analysis was carried out on genomic DNA extracted from venous blood. All 26 exons of *SCN1A* were amplified by polymerase chain reaction (PCR) using flanking intronic primers and standard PCR conditions (primers available upon request). PCR fragments were heat denatured at 95°C for 4 min and slowly cooled to room temperature to form heteroduplex products which were analysed by denaturing high performance liquid chromatography (dHPLC) on the Transgenomic WAVE 3500HT instrument (dHPLC conditions available upon request). Amplicons showing altered dHPLC chromatogram patterns were sequenced in both directions from independent PCR products, on an ABI 3700

sequencer. The final subset of patients (43) was screened by direct sequencing of PCR products (without prior dHPLC screening) by Athena Diagnostics under diagnostic conditions. The numbering for each mutation is taken from the start codon ATG of the full-length *SCN1A* isoform sequence (Genbank accession number AB093548). In cases where an *SCN1A* mutation was detected, the appropriate amplicon from parental DNA (where available) was tested by DNA sequencing to distinguish between *de novo* and familial variants. Mutations or rare variants were distinguished from coding single nucleotide polymorphisms which have previously been reported (Escayg *et al.*, 2001).

Results
Clinical diagnoses

One hundred and eighty-eight patients were recruited from Australia (110), Canada (27), United Kingdom (23), New Zealand (20), Israel (4), USA (3) and Denmark (1) with seizure onset in the first 2 years of life. These included 14 cases who were negative for *SCN1A* mutations on single-stranded conformation analysis in our previous study (Wallace *et al.*, 2003); the eight positive cases and two, who were negative on sequencing, are not included in the data presented here.

Our total cohort contained 66 with SMEI, 36 with SMEB including the various subcategories, 25 with cryptogenic generalized epilepsy, 18 with cryptogenic focal epilepsy, 10 with MAE and 12 with LGS. The remaining cases had a range of other syndromes or were unable to be classified (Table 1).

Table 1 *SCNIA* mutations in patients with epileptic encephalopathies

	Total	*SCNIA* mutation
SMEI	66	52
SMEB	36	25
SMEB-O	16	10
SMEB-SW	14	11
SMEB-M	4	3
ICEGTC	2	1
Cryptogenic generalized epilepsy	25	6
Cryptogenic focal epilepsy	18	4
Myoclonic−astatic epilepsy	10	2
Lennox−Gastaut syndrome	12	1
West syndrome	5	
Idiopathic spasms	1	
Early myoclonic encephalopathy	1	
Progressive myoclonic epilepsy	1	
Alternating hemiplegia of childhood	1	
Unclassified	12	
Total	188	90

SMEI, severe myoclonic epilepsy of infancy; SMEB-SW, SMEI borderland without generalized spike wave; SMEB-M, SMEI borderland without myoclonic seizures; SMEB-O, SMEI borderland lacking more than one feature of SMEI; ICEGTC, intractable childhood epilepsy with generalized tonic−clonic seizures.

Molecular analysis

Of the 188 patients examined, 90 (48%) had *SCN1A* mutations. Ninety-four sequence variants were identified in the 90 mutation positive patients as four children each had two changes. In each child, the putative pathogenic variant was distinguished from the likely non-pathogenic variant; the latter was not included in further analyses (see later and Supplementary Table). The majority of mutations are novel (72/90, 80%), reinforcing the mutational heterogeneity characteristic of *SCN1A*. Of the 90 cases, DNA was available from 76 sets of parents and 73/76 (96%) were *de novo* mutations.

Amino acid alignments of the missense mutations show that they affect conserved domains of the protein in other human alpha channels (SCN2A, SCN3A and SCN8A), chimpanzee, rat, mouse, *Fugu* and *Drosophila* consistent with their interpretation as pathogenic mutations (Supplementary Fig. S1). Moreover, the probability that these missense mutations are pathogenic mutations is supported by their *de novo* origin (in 34/37 cases where parents have been examined) and previously published observations in SMEI.

SMEI

Fifty-two of the 66 (79%) of patients with SMEI had *SCN1A* mutations (Table 1). Forty-four percent (23/52) of the SMEI-related mutations were non-sense or frameshift mutations resulting in protein truncation, 39% (20/52) were missense mutations and the remaining 17% (9/52) were intronic splice donor or splice acceptor site changes. These mutations were spread throughout the gene with the majority of missense mutations (14/20, 70%) localized to the transmembrane regions of the protein, in particular the S5–S6 loop of domain II that forms part of the ion channel pore (Supplementary Table, Fig. 1A). In contrast, 57% (13/23) of truncation mutations were positioned in the intracellular loops of the protein (Supplementary Table, Fig. 1A).

Parental DNA was available for testing for 42/52 SMEI patients who were mutation positive. Analysis of the DNA from these parents confirmed that all 42 mutations were *de novo*.

There were four patients with two sequence variants that posed challenges in clinico-molecular interpretation (Supplementary Table). Patient 2 had two *SCN1A* sequence variants: one was a *de novo* missense change (Y84C) affecting a highly conserved amino acid site (Supplementary Fig. S1) and the second was a splice acceptor site change found to have a maternal origin. The mother was unaffected; the maternal grandfather had a history of convulsions until 7 years but was negative for the splice acceptor site change. There was no further seizure history within this family suggesting that the change within the splice site was probably a benign variant. Therefore this variant was not considered in the determination of mutation frequencies.

Patients 6, 32 and 38 also had two sequence variants detected but parental DNA was unavailable in order to ascertain which variant was *de novo* and thus the likely pathogenic mutation (Supplementary Table). Patient 6 had two intronic mutations detected, both potentially pathogenic. In the absence of parental DNA we can only assume that one is likely to be pathogenic. The intron IVS3-13T→A change was chosen as the most likely variant to affect splicing since it is within the consensus C/T run in the splice acceptor site. Patient 32 had both a missense (E1238D) and an intronic donor splice site mutation. Since the missense change affected a highly conserved amino acid site (Supplementary Fig. S1), this was considered to be the true mutation. Patient 38 had a truncation mutation (R1525X) also seen in Patient 39 with SMEI and in a previous study (Supplementary Table) (Kearney *et al.*, 2006). Patient 38 also had a missense change not as highly conserved as most missense mutations (Supplementary Fig. S1) so the truncation mutation was considered the likely pathogenic mutation. The second variant found in each case has not been included in the mutational analyses.

Simultaneous double mutation in the same patient is a theoretical possibility, as is a *de novo* mutation adversely interacting with a pre-existing rare variant. However, in the absence of definitive evidence from other SMEI cases and
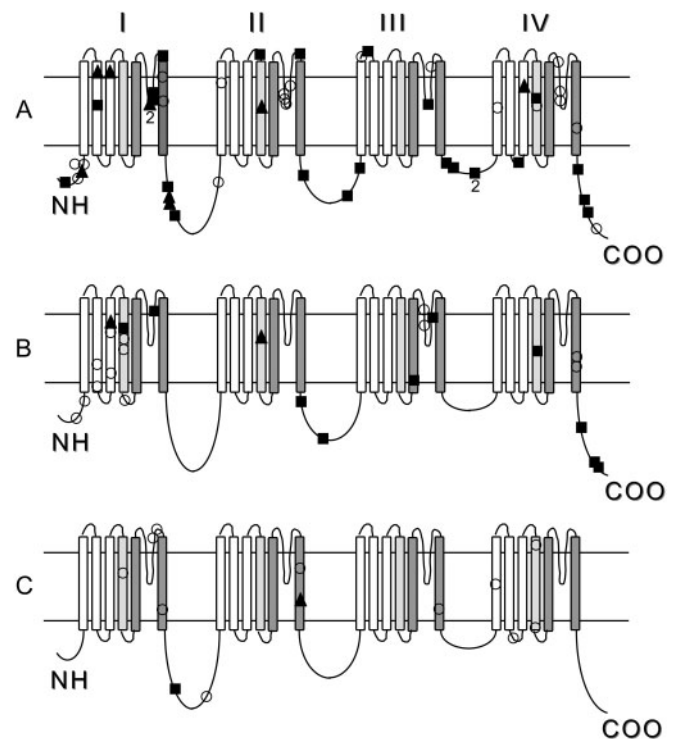

Fig. 1 Schematic representation of mutations in *SCN1A* in patients with (**A**) SMEI, (**B**) SMEB and (**C**) other phenotypes. Refer to Supplementary Table for details. The SCN1A protein consists of four domains designated I–IV, each contains six transmembrane segments designated S1–S6. ■ = truncation, ○ = missense, ▲ = splice-site mutations.

absence of parental DNA to establish *de novo* origin, the most parsimonious explanation is that of a single mutational event unless proven otherwise.

SMEI—borderland

SCN1A mutations were identified in 25/36 (69%) patients with SMEB including all subcategories (Table 1). Over half of these changes were missense mutations (13/25) with 40% (10/25) being truncation mutations; the remaining two were splice-site mutations. The mutations were spread throughout the gene with the majority (18/25, 72%) localized to the transmembrane domain regions. Missense mutations were clustered in the S2–S4 transmembrane segments of domain I (Fig. 1B).

Analysis of parental DNA from 22/25 patients with mutations confirmed 95% (21/22) were *de novo*. Patient 63 had a paternally inherited mutation (A239T). The proband's father had febrile seizures plus (Scheffer and Berkovic, 1997) and the paternal grandmother had unclassified seizures. Both individuals were found to carry the A239T change, which when taken together with the amino acid conservation of this residue (Supplementary Fig. S1), reinforces the status of this variant as a true pathogenic mutation of *SCN1A*. The family had a bilineal family history of seizures as the proband's mother had febrile seizures and did not carry the *SCN1A* mutation (Supplementary Fig. S2).

Cryptogenic generalized epilepsy

Of the 25 patients with cryptogenic generalized epilepsy, six (24%) had mutations. None of the mutations have been previously reported, however the T226M in Patient 78 was also seen in Patient 61 within this cohort with SMEB-O (Supplementary Table). Four mutations arose *de novo*; parental DNA was unavailable for one patient and for Patient 82 the mutation (M973V) was found in her unaffected father. There was no family history of seizures but the amino acid conservation at this site is reasonably strong (Supplementary Fig. S1) providing circumstantial evidence that it is a true mutation. If so, then it must be non-penetrant in the father or else function as a susceptibility allele acting in tandem with other unidentified susceptibility genes responsible for the phenotype in the proband.

The six cases with mutations had heterogeneous phenotypes with onset between 1.5 and 12 months (Table 2). Two had a phenotype with onset in the first 2 months of life and abnormal early development but other features were similar to SMEI. Patient 80 fixed and followed and smiled by 6 weeks when seizures began. Development slowed from 6 weeks: he sat late, walked at 18 months and development stagnated from 2 years. He died at 13 years. Patient 78 had seizure onset at 8 weeks, smiled at 3 months, never sat or acquired words.

The other four cases presented a mixed picture, but generalized spike wave and focal discharges were usually

Table 2 Clinical features of *SCN1A* mutation positive patients with diagnosis other than SMEI and SMEB

Patient	Age at study (years)	Seizure onset (months)	Seizure types	Intellect	Neurological signs	Epilepsy classification	*SCN1A* mutation	Inheritance
78	5	2	GTCS, H, MJ, F, NCS	Severe ID	Increased tone, later generalized hypotonia	CGE	T226M	De novo
79	23	5.5	FS, GTCS	Mild-moderate ID	None	CGE	A395P	De novo
80	14	1.5	GTCS, H, At, MJ, F, SE	ID	None	CGE	V422E	De novo
81	14	12	FS, GTCS, aAb, MJ, SE	ID	Ataxia, intermittent movement disorder	CGE	S626G	ND
82	35	9	FS, GTCS, MJ, F-SG	Low average	None	CGE	M973V	Paternal
83	3	6	FS, GTCS, MJ	Normal	None	CGE	IVS15+1G→T	*De novo*
84	16	7	FS, GTCS, MJ, F, NCS	Mild ID	Mild generalized spasticity	CFE (SIMFE)	F575fsX622	*De novo*
85	5	4.5	F, H, SE	ID	None	CFE (SIMFE)	F1543S	Maternal
86	20	5	GTCS, MJ, F, T	Moderate ID	Ataxia, mild left hemiparesis	CFE (SIMFE)	R1596C	*De novo*
87	5	18	FS, F-SG, SE	Normal	None	CFE	R1657H	*De novo*
88	21	0.75	IS, At, aAb, T, SE, NCS	ID	Mild right hemiparesis	LGS	R1636Q	*De novo*
89	11	4	FS, GTCS, MJ, At, T, H	ID	None	MAE	R393C	*De novo*
90	12	13	FS, F, MA, MJ	Moderate ID	None	MAE	G1480V	*De novo*

FS, febrile seizures; aAb, atypical absence seizures; At, atonic seizures; F, focal seizures (not hemiclonic/unilateral); GTCS, generalized tonic–clonic seizures; H, hemiclonic; IS, infantile spasms; MA, myoclonic–astatic; MJ, myoclonic jerks; NCS, non-convulsive status epilepticus; SE, status epilepticus; SG, secondary generalization; T, tonic seizures; CGE, cryptogenic generalized epilepsy; CFE, cryptogenic focal epilepsy; LGS, Lennox–Gastaut syndrome; MAE, myoclonic–astatic epilepsy; SIMFE, severe infantile multifocal epilepsy; ID, intellectual disability; ND, not done.

seen. The severity of the seizures varied with some only having generalized tonic–clonic seizures, which settled by adult life.

Cryptogenic focal epilepsy

Of 18 patients with cryptogenic focal epilepsy within this cohort of infantile epileptic encephalopathies, four (22%) had mutations (Table 1). Five cases presented with severe infantile multifocal epilepsy with developmental delay and are described later. Three had mutations: two (Patients 84 and 86) arose *de novo* (F575fsX622, R1596C) and one was maternally inherited. The latter (Patient 85) had a putative mutation (F1543S) that was highly conserved (Supplementary Fig. S1) and was carried by her unaffected mother, and may represent a susceptibility allele.

One (Patient 87) had recurrent febrile status epilepticus with onset at 18 months (Table 2). Twenty-four episodes of status epilepticus occurred, some with focal features with variable lateralization. MRI was normal. The patient died at 5 years due to complications of status epilepticus. He had a *de novo* missense *SCN1A* mutation (R1657H).

Severe infantile multifocal epilepsy

Five cases had this phenotype with seizure onset at a mean of 4 months. Of those with *SCN1A* mutations (Patients 84, 85 and 86, Table 2), onset occurred at mean of 5.5 months (4.5, 5 and 7 months) compared with 6- and 8-week onsets in the other two cases. Each child had multiple types of focal seizures with varying semiology. EEG studies showed abundant multifocal epileptiform activity typically with no (or exceptional) generalized or bilaterally synchronous discharges. The three patients with mutations had MRI brain studies; two showed mild atrophy. The remaining two had CT brain scans; one showed mild right sided atrophy.

Developmental delay became evident in all cases. In the two cases that were mutation negative, seizures began at 6 and 8 weeks concurrent with the recognition that developmental delay was present. In the three cases with *SCN1A* mutations, early development was normal with developmental slowing noted at the ages of 16 months, 3–4 years and 6 years even though seizure onset occurred at 4.5, 5 and 7 months, respectively (Table 2). Developmental outcome was poor with intellectual disability ranging from mild (one case, mutation positive: Patient 85), moderate (three cases, two had mutations: Patients 84 and 86) to severe (one case).

Other phenotypes

De novo SCN1A mutations were identified in 2/10 patients with MAE (Patients 89 and 90) and 1/12 patients with LGS (Patient 88) (Tables 1 and 2). No mutations were identified in patients with West syndrome, idiopathic spasms, early myoclonic encephalopathy, progressive myoclonic epilepsy, alternating hemiplegia of childhood or the 12 cases that could not be classified.

Discussion

The sodium channel alpha 1 subunit gene, *SCN1A*, is currently the most clinically relevant epilepsy gene. Mutations in *SCN1A* are an important cause of SMEI and SMEB and its subset ICEGTC (Claes *et al.*, 2001; Mulley *et al.*, 2005). Recently we showed that so-called 'vaccine encephalopathy' should be regarded as SMEI/ SMEB on clinical and molecular grounds (Berkovic *et al.*, 2006). Whilst *SCN1A* was originally associated with a small proportion of patients with the mild phenotypes characteristically seen in the GEFS+ syndrome (Escayg *et al.*, 2000; Mulley *et al.*, 2005), mutations within this gene have been identified far more often in patients with more severe forms of epilepsy. This study examines epileptic encephalopathies beginning early in life and expands the phenotypic spectrum of *SCN1A* defects beyond that previously recognized, to now include patients with cryptogenic generalized epilepsy and cryptogenic focal epilepsy.

The majority of mutations identified in the 90 children in this study were novel (72/90, 80%), whereas 18 (20%) had been previously published (Claes *et al.*, 2001; Ohmori *et al.*, 2002; Sugawara *et al.*, 2002; Fujiwara *et al.*, 2003; Nabbout *et al.*, 2003a; Wallace et al., 2003; Fukuma *et al.*, 2004; Mulley *et al.*, 2005; Kearney *et al.*, 2006; Mancardi *et al.*, 2006; Marini *et al.*, 2006). This expanded list of mutations, taken together with those reviewed by Mulley *et al.* (2005), provides an essential mutational database for use as an interpretative aid for diagnostic laboratories offering *SCN1A* mutation testing. Unlike some disorders where mutations are largely concentrated in 'hot spots', the mutations within *SCN1A* are widely distributed throughout the gene.

Parental DNA was available in 84% (76/90) of cases of which 96% (73/76) arose *de novo* and 4% (3/76) were familial. Familial *SCN1A* mutations have been previously reported in around 5% of SMEI where family members have mild GEFS+ phenotypes, as we observed here (Supplementary Fig. S2) (Fujiwara *et al.*, 2003; Nabbout *et al.*, 2003a; Mulley *et al.*, 2005). In these probands, it is likely that their disorder has a multifactorial basis where *SCN1A* is a major but not the sole contributing gene. This would explain the marked disparity in phenotypic severity between the proband and their relatives. This model could explain probands 63, 82 and 85 where the parent was unaffected or had a mild phenotype. It is worth noting that these probands had a range of phenotypes including cryptogenic generalized and cryptogenic focal epilepsies.

Given the current state of knowledge, the majority of *SCN1A* mutations remain novel. This creates a challenge in determining whether new variants are pathogenic or not. Where the variant is *de novo* or results in truncation of the protein, then the likelihood of it being pathogenic is

extremely high; 79 (88%) of our 90 positive cases fitted these criteria. In cases with missense changes, where DNA from parents is unavailable, or where an unaffected transmitting parent is identified, the case for pathogenicity rests on circumstantial evidence provided by evolutionary conservation of protein structure. Definitive functional studies are rarely available for this particular ion channel. In the four cases with two rare *SCN1A* variants, one of the two was assessed as more likely to be relevant to the observed phenotype. In cases where an unaffected transmitting parent is identified, these changes may be incidental benign variants, incompletely penetrant pathogenic variants or represent a susceptibility allele that contributes to the phenotype in a polygenic manner. Another alternative is that the variant has a major effect on the proband, whereas the transmitting parent has unrecognized protective molecular mechanisms.

SMEI

This study reinforces the high frequency of *SCN1A* mutations in patients with SMEI. The initial report described mutations in 7/7 cases (Claes *et al.*, 2001). Subsequently, large series from a number of centres have reported mutations in 61–87% cases consistent with our finding of 79% reported here (Ohmori *et al.*, 2002, 2003; Sugawara *et al.*, 2002; Fujiwara *et al.*, 2003; Fukuma *et al.*, 2004). Lower mutation rates of 35% (33/93) and 33% (55/169) have been reported (Nabbout *et al.*, 2003*a*; Suls *et al.*, 2006) and of 33% (8/24) by our laboratory (Wallace *et al.*, 2003). The latter study used single-strand conformation analysis for mutation detection, a rapid screening technology less sensitive than dHPLC used here. DHPLC has >96% sensitivity and specificity (Xiao and Oefner, 2001). Additional direct sequencing was performed in five cases (two negative). Fourteen of the remaining negative SMEI cases from our study were tested by dHPLC (3 cases) or direct sequencing (11 cases) here. Eight mutations were identified (two by dHPLC and six by sequencing), bringing the mutation rate to 16/24 (66%) for those cases reported in our original study (Wallace *et al.*, 2003). Of our *SCN1A* mutation negative SMEI cases on dHPLC, 2 of 13 (15%) were subsequently found to have whole exon deletions detected by multiple ligase-dependent probe amplification (Mulley *et al.*, 2006). Other SMEI cases lacking point mutations have been shown to have microdeletions including the *SCN1A* gene (Madia *et al.*, 2006; Suls *et al.*, 2006).

SMEB

We found 69% of our SMEB cases had *SCN1A* mutations. This figure is higher than the 26% reported by Fukuma *et al.* (2004) and more in keeping with the 88% mutation rate of Ohmori *et al.* (2003). The majority of SMEB mutations detected in this study were novel changes (17/25, 68%), with eight mutations being previously reported in patients with SMEI (Claes *et al.*, 2001; Ohmori *et al.*, 2002; Sugawara *et al.*, 2002; Fujiwara *et al.*, 2003; Nabbout *et al.*, 2003*a*; Wallace *et al.*, 2003; Fukuma *et al.*, 2004; Kearney *et al.*, 2006; Mancardi *et al.*, 2006; Marini *et al.*, 2006).

SMEB is distinguished from SMEI by the absence of specific features. The question of whether myoclonic seizures are an essential component of a SMEI phenotype remains controversial (Ogino *et al.*, 1988; Commission on Classification and Terminology of the International League Against Epilepsy, 1989; Ohmori *et al.*, 2003; Fukuma *et al.*, 2004; Dravet *et al.*, 2005). Dravet and colleagues observed that myoclonic seizures may be segmental or occur immediately prior to convulsive seizures and they postulate that subtle myoclonus may be missed (Dravet *et al.*, 2005). Our data suggest that myoclonic seizures are not obligatory as three of four patients with an SMEI phenotype lacking only obvious myoclonic seizures (SMEB-M) carried a *SCN1A* mutation. Similarly, generalized spike-wave activity is considered the EEG hallmark of SMEI, but we found that 11/14 (79%) of our patients with a SMEI picture without demonstrated generalized spike-wave activity (SMEB-SW) had mutations.

Our findings in SMEB have important implications for the 'lumpers and splitters' debate. Whilst Ohmori and co-workers (2003) found a higher mutation rate in SMEB (88%) than SMEI (72%), our larger study shows the reverse. Moreover, three mutations are associated with both SMEI and SMEB (Patients 4 and 54, 8 and 59, 48 and 74) in this study. Similarly, eight cases have a mutation previously associated with the alternate phenotype (Supplementary Table). The recent ILAE classification proposal suggests the new name of Dravet syndrome for SMEI (Engel, 2001). In terms of clinical utility, we suggest that it may be more helpful to conceptualize SMEI and SMEB as a spectrum and incorporate both under the eponym of Dravet syndrome. This would also resolve the inaccuracy in terminology arising from the absence of myoclonic seizures in some cases of SMEI despite 'myoclonic' being part of the syndrome's name.

SCN1A mutations in SMEI and SMEB

Our data show similar results to those previously summarized in our review of *SCN1A* mutations (Mulley *et al.*, 2005), with mutations comprising 43% (33/77) truncation and 43% (33/77) missense changes. The proportion of missense (39% versus 52%) and truncation (44% versus 40%) mutations is similar in SMEI and SMEB. Our new data fail to fully confirm previous observations of a predilection for missense mutations to occur in the ion channel pore region (Kanai *et al.*, 2004), with only 15/33 (46%) in this region. Previous studies suggested clustering of missense mutations in SMEI in the S5–S5 loops of domain I and II (Mulley *et al.*, 2005) but here, clustering in domain I was not seen (Fig. 1A).

No consistent pattern of clustering has emerged in SMEB although 18/25 mutations were located in the transmembrane domains (Fig. 1B). Here, clustering of mutations was noted in the S2–S4 transmembrane segments of domain I, in contrast to patterns seen previously where clustering in domain II was observed (Mulley *et al.*, 2005). More data are required in order to establish if a true pattern of clustering exists.

Broader phenotypes of *SCN1A* mutations (Table 3)

The specific generalized epilepsy syndromes of MAE and LGS had a low yield of mutations with 2/10 and 1/12 positive cases respectively confirming that *SCN1A* is rarely associated with these syndromes (Wallace *et al.*, 2001; Nabbout *et al.*, 2003b; Ebach *et al.*, 2005). The nosological boundaries between these disorders, SMEI, SMEB and other cryptogenic generalized epilepsies are blurred. Indeed, in the large group of patients with cryptogenic generalized epilepsy of early onset where a more specific syndromal diagnosis could not be reached, 6/25 had *SCN1A* mutations. Two patients had a phenotype with features similar to SMEI but had onset in early infancy with abnormal early development and a more severe course. Others had heterogeneous phenotypes of generalized epilepsy with intellectual disability including those previously recognized in GEFS+ families (Scheffer and Berkovic, 1997; Singh *et al.*, 1999).

In patients classified as cryptogenic focal epilepsy, we identified a clinical subgroup who presented with a devastating multifocal epileptic encephalopathy. Of the five cases, three had *SCN1A* mutations. We designated this group severe infantile multifocal epilepsy (SIMFE) as onset is in the first year of life and multiple seizure types occur, with the most prominent being focal seizures. Multiple types of focal seizures occur including complex partial seizures of temporal lobe origin and hemiclonic

seizures. Video-EEG telemetry showed that the variation in seizure semiology was not due to seizure spread patterns. Focal myoclonus may occur or even be brought out by specific anti-epileptic drugs known to exacerbate myoclonic seizures, such as vigabatrin. Patients may also have convulsive or non-convulsive status epilepticus, tonic seizures with focal features and tonic–clonic seizures. Interictal EEGs show abundant multifocal epileptiform discharges. These individuals do not have generalized spike-wave activity on EEG. Their MRI brain scans are normal or show non-specific features. They usually have normal early development followed by cognitive decline, with the refractory seizure disorder culminating in intellectual disability. Abnormal neurological signs such as ataxia and spasticity may evolve. The factor that distinguishes these children from SMEI is the absence of generalized absence and myoclonic seizures, generalized spike-wave activity on EEG, and that their cognitive decline may be later than the second year of life. These children had a severe, progressive and hitherto puzzling phenotype, where extensive investigations had been performed searching for an aetiology such as muscle biopsy, lumbar puncture and liver biopsy.

Similar cases are described in the literature by many authors (Noriega-Sanchez and Markand, 1976; Markand, 1977; Blume, 1978; Malik *et al.*, 1989; Ohtsuka *et al.*, 1990, 2000; Burnstine *et al.*, 1991; Ohtahara *et al.*, 1995; Nabbout and Dulac, 2003; Yamatogi and Ohtahara, 2003). Some clinicians regard this phenotype as being the later evolution of a 'burnt out' symptomatic generalized epilepsy, but these patients never have the EEG signature of generalized spike-wave activity. The phenotype could also be regarded as part of 'severe epilepsy with multiple independent spike foci' described by Ohtahara and colleagues where generalized minor seizures are also emphasized (Ohtsuka *et al.*, 1990; Ohtahara *et al.*, 1995; Yamatogi and Ohtahara, 2003, 2006). This group incorporates a heterogeneous array of causes

Table 3 Epileptic encephalopathies with *SCN1A* mutations

	SMEI (n = 66)	SMEB (n = 36)	CGE (n = 25)	CFE (n = 13)	SIMFE (n = 5)
Average age seizure onset (months)	5.5	6	9.5	8	4
Clinical features					
Hemiclonic and/or generalized convulsions	Always	Always	Often	Often	Often
Myoclonic seizures	Always	Often	Often	Occasional	Often
Other focal seizures	Often	Often	Occasional	Always	Always
Other generalized seizures	Often	Often	Often	Occasional	Rare
EEG					
Generalized spike wave	Always	Occasional	Often	Rare	No
Multifocal epileptiform activity	Occasional	Occasional	Occasional	Occasional	Always
SCN1A mutations	52 (79%)	25 (69%)	6 (24%)	1 (8%)	3 (60%)
Truncation	23	10	–	–	1
Missense	20	13	5	1	2
Splice site	9	2	1	–	–

SMEI, severe myoclonic epilepsy of infancy; SMEB, SMEI borderland; CGE, cryptogenic generalized epilepsy; CFE, cryptogenic focal epilepsy; SIMFE, severe infantile multifocal epilepsy.

such as tuberous sclerosis and birth asphyxia. In contrast, SIMFE encompasses those patients hitherto without a known cause, with 3/5 found to have mutations of *SCN1A*. SIMFE is an important group of patients with a devastating epileptic encephalopathy who are presently difficult to classify. The discovery of *SCN1A* mutations as the basis of their disorder avoids further potentially invasive investigations for alternative causes and assists in targeting therapy. For example, anti-epileptic drugs that exacerbate myoclonic seizures, such as vigabatrin and tiagabine, should be avoided.

This extensive study of the role of *SCN1A* mutations in epileptic encephalopathies beginning in the first year of life has, not surprisingly, expanded the phenotypic spectrum. Disorders are initially identified in a 'pure cohort' with a specific group of essential features. As the molecular basis is determined, phenotype–genotype correlation results in broadening of the phenotypic spectrum to include milder cases or seemingly unrelated disorders. This is just beginning to be possible in epileptology, as *SCN1A* is the first gene shown to have a role in epilepsies previously regarded as cryptogenic. An important finding is that children with an epileptic encephalopathy with multifocal features in the setting of normal MRI may have *SCN1A* mutations as may children with cryptogenic generalized epilepsy. A strong indicator for *SCN1A* analysis is an epileptic encephalopathy with seizure onset before 1 year of age, even if cognitive decline does not occur for several years thereafter. The social and economic benefit in making a definitive diagnosis in children with epileptic encephalopathies cannot be underestimated. Neurologists continue to perform investigations looking for an aetiology in children with cryptogenic encephalopathies such that establishing a definitive molecular diagnosis is cost-effective. More importantly, families are very grateful for a specific diagnosis especially with the treatment and genetic counselling implications that a *SCN1A* mutation carries.

Supplementary material
Supplementary material is available at *Brain* Online.

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