Temporal lobe epilepsy and GEFS\(^+\) phenotypes associated with SCN1B mutations

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SCN1B, the gene encoding the sodium channel \(\beta\) 1 subunit, was the first gene identified for generalized epilepsy with febrile seizures plus (GEFS\(^+\)). Only three families have been published with SCN1B mutations. Here, we present four new families with SCN1B mutations and characterize the associated phenotypes. Analysis of SCN1B was performed on 402 individuals with various epilepsy syndromes. Four probands with missense mutations were identified. Detailed electroclinical phenotyping was performed on all available affected family members including quantitative MR imaging in those with temporal lobe epilepsy (TLE). Two new families with the original C121W SCN1B mutation were identified; novel mutations R85C and R85H were each found in one family. The following phenotypes occurred in the six families with SCN1B missense mutations: 22 febrile seizures, 20 febrile seizures plus, five TLE, three other GEFS\(^+\) phenotypes, two unclassified and ten unaffected individuals. All individuals with confirmed TLE had the C121W mutation; two underwent temporal lobectomy (one with hippocampal sclerosis and one without) and both are seizure free. We confirm the role of SCN1B in GEFS\(^+\) and show that the GEFS\(^+\) spectrum may include TLE alone. TLE with an SCN1B mutation is not a contraindication to epilepsy surgery.

**Keywords:** Epilepsy; GEFS\(^+\); TLE; sodium channel; genetics

**Abbreviations:** CPS = complex partial seizures; FS = febrile seizures; FS\(^+\) = febrile seizures plus; GEFS\(^+\) = generalized epilepsy with febrile seizures plus; GTCS = generalized tonic–clonic seizures; HS = hippocampal sclerosis; MAE = myoclonic–astatic epilepsy; SMEI = severe myoclonic epilepsy of infancy; SSCP = single-stranded conformation polymorphism analysis; TLE = temporal lobe epilepsy


**Introduction**

Generalized epilepsy with febrile seizures plus (GEFS\(^+\)) is a familial epilepsy syndrome characterized by heterogeneous phenotypes including febrile seizures (FS), febrile seizures plus (FS\(^+\)), mild generalized epilepsies and severe epileptic encephalopathies including myoclonic–astatic epilepsy (MAE) and severe myoclonic epilepsy of infancy (SMEI) (Scheffer and Berkovic, 1997; Singh et al., 1999, 2001). Focal epilepsies occur less commonly including temporal lobe epilepsy (TLE) (Singh et al., 1999; Abou-Khalil et al., 2001) and frontal lobe epilepsy (Baulac et al., 1999). GEFS\(^+\) was recognized through the study of large families with multiple individuals with seizure disorders where there was clinical and genetic evidence of a major gene of dominant effect; other modifier genes presumably contribute to the marked phenotypic variability. It is likely, however, that GEFS\(^+\) is more commonly multifactorial with complex genetics involving more than one gene of small to moderate effects (Scheffer et al., 2005).
Mutations have been identified in 10–15% of autosomal dominant GEFS∗ families. These include mutations in the sodium channel regulatory β1 subunit gene, SCN1B (Wallace et al., 1998, 2002; Audenaert et al., 2003) and the pore-forming α1 subunit gene SCN1A (Escayg et al., 2000; Wallace et al., 2001b) as well as the GABA<sub>A</sub> receptor γ2 subunit gene GABRG2 (Baulac et al., 2001a; Wallace et al., 2001a; Harkin et al., 2002). Although SCN1B was the first gene associated with GEFS∗, only two different SCN1B mutations have been reported to date. These include the original C121W missense mutation identified in two unrelated Australian families (Wallace et al., 1998, 2002) and a splice acceptor site mutation in a Belgian kindred leading to utilization of a cryptic splice site and associated loss of five amino acids (Audenaert et al., 2003). In contrast, 15 SCN1A missense mutations have been identified in families with GEFS∗ affecting various parts of the protein (Mulley et al., 2005). The SMEI phenotype is sometimes observed in dominant GEFS∗ families, however in contrast to familial GEFS∗, 90% of SMEI cases due to SCN1A mutations arise de novo with over half involving truncation of the protein (Claes et al., 2001, 2003; Ohmori et al., 2002; Sugawara et al., 2002; Fujisawa et al., 2003; Nabbut et al., 2003; Wallace et al., 2003; Mulley et al., 2005).

Here, we screened probands with generalized epilepsies, FS and other seizure disorders for mutations of SCN1B. We report four new families with GEFS∗ and SCN1B mutations including two novel mutations. We present an analysis of phenotypes associated with different SCN1B mutations, with particular emphasis on TLE phenotypes not previously associated with SCN1B mutations.

**Material and methods**

**Patient ascertainment and determination of phenotypes**

A group of 402 unrelated probands with seizure disorders were screened for SCN1B mutations. The group included 24 individuals with FS, 74 with GEFS∗ phenotypes, 152 with classical idiopathic generalized epilepsies, 127 with focal epilepsies and 25 with other syndromes. Subjects were ascertained from our family and twin epilepsy genetics research database and through routine clinical practice. Seizure histories were sought on all available putatively affected and some unaffected family members using a validated seizure questionnaire (Reutens et al., 1992). Histories were corroborated by questioning parents and eyewitnesses where possible. Strenuous attempts were made to review all previous medical records and available electroclinical data including video-EEG telemetry and neuroimaging studies. Three patients with TLE underwent video-EEG monitoring, interictal 18-fluorodeoxyglucose-PET and interictal SPECT (single photon emission computed tomography) and two had ictal SPECT studies in addition to MRI studies (see later). Seizure types were classified according to the international classification (Commission of Classification and Terminology of the International League Against Epilepsy, 1981, 1985, 1989; Engel, 2001).

Phenotypes in probands and family members were classified according to the international classification of epileptic syndromes (Commission of Classification and Terminology of the International League Against Epilepsy, 1989) or as described previously in the case of GEFS∗ phenotypes (Scheffer and Berkovic, 1997; Singh et al., 1999). FS were defined as generalized convulsive seizures occurring with fever between 3 months and 6 years. FS∗ was defined as FS that continued after 6 years and/or afebrile generalized tonic–clonic seizures (GTCS) before 6 years. This study was approved by the Human Research Ethics Committee of Austin Health and informed consent was obtained from all subjects or their parents or guardians in the case of minors.

**Single-stranded conformation polymorphism (SSCP) analysis, sequencing and genotyping**

The coding region and flanking intronic regions of SCN1B were screened for mutations using single-stranded conformation polymorphism (SSCP) analysis using a Gelscan 2000™ (Corbett Research) fluorescent detection system. PCR products showing conformational changes were sequenced using an ABI BigDye Terminator Ready Reaction kit and analysed on an ABI 3700 genetic analyser. Mutation nomenclature is based upon the NCBI mRNA sequence with Genbank accession number NM_001037. Mutation detection in families E and F has previously been described (Wallace et al., 1998, 2002). Ninety-six blood bank controls were tested for the SCN1B changes found.

Zygosity of a single twin pair in family B was determined by parental questionnaire and evaluation of 10 highly polymorphic DNA markers. The microsatellite markers D19S225, D19S425, D19S876 and D19S876 surrounding SCN1B were analysed. Genotyping of DNA samples was carried out by standard manual methods (Phillips et al., 1995).

**MRI studies**

Families, A, B and E (Figs 1 and 2) included five individuals with TLE. All had MRI scans, and MRI was also performed in A-II-1, the mother of TLE patient A-III-2; and B-III-5, the monozygous twin of B-III-4.

All TLE patients underwent a dedicated epilepsy protocol, and their images were qualitatively assessed by a neuroradiologist. Quantitative assessment of the hippocampal volumes was based on a 3D T1-weighted sequence, following our established protocol (Watson et al., 1992; Briellmann et al., 1998). It was performed in A-III-2 (based on images acquired at a 1.5 T GE scanner), in A-III-3, A-II-2 and A-II-1 (1.5 T Siemens scanner) and in B-III-4, B-III-5 (3 T GE scanner). In A-II-2, the MRI was repeated after 3 years (3 T GE scanner).

**Results**

**SCN1B mutational analysis**

Of the 402 individuals studied, mutations were identified in four unrelated probands: two had FS and TLE, one had FS∗ and one had FS. All four were heterozygous point mutations in SCN1B that alter the amino acid sequence and are not present in the control population (n = 96). All mutations occurred in probands with a family history of seizures consistent with GEFS∗ and affected family members were subsequently genotyped in order to track the coinheritance of phenotype with genotype. No mutations were found in...
the remaining probands with GEFS\(^+\) including 20 with MAE and 11 pwith SMEI (all 11 were later shown to have SCN1A mutations), or in those with classical idiopathic generalized epilepsies, focal epilepsies or other syndromes.

In families A and B, mutational analysis revealed a base change in exon 3 (c.363C→G) that segregated with GEFS\(^+\) phenotypes (Fig. 1). This mutation, identified in the previously reported families E and F (Fig. 2), leads to the

**Fig. 1** Pedigrees of new families with SCN1B mutations.
replacement of a highly conserved cysteine residue with tryptophan at position 121 of the protein (C121W) (Fig. 3). The mutation disrupts a putative disulphide bridge, which might maintain an immunoglobulin-like fold in the extracellular domain. All available affected members of the families carried the mutation. We found no genealogical evidence to suggest that families A, B, E and F are related to one another, however, they share a common haplotype.

Fig. 2 Pedigrees of families previously reported with C121W mutation in SCN1B (Wallace et al., 1998, 2002).
extending 2.41 Mb from marker D19S213 to D19S224 suggesting a founder effect mutation (Wallace et al., 2002).

Two different missense mutations of the highly conserved arginine amino acid at position 85, also located in the extracellular domain of SCN1B (Fig. 3), segregated with four studied affected individuals in family C and all affected individuals in family D (Fig. 1) except for one distantly related individual whose affected status could not be confirmed. R85C, where cysteine replaces arginine, was found in family C and a histidine substitution (R85H) was identified in family D. These mutations are likely to be significant as R85 is evolutionarily conserved in related sodium channel subunits and in different species (Fig. 4).

**Phenotypic analysis of each family**

**Family A**

Family A (Fig. 1A, Table 1), an Australian family whose eldest living generation emigrated from the United Kingdom, comprised six affected family members. Two individuals (A-II-1 and A-III-4) had FS with onset at ages 3 and 3.5 years and offset at 5 and 3.5 years, respectively. The MRI of A-II-1 was qualitatively and quantitatively normal. One individual (A-III-1) had FS+, experiencing two seizures at 1 and 2 years of age, only one was associated with fever.

Two individuals (A-II-2 and A-III-3) had FS or FS+ followed by TLE. The proband (A-III-3) had febrile status epilepticus at 12 months with three further episodes of febrile status epilepticus by 24 months. She had six afebrile GTCS in the next year and developed complex partial seizures (CPS) of temporal lobe origin at 5.5 years. Her aura comprised a ‘yucky feeling’ with epigastric nausea evolving to a frontal headache, often with loss of awareness, prominent oral and manual automatisms, head and eye deviation to the left and clonic activity of her right arm. Convulsive status epilepticus recurred at 6 and 7 years. Her MRI showed severe bilateral hippocampal sclerosis (HS). She had bilateral, symmetrical reduction of hippocampal volumes and an increase of the T2-weighted signal (Table 1). There were no other MR abnormalities.

Her father (A-II-2) had six FS as a child in the United Kingdom and was thought to have bacterial meningitis at 3 years (records not available). He had a late convulsion with fever at 11 years. Simple partial seizures of temporal lobe origin began at 16 years. He sustained a head injury following a motorbike accident at 21 years with loss of consciousness for 4 h. At 26 years, CPS developed and he remained refractory to multiple antiepileptic drugs at 42 years. He had MRI scans at 39 and 42 years which showed non-progressive, non-specific, scattered, diffuse white matter hyperintensities. There was no epileptogenic lesion. In particular, both hippocampi were normal on quantitative and qualitative assessment (Table 1). He underwent right anterior temporal lobectomy at 43 years and is seizure-free 25 months post-operatively. Histology showed non-specific findings including reactive astrocytosis, molecular layer and minor neocortical gliosis with increased perivascular spaces, but no features of HS.

The proband’s cousin (A-III-2) developed partial seizures at 2.3 years without preceding convulsions with fever. His habitual attacks began with a cluster of CPS associated with a fever of 38.8°C. His CPS sometimes began with an aura where he would cling to his mother and say ‘sick Mum’. Behavioural arrest then followed with eye deviation to the left, oral automatisms, cyanosis, dry retching, pallor and occasional urinary incontinence. The seizures lasted 1 min with post-ictal drowsiness. He had nine further clusters with

**Table 1**

<table>
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<th>HCV left</th>
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<td>34</td>
<td>1676†</td>
<td>2756</td>
<td>0.61</td>
</tr>
</tbody>
</table>

*Controls not available for this age group, volumes considered normal.
†Volume abnormal.
one to seven CPS per cluster; four clusters were associated with intercurrent infection or fever; the remaining five clusters occurred without fever. He was fully controlled on carbamazepine and sodium valproate from 5.4 years. He has never had convulsive FS or secondarily GTCS. His EEG at 3 years showed an excess of posterior slow activity and focal slow activity in the left fronto-temporal region. His MRI scan performed at the age of 3 years, was quantitatively and qualitatively normal (Table 1).

**Family B**

Family B (Fig. 1B, Table 1), an Australian family, contained nine affected family members over three generations; three were unavailable for genetic testing. All affected individuals experienced FS (range 1–10 seizures) with mean age of onset of 2.1 ± 1.5 years (range 0.75–3.5 years).

The 37-year-old proband (B-III-4) was a monozygotic twin. Both twins had approximately 10 FS beginning at 9 months; there was no documentation to establish if any attacks were prolonged. CPS developed at 25 years in B-III-4, and were refractory to treatment. The MRI showed right HS with reduced volume and increased T2-weighted signal (Table 1). There were no other lesions. The proband has been seizure free for 3 years following right anterior temporal lobectomy at 34 years except for an occasional premenstrual aura of déjà vu. Histopathology showed classical features of HS. Her monozygotic twin (B-III-5) had no afebrile seizures; her MRI was normal on qualitative and quantitative assessment.

**Family C**

Family C (Fig. 1C) was an Australian family with 20 affected family members over five generations. Only four affected members of this family were available for study (C-III-6, C-III-14, C-III-17 and C-IV-6). Three had FS with onset of seizures at 1.25–2 years and offset at 7–12 years; they experienced 5–8 seizures each, all febrile. The fourth individual (C-III-6) had a single FS at 12 months and a single unreported event at 25 years. There is an extensive family history of GEFS, with 14 additional affected individuals reportedly experiencing FS (2), FS+ (7) and some more complex phenotypes; however, accurate details were not available. One unaffected woman (C-III-7) had children with phenotypes consistent with GEFS, however she did not carry the mutation.

**Family D**

Family D (Fig. 1D), whose second generation emigrated from Italy, contained nine affected family members over three generations. Detailed histories were available for five of the eight living affected family members. Two individuals (D-V-1 and D-V-4) had FS with 1 or 2 seizures, commencing at 2–3 years. Three had FS+ (D-V-3, D-V-5 and D-V-6) with FS onset between 4 months and 9 years and offset between 4.5 and 10 years. They had 3–5 seizures each; all but one seizure was febrile. One additional individual (D-IV-5) had probable FS+ as her seizures continued until 12 years, but no information was available regarding her age of onset, number of seizures or presence of fever.

**Families E and F**

The phenotypes of families E and F (Fig. 2) have been previously published (Wallace et al., 1998, 2002).

**Genotype-phenotype correlation**

The clinical patterns with the different SCN1B mutations were similar, with the major phenotypes being FS and FS+. The only difference was that confirmed TLE was exclusively associated with the C121W mutation, but larger numbers of cases with R85 mutations are needed before regarding this as a definitive association.

**R85 SCN1B mutations**

In families C and D, 11 affected individuals had novel mutations of the same amino acid in SCN1B. The remaining 17 individuals were unavailable for testing. One individual (D-IV-I) with unconfirmed events did not carry the mutation. Of the family members with mutations, three had FS and seven had FS+. One individual was unclassified. Mean age of onset of FS+ was 3.0 ± 3.0 years (0.5–9 years) and offset was at 8.1 ± 3.2 years (range 3–12 years). There were no family members with confirmed TLE. One individual (C-IV-11) was reported to have a history of probable complex seizures suggestive of TLE, however this individual and her immediate family could not be studied. As our data were limited and we do not have molecular confirmation, it is not clear if TLE is a phenotype of R85 mutations.

**C121W SCN1B mutation**

Phenotypic analysis of all families with the C121W SCN1B mutation included families A and B and the two previously published families E and F (Wallace et al., 1998, 2002). There were 41 affected individuals with the C121W mutation. Nineteen individuals had FS alone, 13 had FS+ alone, 4 had FS or FS+ with later TLE, 3 had other GEFS+ phenotypes (in family E, 1 had FS+ and absences, 1 had afebrile GTCS, atonic and absence seizures; in family F, 1 had MAE), 1 had TLE not preceded by FS and 1 had unclassified epilepsy. Mean age of onset of FS was 2.4 ± 1.1 years (range 0.75–5 years). Mean onset of FS+ was 2.5 ± 3.2 years (range 0.5–13 years) with mean offset at 8.2 years ± 4.7 years (range 2–18.5 years).

**TLE phenotypes**

Five individuals had confirmed TLE; all belonged to families with the C121W mutation. Three had preceding FS and one had preceding FS+. One boy A-III-2 had never had a convulsive seizure although his first cluster of CPS occurred with fever. The mean age of onset of temporal lobe seizures
was 10.8 ± 9.5 years (range 2.3–25 years). Febrile status epilepticus only occurred in one patient (A-III-3).

**MRI studies**

Two individuals (Table 1) had HS on visual inspection; bilateral in A-III-3 who had febrile status epilepticus, and unilateral in B-III-4 who did not have a history of status epilepticus. The remaining three individuals had normal hippocampi (including E-VI-30 on qualitative analysis). Quantitative assessment of hippocampal volumes confirmed severe bilateral HS in the proband A-III-3 and right HS in B-III-4; two individuals had normal volumes with normal hippocampal volume ratios. MRI studies of A-II-1, mother of the boy who had TLE, and the monozygotic co-twin B-III-5 were normal.

**Discussion**

The importance of sodium channel gene defects in the generalized epilepsies has been emphasized by the confirmation of familial mutations in two different subunit genes, SCN1A and SCN1B, in GEFS* families. Here we report four new families with mutations in the β1 subunit gene SCN1B bringing the total number of known families with mutations to seven. Four different mutations have now been identified: the original C121W mutation (in four families) (Wallace et al., 1998, 2002; this report), the IVS2-2A→C transversion in the splice acceptor site of exon 3 predicted to cause a deletion of five amino acids (Audenaert et al., 2003), and two more novel mutations reported here, affecting the same R85 codon. The finding of additional families with mutations in SCN1B, including novel mutations in the extracellular loop, confirms the significant role of SCN1B in GEFS*. These results are likely to underestimate the significance of SCN1B in GEFS* because SSCP is a rapid mutation screening technology with a sensitivity of around 80% (Hayashi and Yandell, 1993). The families detected from the 402 index cases illustrate the phenotypic spectrum of SCN1B defects that notably includes five individuals with TLE, in line with observations of partial epilepsies in SCN1A related families.

In families C and D with novel SCN1B mutations, all available well-characterized individuals had FS or FS*. Indeed, their ages of onset were comparable with onsets of FS or FS* in families carrying the C121W mutation. In the previously published Belgian family with the splice site mutation, the only phenotype other than FS or FS* was early onset absence epilepsy without preceding FS (Audenaert et al., 2003). Early onset absence epilepsy was not observed in any of the other SCN1B families. In contrast, families with the C121W mutation also contained individuals with more complex phenotypes: three had generalized seizures including GTCS, absences and atonic seizures and five had TLE. This difference in phenotypic heterogeneity may be due to the relatively small numbers of individuals with the R85 mutations available for study, or alternatively, the C121W mutation may result in greater phenotypic diversity perhaps by being more sensitive to the effect of modifier genes. This will be resolved with additional R85 mutant families.

**TLE in GEFS**

Family A contained a striking number of individuals with TLE, possibly influenced by familial modifiers in the genetic background. TLE has been previously reported in GEFS* families, where it is considerably less common than generalized seizure types and typically follows FS or FS* (Singh et al., 1999; Abou-Khalil et al., 2001; Wallace et al., 2002). This led us originally to conjecture that TLE in such patients occurred as a sequela to FS (Singh et al., 1999) since an association between prolonged FS and TLE is well recognized (Maher and McLachlan, 1995; Hamati-Haddad and Abou-Khalil, 1998). Our hypothesis was supported by the report of a large family with GEFS* and a K1270T SCN1A mutation, in which five individuals had partial seizures of temporal lobe origin (Abou-Khalil et al., 2001). Of these five patients, three had complex FS (prolonged, repetitive or focal seizures), one had unknown FS features and one had simple FS. In two small Japanese families with SCN1A mutations, all individuals with partial epilepsy had preceding FS; seizure semiology was not well documented (Sugawara et al., 2001). Similarly, in a French family with FS and TLE mapped to two loci, all individuals with TLE had preceding simple FS although none showed hippocampal abnormalities (Baulac et al., 2001b). In contrast a Belgian family was reported with TLE and FS where the two disorders did not always co-occur in the same individual; this family has been mapped to chr12q23 (Depondt et al., 2002; Claeys et al., 2004).

The key feature of family A is that one boy (A-III-2) with TLE did not have convulsive FS prior to the onset of TLE. Although FS can present as focal CPS [Camfield et al. (http://www.ilae-epilepsy.org/Visitors/Centre/cfl/febrile_convulsions.cfm)], this boy went on to have several clusters of afebrile CPS with temporal lobe manifestations suggesting that his original presentation may be more aptly diagnosed as TLE provoked by fever, rather than FS. He did not have HS. This observation implies that TLE is a phenotype of the C121W SCN1B mutation. Thus, GEFS* phenotypes may include TLE in its own right, not simply as a secondary effect of damage from generalized seizures. TLE has now been observed in GEFS* kindreds with mutations of SCN1B, SCN1A and GABRG2, indicating that TLE is not specific to SCN1B mutations (Abou-Khalil et al., 2001; Wallace et al., 2001a).

The finding of focal epilepsies such as TLE and frontal lobe epilepsy in GEFS* challenges the name of the familial epilepsy syndrome generalized epilepsy with FS* as previously pointed out (Ito et al., 2002). The concept of GEFS* arose from families who have predominantly generalized seizure disorders and FS, and this observation remains true in the vast majority of GEFS* families. Focal features can be seen in a number of idiopathic generalized epilepsy...
 syndromes yet this does not undermine the clinical utility of the concept of a generalized epilepsy syndrome. Thus it is important to recognize that focal epilepsies are one of the many phenotypes which characterize the familial GEFS+ spectrum associated with known ion channel mutations (Scheffer et al., 2005).

The finding of individuals with refractory TLE in SCN1B families suggests that there are likely to be other genes or environmental contributors involved in the genesis of these more complex phenotypes. Two individuals had relatively mild TLE (A-III-2, E-VI-30), whereas three (A-II-2, A-III-3, B-III-4) had refractory epilepsy. Of these three, two had HS confirmed on qualitative and quantitative imaging. One patient (B-III-4) had typical unilateral HS indistinguishable from the usual pattern of HS. Her excellent outcome from anterior temporal lobectomy is consistent with that usually seen for HS (McIntosh et al., 2001). The other patient (A-III-3) had bilateral HS after febrile status epilepticus and was not a surgical candidate.

A puzzling issue is why only one monozygotic twin developed TLE with HS, despite both twins having FS. There is a longstanding ‘chicken-and-egg’ debate regarding whether prolonged FS lead to hippocampal damage or whether there are pre-existing abnormalities that predispose the child to a prolonged FS in the first place (Falconer et al., 1964). The early childhood history in the pair reported here was not sufficiently clear to determine if there were discriminating features of the seizures to potentially account for the evolution of HS in only one twin. In our previous report of monozygotic twin pairs discordant for HS, there was a clear association with prolonged FS (Jackson et al., 1998).

In addition to antenatal and post-natal acquired factors, it is possible that the twins presented here have genetic causes underlying the disparity with regard to the evolution of HS (Machin, 1996). Although monozygotic twins are considered `identical`, post-zygotic molecular changes may occur that could influence the development of HS and associated epilepsy phenotypes (Singh et al., 2002). This could determine the circuitry that predisposes first to prolonged rather than simple FS and more importantly, to the development of HS.

The question of whether epilepsy surgery should be contraindicated in patients with a known genetic defect, which is present diffusely in the brain, is controversial. Here we observed excellent surgical outcomes in two cases including individual A-II-2 where HS was proven absent. This is the first evidence that the presence of a GEFS+ gene mutation is not a contraindication to epilepsy surgery.

**Functional effects of SCN1B mutations**

There are now four mutations of SCN1B identified in GEFS+: three missense and an in-frame deletion which all localize to the extracellular loop (Fig. 3). The C121W SCN1B mutation putatively disrupts a disulphide bridge in the extracellular domain of the protein, a region critical for channel gating kinetics. Functional studies of the mutant C121W β1 subunit coexpressed with a subunits in (*Xenopus laevis*) oocytes showed a loss of function with a slowed time course of inactivation compared with wild type β1 subunits (Wallace et al., 1998). Similar studies in mammalian cells yielded contradictory results (Meadows et al., 2002; Tammaro et al., 2002). Examination of whole-cell sodium currents showed less run down during high-frequency channel activity with the mutant β1 subunit, reflecting a higher level of channel repriming between episodes of channel activation that could potentially lead to hyperexcitability (Meadows et al., 2002).

In addition to its effects on the electrophysiological behaviour of sodium channels, the β1 subunit exhibits cell adhesion properties such as aggregation and recruitment of cell adhesion molecules such as ankyrin to points of cell to cell contact (McEwen and Isom, 2004; McEwen et al., 2004). These actions may be important for sodium channel subcellular localization and therefore cell excitability. The mutant C121W β1 subunit disrupted cell adhesion (Meadows et al., 2002). The conserved disulphide bond disrupted by the C121W mutation is considered critical for homophilic β1–β1 interactions or heterophilic interaction between β1 and contactin, both resulting in increased levels of cell surface sodium channels (Kazarinova-Noyes et al., 2001; McEwen and Isom, 2004). Overall, the C121W mutation leads to loss of β1 mediated functional modulation and cell adhesion.

The key issue is how these functional deficits determined in various expression systems translate to *in vivo* models. Studies of sodium channel β1(+/−) mice confirm the key role of the β1 subunit in regulation of sodium channel density and localization, axioglial communication and action potential conduction (Chen et al., 2004). More information will be derived from transgenic mice expressing the exact mutations found in man.

There are now seven GEFS+ families with four different mutations of SCN1B. The C121W mutation is recurrent and may be due to a founder mutation. Interestingly the mutation of R85 codon is also recurrent, although the latter is to different amino acid residues. This is reminiscent of the molecular findings in autosomal dominant nocturnal frontal lobe epilepsy where there are recurrent site-specific mutations of the nicotinic acetylcholine receptor subunit CHRNA4 (Steinlein et al., 1995; McLellan et al., 2003) and a recurrent same codon mutation to different amino acid residues of CHRN2B (De Fusco et al., 2000; Phillips et al., 2001). Thus, the pattern for SCN1B and the CHRN subunits differs from the other commonly mutated epilepsy genes SCN1A, SCN2A and KCNQ2 with the alternative pattern of novel mutations spread throughout the gene (Singh et al., 2003; Berkovic et al., 2004; Mulley et al., 2005).

In addition to the phenotypic heterogeneity typically seen in GEFS+, three families with the C121W mutation have individuals with TLE. The TLE phenotype is itself heterogeneous encompassing mild and refractory forms, and
a variable association with HS and FS or FS*. The finding of novel mutations in the extracellular loop of the SCN1B protein emphasizes the importance of the normal functioning of this regulatory domain against sodium channel related seizures. Further attention to the role of SCN1B in GEFS will be important in characterizing the full extent of the phenotypic spectrum associated with SCN1B mutations and in understanding the complex inheritance involved in the more severe phenotypes where additional modifier genes may be involved.

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