

A novel *SCN1A* mutation in a cytoplasmic loop in intractable juvenile myoclonic epilepsy without febrile seizures

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ABSTRACT – Generalised (genetic) epilepsy with febrile seizures plus (GEFS+) is a familial epilepsy syndrome with various phenotypes. The majority of individuals with GEFS+ have generalised seizure types, in addition to febrile seizures (FS) or febrile seizures plus (FS+), defined as either continued FS after 6 years of age or afebrile seizures following FS. A 27-year-old man with no history of FS/FS+ experienced intractable generalised convulsive seizures. The patient's father had a history of similar seizures during puberty and the patient's siblings had only FS. No individual in the family had both generalised seizures and FS/FS+, although GEFS+ might be considered to be present in the family. Analysis of *SCN1A*, a sodium channel gene, revealed a novel mutation (c.3250A>T [S1084C]) in the cytoplasmic loop 2 of *SCN1A* in both the patient and his father. Most previously reported *SCN1A* mutations in GEFS+ patients are located in the conserved homologous domains of *SCN1A*, whereas mutations in the cytoplasmic loops are very rare. *SCN1A* gene analysis is not commonly performed in subjects with generalised seizures without FS. *SCN1A* mutation may be a clinically-useful genetic marker in order to distinguish GEFS+ patients from those with classic idiopathic generalised epilepsy, even if they present an atypical clinical picture.

Key words: *SCN1A* gene, novel mutation, intractable, IGE, JME, GEFS+

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Idiopathic generalised epilepsy (IGE) is classified into classic IGE and the recently proposed “generalised (genetic) epilepsy with febrile seizures plus” (GEFS+). By definition, GEFS+ is a familial epilepsy syndrome and is diagnosed on the

basis of more than one individual within a family with a history of manifestations associated with the GEFS+ spectrum. The GEFS+ spectrum consists mostly of generalised seizures commonly combined with FS or febrile seizures plus (FS+) in

the same individual. FS+ is defined as either continued FS after the age of 6 years or afebrile seizures following FS (Scheffer & Berkovic, 1997). Various point mutations in the sodium channel voltage-gated type I alpha subunit (*SCN1A*; MIM 182389, GenBank AB093548) are the genetic cause of certain GEFS+ phenotypes. The GEFS+ spectrum can range from benign phenotypes, such as febrile seizures (FS), to severe epileptic encephalopathies, such as Dravet syndrome.

We present a patient whose initial diagnosis was believed to be juvenile myoclonic epilepsy (JME). The patient did not have FS/FS+, but his two siblings had only FS. His father and paternal aunt had generalised convulsive seizures alone without FS/FS+. No family member had both epilepsy and FS. However, the possibility of the presence of GEFS+ within the family was considered because the proband's seizures were very poorly controlled, in contrast to classic IGE. Genetic analysis of the *SCN1A* gene showed that the patient and his father had a novel point mutation in the cytoplasmic loop. Thus, their diagnosis as a family was considered to be GEFS+. With the exception of Dravet syndrome, the presence of *SCN1A* missense mutations in patients without any characteristics of the GEFS+ spectrum is very rare. This family may demonstrate the clinical divergence of patients with *SCN1A* missense mutations as well as the clinical variation within the GEFS+ spectrum.

Case study

A 27-year-old Japanese man with no history of FS was regularly treated for frequent generalised tonic-clonic seizures (GTCSs) and presumed myoclonic seizures, in combination. The first GTCS occurred at 14 years of age. The seizures often occurred while he was watching television, with flickering images, suggesting photo-sensitivity. Valproate (VPA) was effective and the seizures diminished within days. At the age of 17 years, GTCSs reappeared and he developed other repetitive myoclonic jerks in his arms, which occurred mostly in the morning. When carbamazepine (CBZ) was administered, his seizure control worsened and CBZ was discontinued. Myoclonic jerks occurred almost every day, and the GTCS frequency gradually increased to more than one a month, when he was aged 27 years and visited our hospital. Based on his medical history, his diagnosis was believed to be JME. He was receiving VPA and clobazam (CLB) upon admission. Blood tests were normal and brain MRI showed neither atrophy nor any structural abnormality. His intelligence quotient scores were within the average range. An EEG showed paroxysmal generalised polyspike-and-wave complexes with myoclonic seizures, one of which evolved into a GTCS during a routine EEG examination.

Abnormally enhanced, somatosensory, early cortical evoked potentials, in response to median nerve stimulation (giant SEPs), were not elicited.

He had a positive family history of both generalised convulsive seizures and typical FS, but neither of these occurred in combination in the same individual. His father and paternal aunt showed a similar phenotype; they had only generalised convulsive seizures. On the other hand, his two sisters had a history of only typical FS (*figure 1*). None of the family members had both epilepsy and FS. There was an autosomal dominant genetic trait of epileptogenicity in the family; thus, the possibility of GEFS+ within the family was considered. In addition, contrary to typical JME, the proband's seizures were very poorly controlled.

Written informed consent for genetic analysis was obtained from the patient and his parents, but not from the patient's sisters. The sisters previously had FS but currently have no symptoms and are of child-bearing age. The family did not wish the sisters to undergo gene testing. Molecular screening was carried out by direct sequencing of the 26 exons of *SCN1A* using DNA from blood cells. A heterozygous missense mutation at c.3250A>T (S1084C) in the *SCN1A* gene of the patient and his father were identified. S1084C is located in the cytoplasmic loop 2, which links homologous domains II and III (*figure 2A*). The putative impact of the amino acid substitution resulting from this gene mutation on the structure and function of the protein was assessed with PolyPhen-2 (prediction of functional effects of human nsSNPs; available at <http://genetics.bwh.harvard.edu/pph/>). This substitution was considered likely to be damaging, with a score of 0.991 (sensitivity: 0.71; specificity: 0.97).

GEFS+ was considered to be present within the family. Since the father had a history of treatment with phenobarbital (PB) since the age of 20 years and his seizures were well controlled, we chose PB as an add-on AED for our patient; his seizure frequency and myoclonic jerks decreased remarkably afterwards.

Discussion

We identified a novel *SCN1A* mutation (S1084C), located in the cytoplasmic loop 2, in a patient with clinically intractable JME (*figure 2B*). The family exhibited a familial classic IGE-like epilepsy syndrome which should be added to the spectrum of large phenotypic variation associated with *SCN1A* mutation.

In general, *SCN1A* mutations in GEFS+ patients are present in one of the four conserved homologous domains, whereas mutations in the loop region are comparatively less common (Zuberi *et al.*, 2011). Other mutations have been identified in loop 2 (41 mutations; 14 of which are missense mutations), however,

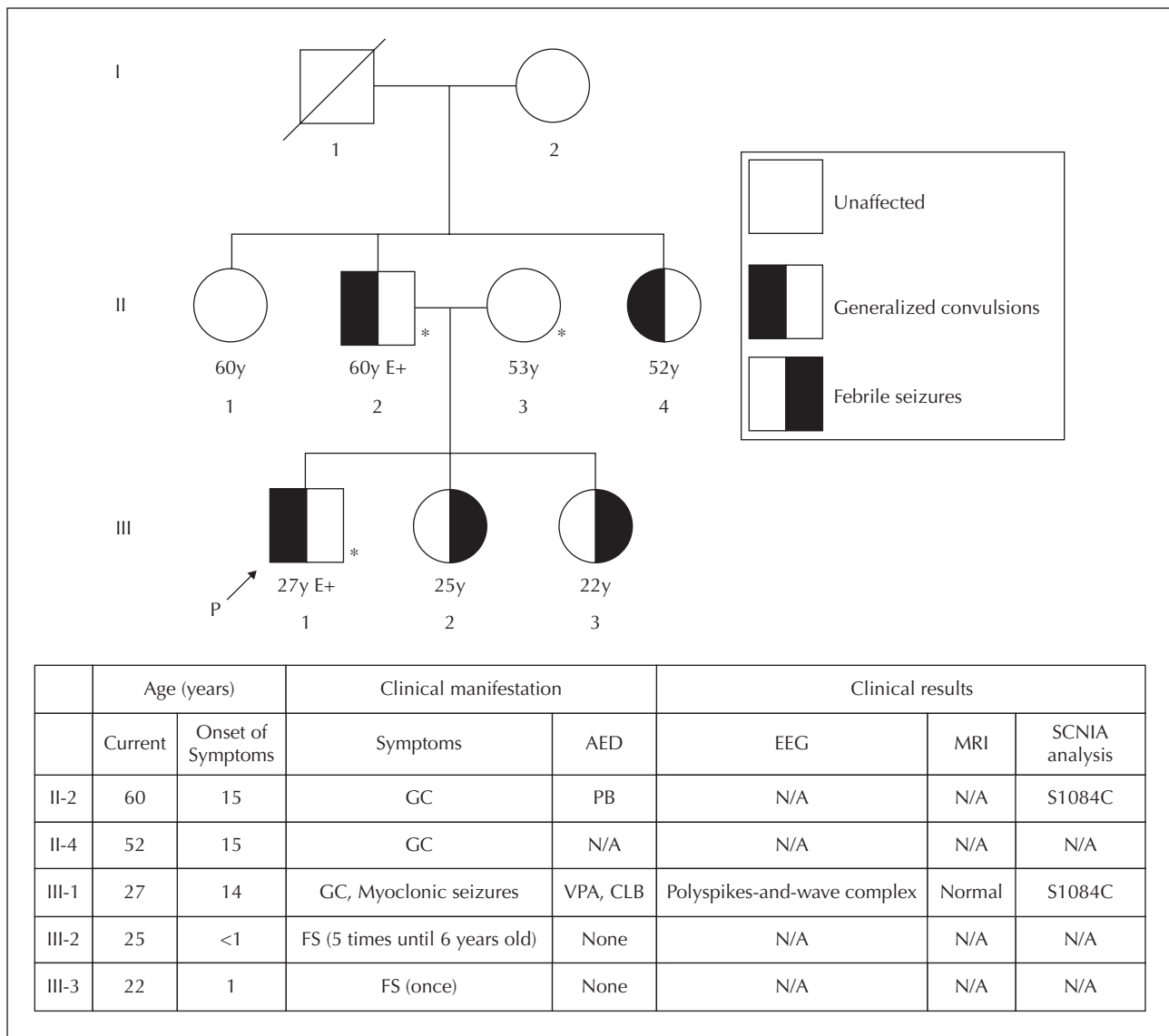


Figure 1. The pedigree and clinical features of the GEFS+ family.

Arrow: the patient; number under each symbol: age; asterisk: subject examined; E+: positive evaluation of gene analysis.

GC: generalised convulsions; FS: febrile seizures; N/A: not available; PB: phenobarbital; VPA: valproate; CLB: clobazam.

The patient's two siblings had only FS. Subject III-2 had five FS and III-3 a single FS. The patient, his father, and paternal aunt had only generalised convulsions.

most of them cause Dravet syndrome (*SCN1A* Variant Database; available at <http://www.molgen.vib-ua.be/SCN1AMutations/>). With regards to Nav 1.1 function, a cytoplasmic loop could be functionally less important relative to the homologous domains. The cytoplasmic loops could possibly act as phosphorylation sites. Point mutations in the loops might cause altered sodium channel (Nav1.1) function and neuronal hyperexcitability (Smith and Goldin, 1997). Loop 2 is also important as an ankyrin G binding site, which is essential for targeting sodium channels to the axon initial segment and nodes of Ranvier (Rasband, 2010). The

S1084C mutation is very close to the ankyrin G binding site and may interfere with its functions, moreover, we confirmed that S1084C may potentially result in damaging functional abnormalities using PolyPhen-2. Among the 24 previously reported *SCN1A* mutations in GEFS+ patients, identified from the *SCN1A* infobase (available at <http://www.scn1a.info/>), only one other missense mutation in a loop region has been reported. Escayg *et al.* (2001) identified a W1204R missense mutation in *SCN1A*, located in the cytoplasmic loop 2, similar to our case. In their study, a German GEFS+ family was reported, in which most of the members

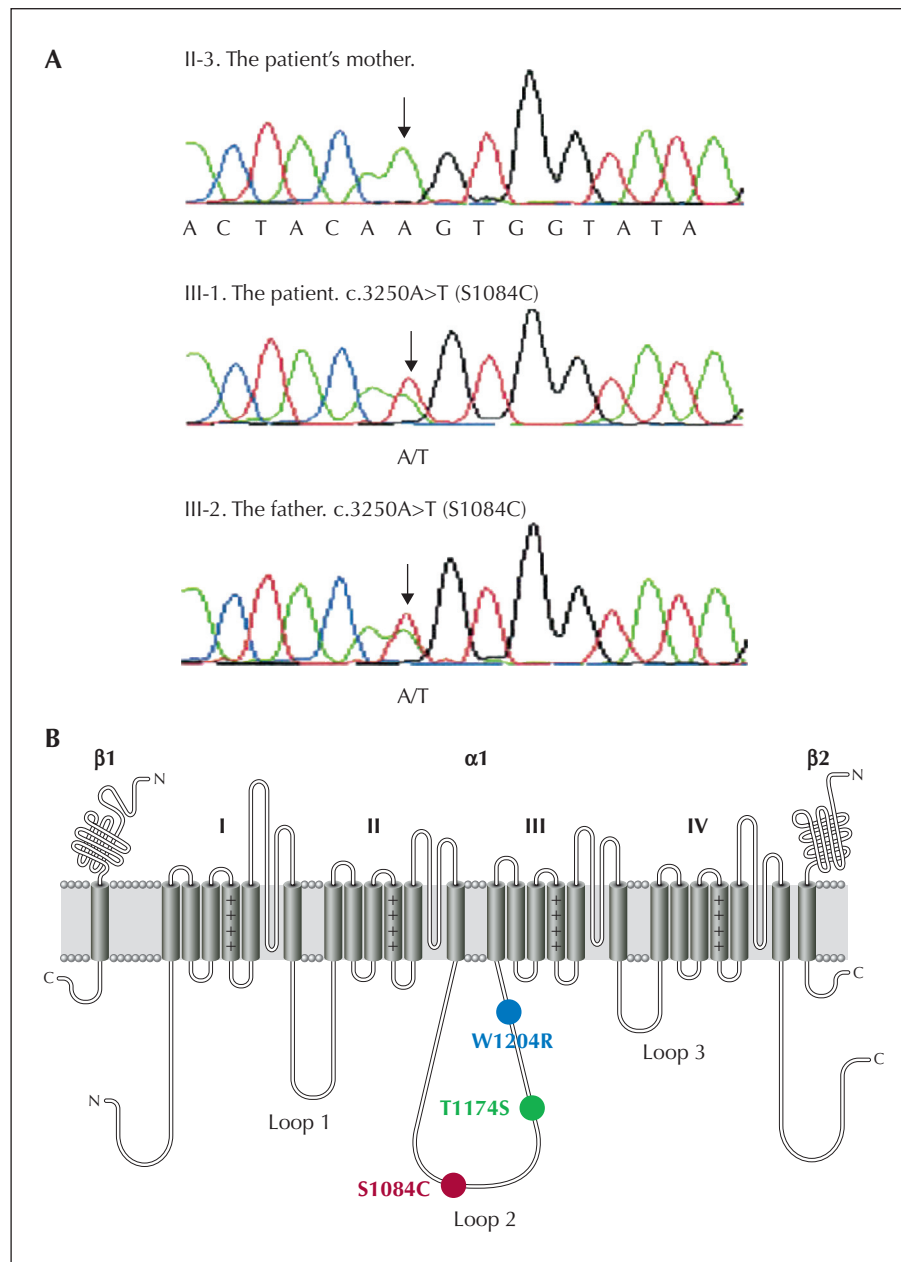


Figure 2. (A) The novel mutation in *SCN1A* genomic DNA. The patient and his father had a heterozygous mutation consisting of an adenine-to-thymine substitution within codon 3250 (S1084C). (B) Schematic diagram of voltage-gated sodium channel alpha and beta subunits. The position of the novel mutation S1084C (red circle) and previously reported mutations are depicted; T1174S (green circle) and W1204C (blue circle). The four homologous domains in the alpha subunit are labelled I-IV, with each domain containing six transmembrane segments. These mutations were in the cytoplasmic loop 2, linking the homologous domains II and III. Figure modified with permission from Hirose *et al.* (2002).

demonstrated the characteristics associated with the GEFS+ spectrum, however, curiously, the proband's initial diagnosis was also JME and he did not have FS/FS+. In the context of studies to date, the mutation T1174S is of particular interest among those identified in loop 2, and is implicated in different phenotypes including JME and familial hemiplegic migraine (Escayg *et al.*,

2001; Gargus and Tournay, 2007; Yordanova *et al.*, 2011; Cestele *et al.*, 2013). The functional effect of T1174S was studied by expressing the protein in cell lines and it was hypothesized that a loss of function is the cause of epileptogenicity (Cestele *et al.*, 2013). It is noteworthy that two other missense mutations in loop 2 (W1204R and T1174S) have been previously

reported to be associated with a JME phenotype, and these were furthermore identified in patients with different ethnic origins. Thus, in addition to these two mutations, we have identified a similar mutation in loop 2 in this GEFS+ family. Further data is required in order to evaluate the association between these phenotypes and loop 2 missense mutations.

Since the *SCN1A* mutation alters $Na_v1.1$ activity, AEDs that block sodium channels (e.g. CBZ, oxycarbamazepine, phenytoin, lamotrigine) should theoretically be avoided in *SCN1A*-related syndromes. On the other hand, VPA, stripentol, levetiracetam, and topiramate reportedly provide good control for these syndromes (Delgado-Escueta and Bourgeois, 2008). Thus, the genetic diagnosis provided us with information that was useful in choosing an appropriate add-on AED, avoiding arbitrary AEDs that could aggravate the patient's seizures. In our case, PB, which the patient's father was taking, with good seizure control, was also greatly effective. PB does not modulate sodium channel function and, thus, it is reported to be effective for *SCN1A*-related seizure disorders (Miller and Sotero de Menezes, 1993).

SCN1A analyses have been widely conducted in patients with FS/FS+ and those with intractable childhood epilepsy (Hirose *et al.*, 2013). However, testing in patients with classic IGE is not yet commonly performed. JME is a comparatively benign form of classic IGE, but about 15% of JME cases are reported to have persisting seizures despite adequate therapy and healthy lifestyles (Gelisse *et al.*, 2001). Some individuals with GEFS+, in the broad sense, might be misclassified with the more common syndromes of classic IGE. In certain cases of intractable classic IGE, when the family history of generalised seizures or FS is present, it would be useful to consider *SCN1A* gene mutation as well as the differential diagnosis of GEFS+. *SCN1A* mutation may be a clinically useful genetic marker for detecting GEFS+ patients among classic IGE cases, even if they present an atypical clinical picture. □

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