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De novo SCN1A pathogenic variants in the GEFS+ spectrum: Not always a familial syndrome

Running title: GEFS+ *de novo SCN1A* pathogenic variants

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Summary

Genetic epilepsy with febrile seizures plus (GEFS+) is a familial epilepsy syndrome characterised by heterogeneous phenotypes ranging from mild disorders such as febrile seizures

to epileptic encephalopathies (EEs) such as Dravet syndrome (DS). While DS often occurs with *de novo SCN1A* pathogenic variants, milder GEFS+ spectrum phenotypes are associated with inherited pathogenic variants. We identified seven cases with non-EE GEFS+ phenotypes and *de novo SCN1A* pathogenic variants, including a monozygotic twin pair. Febrile seizures plus (FS+) occurred in six patients, five of whom had additional seizure types. The remaining case had childhood-onset temporal lobe epilepsy without known febrile seizures. While early development was normal in all individuals, three later had learning difficulties and the twin girls had language impairment and working memory deficits. All cases had *SCN1A* missense pathogenic variants which were not found in either parent. One pathogenic variant had been previously reported in a case of DS, and the remainder were novel. Our finding of *de novo* pathogenic variants in mild phenotypes within the GEFS+ spectrum shows that mild GEFS+ is not always inherited. *SCN1A* screening should be considered in patients with GEFS+ phenotypes as identification of pathogenic variants will influence antiepileptic therapy, and prognostic and genetic counseling.

Key Words: Genetic epilepsy with febrile seizures plus, *SCN1A*, *De novo* pathogenic variant, Febrile seizures, Dravet syndrome.

Introduction

Genetic epilepsy with febrile seizures plus (GEFS+) is a familial epilepsy syndrome characterised by heterogeneous phenotypes. The most common is febrile seizures (FS), followed by febrile seizures plus (FS+) in which febrile and afebrile tonic-clonic seizures occur in early childhood. FS or FS+ may also be accompanied by generalized or focal seizures. At the severe end of the GEFS+ spectrum lie the developmental epileptic encephalopathies, epilepsy with myoclonic-atonic seizures (MAE) and Dravet syndrome (DS).[1] The mechanisms underlying phenotypic heterogeneity within families are not understood but may involve variable expressivity of a single causative gene, modifying genes or epigenetic factors. Pathogenic variants in *SCN1A*, *SCN1B*, *SCN9A*, *STX1B* and *GABRG2* have been associated with GEFS+;[2]

with *SCN1A* the most commonly implicated gene, found in approximately 11% of large families.[3]

SCN1A pathogenic variants have primarily been identified in individuals with GEFS+ phenotypes in the setting of autosomal dominant families and as *de novo* events in the epileptic encephalopathies.[4] DS is a developmental epileptic encephalopathy that presents with febrile seizures in the first year of life followed by developmental arrest or regression and multiple types of seizures. More than 80% of patients with DS have *SCN1A* pathogenic variants, of which 90% occur *de novo*.[5] *De novo SCN1A* pathogenic variants are also rarely found in severe infantile epileptic encephalopathies such as epilepsy of infancy with migrating focal seizures.[6] Here, we report seven patients with *de novo SCN1A* pathogenic variants causing milder GEFS+ phenotypes, illustrating that this genetic syndrome is not necessarily familial or inherited.

Methods

We reviewed our Epilepsy Genetics Research Program database to identify individuals with GEFS+ phenotypes and *de novo SCN1A* pathogenic variants. Patients with the epileptic encephalopathies of DS and MAE were excluded. Individuals underwent detailed phenotyping and review of their EEG and imaging studies, where available. The biological significance of identified variants was assessed using *in silico* methods.

The Human Research Ethics Committee of Austin Health approved the study (Project No. H2007/02961). Written informed consent was obtained from all participants or parents/legal guardians in the case of minors or those with intellectual disability.

Results

Seven cases were identified with a *de novo SCN1A* pathogenic variant, including a pair of monozygotic twins. All pathogenic variants were identified through single gene testing.

Six cases had phenotypes of FS+, together with other seizure types in five (Table). These included absence (4), hemiclonic (3) and focal seizures with evolution to a bilateral tonic-clonic seizure (4). The remaining subject (case 4) had temporal lobe epilepsy with childhood onset without known febrile seizures, but had a child with FS+ (see below). Five of the patients had drug-resistant epilepsy; details of medication response for each case are given in Table. Early development was normal in all cases, but five later developed learning difficulties, and the twin girls (cases 6, 7) had language impairment and deficits in working memory on formal speech

pathology assessment at age five years. MRI brain studies did not show significant abnormalities in four patients.

There was no family history of seizures in the preceding generations of three probands. Case 2 had a maternal uncle with FS and the twins (cases 6, 7) had a maternal uncle with unconfirmed FS. Two probands (cases 4, 5) had affected children who carried the same variant (Figure 1). Case 4 married her maternal first cousin and had a son with refractory febrile and afebrile seizures (including tonic and focal clonic) from two months of age, diffuse slowing on EEG, and moderate intellectual disability. A second son had borderline intellect and febrile seizures from 12 months until 6.5 years. Case 5 had a son who had febrile seizures at 13 months and subsequently developed absence, myoclonic and afebrile generalized tonic-clonic seizures, occasionally provoked by flashing lights. He had normal intellect at 19 years with refractory seizures.

Sanger sequencing revealed *SCN1A* missense pathogenic variants in all cases, which were not present in their parents. The pathogenicity of variants met criteria defined in the 2015 ACMG/AMP guidelines;[7] based primarily on being *de novo*, absence in the Exome Aggregation Consortium (ExAC) database, and supported by *in silico* predictions (supplementary material). All pathogenic variants were novel, with the exception of case 4 (p.A104V) which was previously reported in a case of DS (supplementary material).[8] Four pathogenic variants were located in the pore-forming regions of SCN1A (p.G329A, p.A394D, p.H1393D, p.A1429V), with one each in the N-terminal (p.A104V) and voltage sensor (p.R859H) regions (Figure 2).

Discussion

While *de novo* pathogenic variants are increasingly recognised as causing a wide array of epileptic encephalopathies with devastating consequences, their role in milder epilepsies is just beginning to emerge. The prototypic epileptic encephalopathy of DS is associated with *de novo SCN1A* pathogenic variants in contrast to the inherited pathogenic variants found in dominant GEFS+ families. Our series of seven patients adds to previous reports of one or two cases with *de novo SCN1A* pathogenic variants that have phenotypes such as FS+ that fall within the GEFS+ spectrum (supplementary table). This suggests that *de novo* pathogenic variants may arise more frequently in GEFS+ phenotypes than previously appreciated.

This leads to a departure from our original concept that GEFS+ is a familial epilepsy syndrome and suggests that a family history is *not* essential to make a diagnosis of GEFS+, and emphasizes

that GEFS+ phenotypes can be diagnosed in sporadic individuals. There are many genetic disorders which were considered to be exclusively familial until the role of *de novo* genetic change was identified. Familial adenomatous polyposis is an excellent example of a phenotype initially described in families, though we now know that up to 25% of identified cases are due to *de novo* pathogenic variants.[9] It is exciting therefore to see a range of GEFS+ phenotypes that occur due to *de novo* pathogenic variants.

Of our seven cases, only case 5 had FS+ alone, with the remaining six having more complex phenotypes. They commonly presented with FS+ and developed afebrile absence and/or focal motor seizures. Although development was not always normal, all individuals were capable of living independently and having children of their own, if they had not done so already. Family history was negative for FS, FS+ and other GEFS+ phenotypes in most cases; though cases 2, 6 and 7 had at least possible FS in one of their parents' siblings. This suggests the presence of additional genes related to epilepsy in these families.

Inheritance may also be complicated in case 5. First, the proband had a sister with FS who was not available for genetic testing. Although the parents tested negative for *SCN1A* pathogenic variant, one may have had low-level mosaicism not detected by standard Sanger sequencing, and transmitted the pathogenic variant to both the proband and her sister. Further complicating matters, the proband's husband and his sister both had FS. Given that the proband's son was more severely affected, he may have inherited modifier genes from his father in addition to the maternally inherited *SCN1A* pathogenic variant, which contributed to his refractory phenotype.

The association of mild GEFS+ phenotypes with *de novo SCN1A* pathogenic variants suggests that clinical testing guidelines deserve re-evaluation. In 2013, the International League Against Epilepsy Genetics Commission confirmed the need for *SCN1A* testing in cases of DS but recommended that "*infants and children with GEFS+ should not be advised to undergo SCN1A testing as it will neither influence management nor provide information regarding the patient's prognosis*".[10] Based on this guideline, *SCN1A* testing would not be indicated for the patients we have reported here.

That GEFS+ management would not change with identification of an *SCN1A* pathogenic variant is partially based on the belief that *SCN1A*-associated GEFS+ is inherited in an autosomal dominant manner. This is not always the case, as illustrated by our *de novo* cases in which a *SCN1A* pathogenic variant only came to light once they had affected children (as in cases 4, 5).

Recessive inheritance has also recently been reported with homozygous *SCN1A* pathogenic variants in two consanguineous families, providing another instance in which a sporadic case should be tested.[11] Furthermore, the rate of parental mosaicism in cases of apparent *de novo* *SCN1A*-associated DS may be as high as 10%.[12] Mosaic parents of individuals with DS typically exhibit milder phenotypes on the GEFS+ spectrum.[13]

Recognition of the potentially complex *SCN1A* heritability patterns in GEFS+ suggests that *SCN1A* testing may have significant utility across a broader spectrum of phenotypes than just DS. Patients and their families can benefit from genetic counseling. In light of these findings, we propose that *SCN1A* testing be considered in all individuals with FS+ or DS, as well as in familial cases consistent with GEFS+.

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We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Figure Legends

Figure 1 – Pedigrees for cases. Arrows indicate the individual in which the *de novo* pathogenic variant in *SCN1A* occurred. * Absence seizures had early onset in these patients (18 months). EOEE = Early Onset Epileptic Encephalopathy.

Figure 2 – Location of pathogenic variants in the SCN1A protein. The four domains (I-IV) are shown, each having six transmembrane helices. In each domain, segment 4 (blue) is the voltage sensor and segments 5 and 6 (pink) form the pore of the ion channel. Four variants were found in pore-forming regions (p.G329A, p.A394D, p.H1393D, p.A1429V), one in the N-terminal region (p.A104V) and one in the voltage sensor (p.R859H) region.

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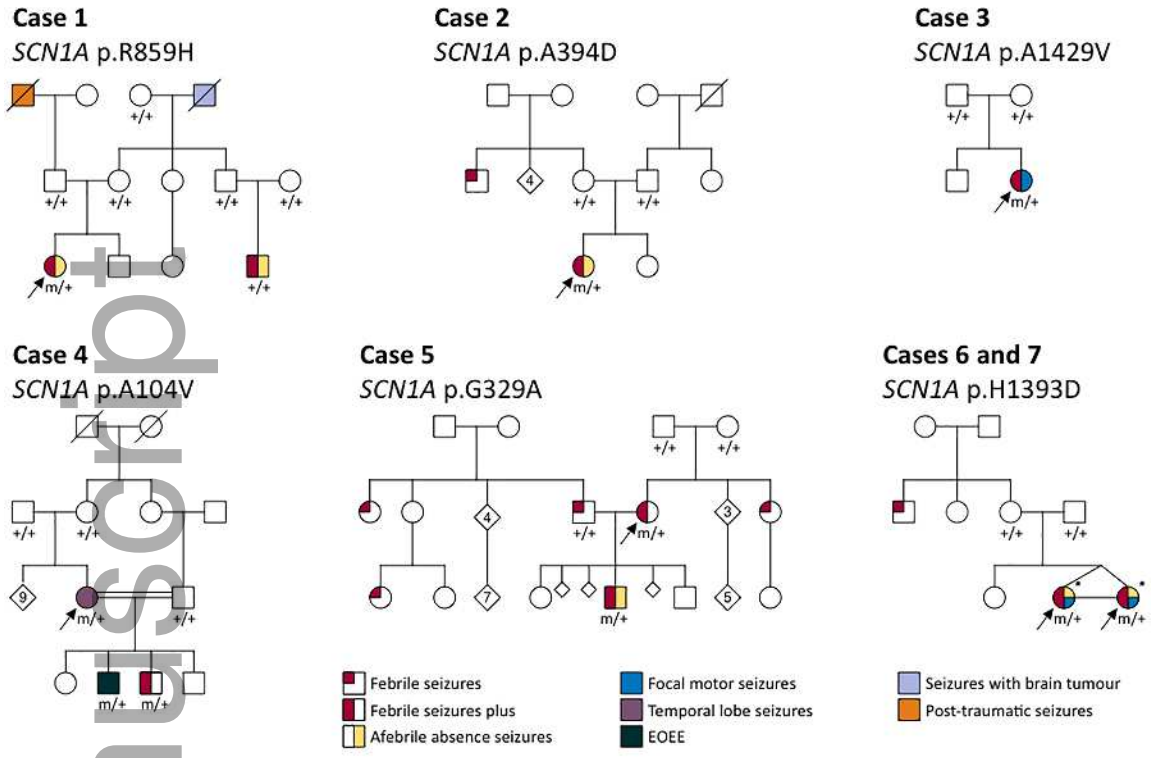
Table – Phenotypic Data. Clinical features of patients with de novo SCN1A mutations and non-DS GEFS+ phenotype. * Cases 6 and 7 are monozygotic twins.

Abbreviations: A = Absence; ASD = Autistic Spectrum Disorder; CAN = Medical cannabis; CBZ = Carbamazepine; CLB = Clobazam; ETX = Ethosuximide; F-EG = Focal with Evolution to Generalized Convulsions; FS = Febrile Seizures; FS+ = Febrile Seizures Plus; FSIQ = Full Scale Intelligence Quotient; GF = Grandfather; GTC = Generalized Tonic-Clonic; H = Hemiclonic; LCM = Lacosamide; LEV = Levetiracetam; LTG = Lamotrigine; M = Myoclonic; MRI = Magnetic Resonance Imaging; PB = Phenobarbital; Sz = Seizure; TLE = Temporal Lobe Epilepsy; TPM = Topiramate; VPA = Valproate; ZNS = Zonisamide.

#/ Sex/ Age	Seizure Onset	Fever-Induced Seizure?	Seizure Types	Development	Brain MRI	Family History	Phenotype	Medication Response	SCN1A Mutation
1/F/28 y	11 m	Y	GTC, A	Mild learning difficulties	Unusual right frontal gyral pattern.	- Paternal GF post-traumatic sz - Maternal GF sz with brain tumour	FS+ with absence	Partial sz control on TPM and VPA. CBZ and LTG ineffective.	c.2576G>A p.R859H
2/F/13 y	5 m	Y	GTC, A	Mild learning difficulties, ASD	Mild prominence of lateral ventricles.	- Maternal uncle FS	FS+ with absence	Sz controlled on VPA but recurred following wean. Sz now controlled on	c.1181C>A p.A394D

								LEV.	
3/F/19 y	6 m	Y	GTC, H, F-EG	Mild learning difficulties. FSIQ 88.	Pineal cyst.	- No FS or epilepsy	FS+ with focal motor seizures	Partial sz control with VPA and LEV. Worsened with LTG.	c.4286C>T p.A1429V
4/F/33 y	5 y	N	F-EG	Normal	Not performed.	- Son severe infantile EE - Son FS+	TLE	Ongoing sz on PB and CBZ.	c.311C>T p.A104V
5/F/46 y	9 m	Y	GTC	Normal	Not performed.	- Son FS+ with absence and myoclonic sz	FS+	Never on anti-epileptic drugs.	c. 986G>C p.G329A
6*/F/7 y	16 m	Y	H, F-EG, A	Language impairment, working memory deficits	Normal.	- Maternal uncle possible FS	FS+ with focal motor and absence	Ongoing sz on ETX, ZNS, TPM, VPA, CLB, CAN, LTG.	c.4177C>G p.H1393D
7*/F/7 y	16 m	Y	H, F-EG, A	Language impairment, working memory deficits	Not performed.	- Maternal uncle possible FS	FS+ with focal motor and absence	Ongoing sz on ETX, ZNS, TPM, VPA, CLB, LCM, LEV, CAN,	c.4177C>G p.H1393D

								LTG.	



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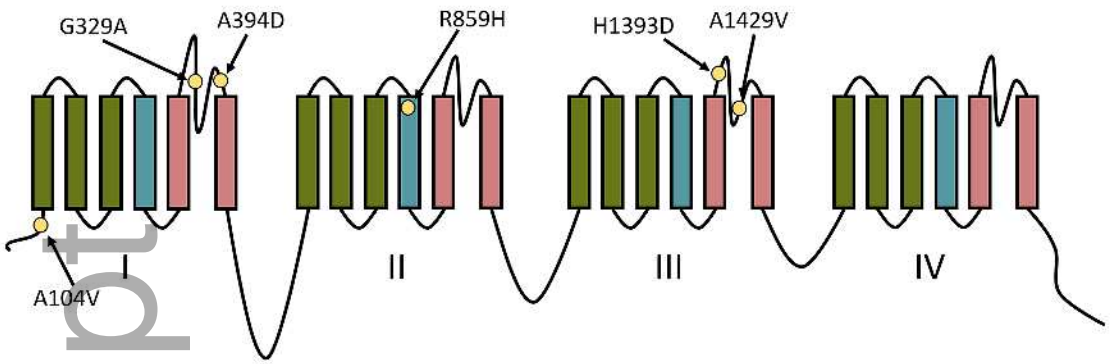

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