De-novo mutations of the sodium channel gene *SCN1A* in alleged vaccine encephalopathy: a retrospective study

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See Reflection and Reaction
page 465

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Summary

Background **Vaccination, particularly for pertussis, has been implicated as a direct cause of an encephalopathy with refractory seizures and intellectual impairment. We postulated that cases of so-called vaccine encephalopathy could have mutations in the neuronal sodium channel α1 subunit gene (*SCN1A*) because of a clinical resemblance to severe myoclonic epilepsy of infancy (SMEI) for which such mutations have been identified.**

Methods **We retrospectively studied 14 patients with alleged vaccine encephalopathy in whom the first seizure occurred within 72 h of vaccination. We reviewed the relation to vaccination from source records and assessed the specific epilepsy phenotype. Mutations in *SCN1A* were identified by PCR amplification and denaturing high performance liquid chromatography analysis, with subsequent sequencing. Parental DNA was examined to ascertain the origin of the mutation.**

Findings ***SCN1A* mutations were identified in 11 of 14 patients with alleged vaccine encephalopathy; a diagnosis of a specific epilepsy syndrome was made in all 14 cases. Five mutations predicted truncation of the protein and six were missense in conserved regions of the molecule. In all nine cases where parental DNA was available the mutations arose de novo. Clinical-molecular correlation showed mutations in eight of eight cases with phenotypes of SMEI, in three of four cases with borderline SMEI, but not in two cases with Lennox-Gastaut syndrome.**

Interpretation **Cases of alleged vaccine encephalopathy could in fact be a genetically determined epileptic encephalopathy that arose de novo. These findings have important clinical implications for diagnosis and management of encephalopathy and, if confirmed in other cohorts, major societal implications for the general acceptance of vaccination.**

Introduction

The sudden occurrence of seizures and developmental regression after vaccination in previously healthy infants led to the implication of a causal link, especially with pertussis vaccination.[1,2] Extensive debate ensued, but subsequent epidemiological studies did not lend support to the view of a causal association between immunisation and permanent brain damage.[3–8] In individual cases, however, the perception of causality can be difficult to challenge, especially if no alternative cause is identified, and has led to successful litigation. Public interest in this issue is high with a vocal minority urging avoidance of vaccination,[9] with the grave consequence of a potential resurgence of preventable serious childhood illnesses.[10] This issue is difficult to clarify largely because the diagnostic features of vaccine encephalopathy have never been defined. Reported cases have an apparent temporal relation to vaccination (varying from <1 day to 14 days) and typically have multiple seizure types with developmental arrest or regression.[2,3,5,8,11–16]

There are various causes of seizures and developmental regression in infancy, some of which have been previously misdiagnosed as vaccine encephalopathy.[17] A particular epilepsy syndrome, severe myoclonic epilepsy of infancy (SMEI), has become increasingly recognised. SMEI begins in the first year of life in previously healthy children. Hemiclonic seizures, which may be long lasting, are characteristic and can be associated with fever. Myoclonic, absence, tonic–clonic, and partial seizures also occur. The epilepsy is refractory and developmental regression ensues.[18,19] The syndrome is associated with more than 100 different mutations in the neuronal sodium channel α1 subunit gene *SCN1A*. Most cases of SMEI have such mutations, although the exact percentage is still debated. Around half the mutations truncate the protein and about 95% are de novo.[20–27]

We noted a similarity between the clinical pattern of SMEI and alleged cases of vaccine encephalopathy. Thus, we postulated that *SCN1A* mutations might underlie such cases where the physician or family believed that vaccination was causal. This finding would imply that the encephalopathy was not fundamentally caused by vaccination, but was due to a genetically determined, age-specific, epileptic encephalopathy.

Methods

Patients

This retrospective study of post-vaccination cases was nested within a larger study of 96 patients with unexplained encephalopathies and seizures beginning in the first year of life. We recruited participants from child neurologists around Australia and New Zealand during 2002 and 2003 for whom clinical details and DNA were obtainable and other causes of epileptic encephalopathies (perinatal,

post-traumatic, post-infectious, metabolic, and structural, etc) were excluded by appropriate metabolic and imaging studies. A few referrals were also accepted from outside Australasia. The study was approved by the Human Research Ethics Committee of Austin Health. Written informed consent was obtained from parents, guardians, or the appropriate government authority.

Cases were systematically classified on the basis of an exhaustive review of medical records from child neurologists, paediatricians, hospitals, and other treating doctors. Source records from initial medical presentations were sought to determine the precise onset details relative to vaccination. No specific neurological phenotype has been described for vaccine encephalopathy, so all cases were coded as vaccine encephalopathy when a relation to vaccination had been previously claimed and our review showed that the first seizure occurred within 72 h of vaccination. The time interval has no agreed definition, but on the basis of the published work we selected the time frame of documented seizure onset within 72 h of vaccination.[3,5,10,12,14,15]

All patients had epileptic encephalopathy (refractory seizures and developmental slowing); febrile seizures and other benign epilepsies were excluded. Epileptic seizures and epilepsy syndrome were diagnosed according to the International League Against Epilepsy classifications.[19,28] For this study, SMEI was diagnosed if all the following characteristics were present: onset in the first year with hemiclonic or generalised seizures; previous normal development; evolution of myoclonic seizures and generalised spike-wave discharges; and subsequent neurological deterioration. In Lennox-Gastaut syndrome, tonic seizures, atypical absences, and slow spike-wave on EEG were regarded as characteristic. Lennox-Gastaut syndrome can evolve from West syndrome with infantile spasms and hypsarrhythmia. The term borderline SMEI (SMEB), introduced by Japanese authors,[18,26] was used for cases without key features of SMEI (eg, lack of generalised spike-wave discharges, lack of myoclonus, few or atypical seizure types).

Procedures

After clinical classification of the epilepsy syndrome, molecular analysis was done on genomic DNA extracted from patients' venous blood samples. All 26 exons of *SCN1A* were PCR amplified with flanking intronic primers and standard PCR conditions (primers available on request). PCR fragments were heat denatured at 95°C for 4 min and slowly cooled to room temperature before being analysed by denaturing high-performance liquid chromatography (dHPLC) on the WAVE 3500HT instrument (Transgenomic, NE, USA). Amplicons showing altered dHPLC chromatogram patterns compared with normal control DNA were sequenced from independent PCR products in both directions on an ABI 3700 sequencer (Applied Biosystems, CA, USA). Numbering of each mutation was taken from the start codon ATG of the full length *SCN1A* isoform sequence (Genbank accession number AB093548). In cases where a mutation was identified, the parents' DNA (if available) was checked for the mutation by direct sequencing.

Sequence changes were identified as mutations rather than as normal polymorphisms if they were not reported as common variants and they resulted in the generation of stop codons or deletions or, for missense mutations, if they resulted in a non-conservative amino-acid change and arose de novo, if parental DNA was available. Specific missense mutations were further validated by excluding them with dHPLC screening from a panel of anonymous Australian blood donors used as the control population.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

14 patients were identified for whom vaccination had been judged as causative of the epileptic encephalopathy and our review confirmed seizure onset within 72 h of vaccination. The patients were aged 2·5–47 years at the time of study (mean 12 years [SD 11]). They were 2–11 months old (5·4 months [2·6]) at the onset of the illness, which followed vaccination by 1–48 h (22 h [15 h]). The vaccines included pertussis in all cases (table).

The first seizure was described as hemiclonic (n=5), generalised clonic or tonic–clonic (n=6), infantile spasms (n=1), tonic (n=1), and unclassified (n=1). The first seizure was definitely associated with fever (>38°C) in five patients, six were afebrile, and in three the temperature was not recorded. Status epilepticus (seizures lasting ≥30 min) occurred at presentation in six cases. All cases had severe epilepsy with multiple seizure types and intellectual disability. Our review of the subsequent clinical course led to diagnosis of SMEI in eight patients, SMEB in four, and Lennox-Gastaut syndrome in two. In the two patients with Lennox-Gastaut syndrome, spasms and hypsarrhythmia occurred early, representing the known evolution from West syndrome. MRI showed no focal lesions and no evidence of destructive or inflammatory processes; scans in all cases were either normal (n=8) or showed varying degrees of diffuse atrophy and delayed myelination (n=6).

Molecular genetic analysis showed heterozygous mutations of *SCN1A* in 11 of 14 cases. These mutations were predicted to lead to truncation of the protein in five cases (three frameshift and two non-sense mutations); the other six were missense mutations (figure).[29,30] A display of evolutionary conservation of the residues where the mutations were found is shown in the webfigure. None of the six missense mutations were identifed in the blood donor control population; a

See Online for webfigure

	Age at study (years)	Age at onset (months)	Seizure onset post vaccination (h)	Vaccine type	First seizure			Later seizures	Epilepsy syndrome	*SCN1A* mutation	De-novo mutation
					Febrile	Status epilepticus	Seizure type				
1	17·5	8	24	2nd TA	N	N	Hemiclonic	Ab, At, H, T, GTCS, SE	SMEI	Frameshift C1354fsX1359	Y
2	2·5	2·5	24	1st TA	Y	N	Hemiclonic	Ab, At, H, M, T, CPS	SMEI	Missense R946H	Y
3	5	3	5	3rd TA	Y	Y	Hemiclonic	H, F, GTCS, SE	SMEB	Frameshift K1077fsX1079	Y
4	4·5	7	48	3rd TA	N	Y	Hemiclonic	Ab, At, H, M, GTCS,	SMEI	Nonsense R1407X	Y
5	4	6	12	3rd TA	N	Y	GC	Ab, F, M, GTCS, SE	SMEI	Missense R1645Q	Y
6	12	3	24	1st TA	Y	Y	GCS	H, M, GCS, GTCS, SE	SMEI	Missense E1238D	Unknown
7	6·5	2	9	1st TA	N	N	GC	Ab, H, M, T, GTCS, SE,	SMEI	Frameshift N1509fsX1511	Y
8	13·5	6	6	3rd TA	N	Y	GTCS	F, M, SGTCS, SE	SMEB	Missense C1396G	Y
9	4·5	7	24	3rd PV	Y	Y	Hemiclonic	H, M, SGTCS, SE,	SMEI	Missense Y413N	Y
10	47	6	24	1st TA	Unknown	Unknown	Unknown	At, F, M, GTCS, SE,	SMEB	Nonsense W384X	Unknown
11	8	4	36	2nd TA	Unknown	N	GCS	F, M, GTCS, SE,	SMEI	Missense F403L	Y
12	16·5	11	24	3rd TA	Y	N	GTCS	Ab, At, M, Sp, GTCS	SMEB	None detected	NA
13	13·5	7	1	3rd TA	Unknown	N	Spasms	Sp, T, At, M, GTCS, SE,	LGS	None detected	NA
14	14·5	2·5	48	1st TA	N	N	Tonic	Sp, T, F, M, GTCS, SE	LGS	None detected	NA

TA=triple antigen (diptheria, pertussis, tetanus); PV=pentavalent vaccine (diptheria, pertussis, tetanus, inactivated polio, and haemophilus); GTCS=generalised tonic-clonic seizures; GCS=generalised clonic seizures; GC=generalised convulsion (uncertain if tonic-clonic or clonic); M=myoclonic seizures; Ab=absences; At=atonic; SE=status epilepticus; CPS=complex partial seizures; H=hemiclonic; SGTCS=secondarily generalised tonic-clonic seizures; F=focal seizures; Sp=spasms; T=tonic; SMEI=severe myoclonic epilepsy of infancy; SMEB=borderland SMEI; LGS= Lennox-Gastaut syndrome; NA=not applicable.

Table: **Clinical characteristics of 14 patients with alleged vaccine encephalopathy**

minimum of 130 and maximum of 149 control samples were successfully screened for each mutation. In nine of the 11 patients with *SCN1A* mutations for whom samples from both parents were available, the mutations were absent in parental DNA and thus arose de novo. In patient six, parental DNA was not available. In patient ten, the mother was tested and did not have the mutation and the father was deceased. This patient had a deceased brother who was also said to have seizures beginning after vaccination, but medical records were destroyed and this could not be verified. Correlation of the clinically diagnosed phenotype with the molecular analyses showed that the sodium channel mutations were confined to the cases diagnosed as SMEI (eight of eight cases) or SMEB (three of four cases) and were absent in patients who had Lennox-Gastaut syndrome.

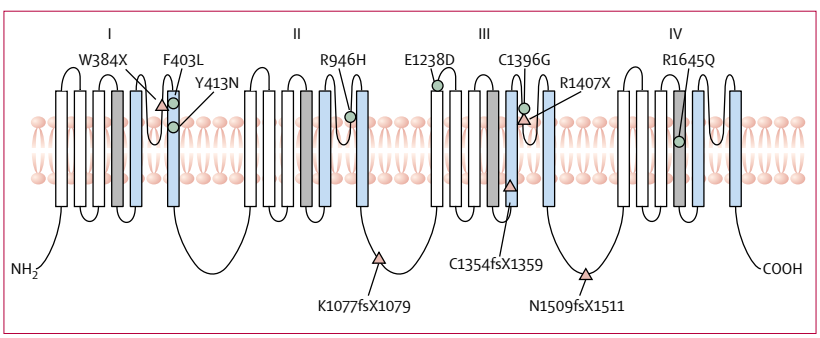

Figure: **Schematic representation of the proposed structure of SCN1A protein**
The protein comprises four homologous domains (I–IV), each with six transmembrane segments. Segments five and six (blue) form the ion channel pore and segment four (grey) is the voltage sensor.[29] The relative location of the six missense mutations (green circles) and five mutations possibly causing protein truncation (pink triangles) in the 11 cases with alleged vaccine encephalopathy are shown. The missense mutations predominantly occurred in the exons coding for the pore forming segments, as previously described in SMEI.[30]

Discussion

In this retrospective cohort of unexplained encephalopathies in the first year of life, 14 patients were judged by clinicians and families to have a vaccine encephalopathy and had documented seizure onset within 72 h of vaccination. With careful electroclinical analysis, we established that the clinical syndrome was SMEI in eight patients and the related syndrome SMEB in four. Eleven patients were shown to have mutations in the sodium channel gene *SCN1A*, which is now a well established finding in SMEI.[20–27] Two of the mutations have been reported before in association with SMEI or SMEB (R946H, R1407X) after nomenclature were standardised to the full length isoform given in Genbank accession number AB093548.[21,26,27] The mutations led to truncation of the protein in five cases, consistent with previous reports of truncation mutations being responsible for about half of SMEI cases with *SCN1A* mutations.[27] The other six patients had missense mutations. All are likely to be pathogenic as they have not been reported in control populations nor were they found in our controls, and the observed missense mutations affect highly conserved amino-acid sites (data not shown), are in regions where SMEI mutations have been previously described,[30] and arose de novo in all cases where both parents were tested.

There is no satisfactory case definition of the specific neurological phenotype in vaccine encephalopathy; indeed, even the temporal relation to immunisation is loose with cases described with onset of symptoms from less than 1 day to 14 days post vaccination.[2,3,5,8,11–16] Although we showed that SMEI or SMEB were important phenotypes in vaccine encephalopathy we were surprised

that no less than 12 of 14 patients were diagnosed as such with careful phenotypic analysis. We do not know if this finding is representative of cases in other centres, but previous reports of seizures in SMEI being associated with vaccination as well as fever lend support to our findings.[5,31,32] The multiple seizure types in SMEI and SMEB can make diagnosis difficult for observers unfamiliar with these disorders; patients can be judged as having an unclassified form of epilepsy and intellectual disability. The discovery of *SCN1A* mutations has led to improved awareness and diagnosis of these severe infantile encephalopathies.[33]

Scientific and medicolegal controversy of vaccine encephalopathy has spanned seven decades.[1,4] We suspect that the nature of cases has changed because of increasingly sophisticated clinical and neurological diagnoses and investigations. Some patients had coma at onset whereas others had seizures with subsequent regression. In the early research, detailed analysis led to the conclusion that some alleged cases were probably due to heterogeneous causes, including viral encephalitis and Reye's syndrome.[17] The molecular delineation of genetic encephalopathies with phenotypes of SMEI and SMEB now seems to be another major piece in the heterogeneous diagnostic puzzle of alleged vaccine encephalopathies.

The genetically determined epilepsy syndromes of SMEI and SMEB typically arise in association with de novo *SCN1A* mutations, presumably due to mutations in the gametes or in the very early post-fertilisation period.[20–24,26,27] In alleged vaccine encephalopathy the assumption of vaccination as a cause has been reinforced by the absence of a family history of severe epilepsy. Now, the molecular findings could explain the nature of the encephalopathy and the usual lack of family history since around 95% of mutations in SMEI occur de novo.[20–27]

SMEI often begins with febrile seizures and fever is frequently associated with seizures early in the clinical course. In the presence of *SCN1A* mutations, vaccination can still be argued to be a trigger for the encephalopathy, perhaps via fever or an immune mechanism. Our experimental design does not address this issue, but the role of vaccination as a significant trigger for the encephalopathy is unlikely for several reasons. First, although vaccination might trigger seizures as shown by the increased risk of febrile seizures on the day of triple antigen or MMR vaccination, there is no evidence of long-term adverse outcomes.[6–8] Second, less than half our patients had documented fever with their first seizure, which indicates that fever is not essential. Third, our neuroimaging data showed no evidence of an inflammatory or destructive process. Finally, truncation and missense mutations reported in conserved parts of *SCN1A* have not been found in many hundreds of healthy patients.[20,22,23,25,26] Thus, individuals with such mutations seem to develop SMEI or SMEB whether or not they are immunised in the first year of life. We do not think that avoiding vaccination, as a potential trigger, would prevent onset of this devastating disorder in patients who already harbour the *SCN1A* mutation.

The mechanism by which *SCN1A* mutations cause SMEI is unknown. Few causative mutations have so far been subjected to functional analysis, and the results are inconsistent; however, these mutations are presumed to cause abnormal neuronal excitability.[34,35] Studies of less severe mutations of *SCN1A* that cause milder phenotypes have also produced conflicting results dependent on the techniques and the model system investigated.[36,37] Definitive data from neuronal systems have yet to emerge. Moreover, because many of the mutations associated with SMEI cause truncation of the protein, these proteins are unlikely to be expressed at the cell surface; thus poorly understood changes to sodium-channel density, stoichiometry, and function might all contribute to the phenotypes observed. Further study in neuronal systems, and ideally whole animal models, is needed to clarify the complex functional effects of *SCN1A* mutations.

We did not find a molecular explanation for three patients with alleged vaccine encephalopathy. These could be chance associations of vaccination with other causes leading to the onset of encephalopathies. Other cases could be due to large deletions or undiscovered mutations in non-translated parts of the *SCN1A* gene or perhaps due to rare mutations in related genes, such as *GABRG2*[38] and *SCN2A*.[39]

Although epidemiological studies have cast doubts on the hypothesis of vaccination as a cause of encephalopathy,[4,7] families, the medical profession, and society remain difficult to reassure of the lack of causality in individual patients in whom vaccination and onset of encephalopathy were coincidental. For individual cases, this problem is particularly significant in the legal setting. For society, fear of adverse consequences of vaccination is a major factor in suboptimum immunisation rates. The identification of a genetic cause of encephalopathy in a particular child should finally put to rest the case for vaccination being the primary cause. Confirmation of our findings by others would be of value in determining their generalisability and the broad societal implications.

Cases of vaccine encephalopathy should be carefully assessed clinically for characterisitcs of SMEI or SMEB, and testing for *SCN1A* mutations should be considered. Correct diagnosis will reassure the family as to the true cause, remove the blame of having vaccinated the child, direct appropriate treatment, and allow realistic planning for prognosis. Specific treatment regimens for seizures in SMEI are emerging with controlled data showing the effectiveness of stiripentol,[40] and uncontrolled open studies suggesting avoidance of lamotrigine[41] and probable benefit of topiramate.[42] Medical and societal energies that have focused on the alleged association with vaccination need to be redirected towards the care of these severely handicapped individuals and towards novel approaches to treat and ultimately prevent these encephalopathies.

Contributors

SFB developed the hypothesis and wrote the first draft. Analysis of clinical data was done principally by IES, JMM, JTP, and SFB, and also by SMZ, ECW, and DSG. Molecular analysis was undertaken by LH, XI, and JCM. All authors critically revised the first draft and approved the final manuscript.

Conflicts of interest

SFB, IES, and JCM have received research support and honoraria from Bionomics Ltd. Bionomics Ltd has licensed a diagnostic test for *SCN1A* mutations.

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