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Missense mutations in sodium channel *SCN1A* and *SCN2A* predispose children to encephalopathy with severe febrile seizures

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ABSTRACT

Objective: Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) is a childhood encephalopathy following severe febrile seizures. The pathogenesis of AESD is considered to be fever-induced seizure susceptibility and excitotoxicity, which may be caused by sodium channel dysfunction in some cases. Here we studied whether mutations in genes encoding sodium channels, *SCN1A* and *SCN2A*, predispose children to AESD.
Methods: We recruited 92 AESD patients in a nationwide survey of acute encephalopathy in Japan from 2008 to 2011. We collected their genomic DNA samples, and sequenced the entire coding region of *SCN1A* and *SCN2A*.
Results: Five out of 92 patients (5.4%) had missense mutations either in *SCN1A* or *SCN2A*. After a preceding infection with fever, all the patients showed status epilepticus at the onset. Hemiconvulsion–hemiplegia was recognized in three patients during the acute/subacute phase. One patient had taken theophylline for the treatment of bronchial asthma just before the onset of AESD. Familial history was not remarkable except one patient with a *SCN1A* mutation (G1647S) whose mother had a similar episode of AESD in her childhood. A different substitution (G1674R) at the same amino acid position, as well as two other *SCN1A* mutations found in this study, had previously been reported in Dravet syndrome. Another *SCN1A* mutation (R1575C) had been detected in other types of acute encephahlitis/encephalopathy. One patient had *SCN2A* mutation, F328V, which had previously been reported in Dravet syndrome. Another *SCN2A* mutation, I172V, was novel. None of the patients were diagnosed with Dravet syndrome or genetic (generalized) epilepsy with febrile seizure plus in the following-up period.
Conclusions: Mutations in *SCN1A* and *SCN2A* are a predisposing factor of AESD. Altered channel activity caused by these mutations may provoke seizures and excitotoxic brain damage.

Introduction

During the acute phase of febrile diseases, some children develop repetitive or prolonged seizures, followed by severe impairment of consciousness. Several distinct syndromes have been described and characterized: fever-induced refractory epileptic encephalopathy in school-aged children (FIRES), idiopathic hemiconvulsion–hemiplegia syndrome (IHHS), and acute encephalopathy with biphasic seizures and late reduced diffusion (AESD). As a generic term to encompass these conditions, Nabbout proposed the term acute encephalopathy with

Abbreviations: AEIMSE, acute encephalopathy with inflammation-mediated status epilepticus; AESD, acute encephalopathy with biphasic seizures and late reduced diffusion; ANE, acute necrotizing encephalopathy; AERRPS, acute encephalitis with refractory, repetitive partial seizures; DS, Dravet syndrome; GEFS+, genetic (generalized) epilepsy with febrile seizure plus; BFNIS, benign familial neonatal-infantile seizures.

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inflammation-mediated status epilepticus (AEIMSE) (Nabbout et al., 2011). AESD is prevalent in Japan, affecting hundreds of children every year (Hoshino et al., 2012), whereas IHHS is encountered worldwide. When IHHS occurs during an infectious disease, it is regarded as a subgroup of AESD (Takanashi et al., 2006).

The main pathogenetic mechanism of AESD is considered to be excitotoxicity, based on the magnetic resonance spectroscopy findings showing an increase in glutamine/glutamate in the cerebral lesions (Mizuguchi et al., 2007; Takanashi et al., 2009). The genetic background of AESD remains to be elucidated. Polymorphisms in genes controlling neuronal excitability are candidates for risk factors of AESD. Recently, polymorphism of genes encoding adenosine receptor 2A (*ADORA2A*) and carnitine palmitoyltransferase II (*CPT2*) has been identified as genetic predisposition for AESD (Shinohara et al., 2013, 2011). However, some AESD patients have no such polymorphism, suggesting the involvement of other genes. Voltage-gated sodium channels are essential for neuronal excitability. We hypothesized that intrinsic susceptibility to seizures may predispose children to AESD and focused on the *SCN1A/SCN2A* genes, whose mutations are known to cause genetic epilepsy characterized by hyperthermia-induced seizures. Mutations in genes encoding a voltage-gated sodium channel subunit protein, *SCN1A*, cause a variety of genetic epileptic syndromes including Dravet syndrome (DS, severe myoclonic epilepsy of infancy) and genetic (generalized) epilepsy with febrile seizures plus (GEFS+) (Escayg et al., 2000; Claes et al., 2001; Escayg and Goldin, 2010). Recently, we and other researchers have reported that some patients with various types of acute encephalopathy have truncation or missense mutations of *SCN1A* (Ohmori et al., 2008; Sakakibara et al., 2009; Takayanagi et al., 2010; Kobayashi et al., 2010; Saitoh et al., 2012). *SCN2A* mutations cause benign familial neonatal-infantile seizures (BFNIS) (Heron et al., 2002; Berkovic et al., 2004), which are usually inherited from an affected parent. Several de novo *SCN2A* mutations have been reported in severer phenotypes such as DS (Shi et al., 2009) and early-onset epileptic encephalopathy including Ohtahara syndrome (Nakamura et al., 2013). On the other hand, missense *SCN2A* mutations have recently been identified in a patient with acute encephalitis with refractory, repetitive partial seizures (AERRPS), a typical syndrome of AEIMSE (Kobayashi et al., 2012), and in a patient with recurrent acute encephalopathy (Fukasawa et al., 2015).

To elucidate the genetic basis of AESD, we conducted an analysis of the *SCN1A* and *SCN2A* genes. This is the first report that evaluated the frequency of sodium channel mutations in a large number of patients with AESD and clarified them as a genetic risk factor.

Methods

Subjects

We recruited patients with AESD from hospitals in Japan during 2008–2011 based on the diagnostic criteria (Hoshino et al., 2012). It was regarded as 'definite' when both the characteristic clinical course (biphasic seizures) and CT/MRI findings (delayed appearance of cerebral cortical edema, distribution of lesions showing lobar or hemispheric involvement and peri-Rolandic sparing, and restricted diffusion of the subcortical white matter (so-called bright tree appearance) were present, 'probable' when either clinical or CT/MRI features were present (Saitoh et al., 2015). Patients diagnosed with definite or probable AESD were included in this study. Ninety-two Japanese patients, 42 male and 50 female aged from five months to six years and eleven months (median, two year and one month) participated in this study. The summary and details of clinical data are shown in Table 1 and Supplementary table, respectively. All patients had their first convulsion, mostly status epilepticus, within 24 h from the onset of fever, followed by impairment of consciousness that improved on the second day in most cases. On the fourth to sixth day of illness, there was a recurrence of convulsions or a cluster of partial seizures, followed again by impairment of consciousness in most cases (82%, Table 1). Such a biphasic clinical course is one of the characteristics of AESD. Typically, cranial MRI was normal on the first to second day of illness, but showed lesions in the cerebral subcortical white matter on the third to ninth day. Pathogens of antecedent infections included human herpesvirus 6 (30 cases), influenza virus (8 cases), respiratory syncytial virus, rotavirus, adenovirus, mumps virus and *Mycoplasma pneumoniae*.

Table 1
Clinical characteristics of patients with AESD ($n = 92$).

	Patients (%)
Female	50 (54%)
Age	
0–6 months	1 (1%)
7–12 months	28 (30%)
13–24 months	32 (35%)
>24 months	31 (34%)
Pathogen of preceding infection	
Human herpesvirus-6	30 (33%)
Influenza virus	8 (9%)
Adenovirus	3 (3%)
Respiratory syncythial virus	2 (2%)
Mumps virus	1 (1%)
Varicella zoster virus	1 (1%)
Mycoplasma pneumoniae	1 (1%)
Others (not identified)	46 (50%)
Duration of first convulsion	
<15 min	19 (21%)
15–30 min	55 (60%)
>30 min	14 (15%)
Not recorded	4 (4%)
Biphasic clinical course	76 (82%)
MRI findings distribution of lesions	
Frontal	21 (23%)
Hemispheric	16 (17%)
Diffuse	34 (37%)
Others	18 (20%)
Not particular	2 (2%)
Not available	1 (1%)
Prognosis (Intellectual/motor)	
Full recovery	16 (17%)/29 (31%)
Mild disability	16 (17%)/21 (23%)
Moderate disability	10 (11%)/9 (10%)
Severe disability	30 (33%)/21 (23%)
Death	0 (0%)
Not determined	15 (16%)/7 (7%)
Not available	5 (6%)

Controls

To search the variants frequency in normal control population, we used Human Genetic Variation Browser (http://www.genome.med.kyoto-u.ac.jp/SnpDB (Japanese Genetic Variation Consortium, A reference database of genetic variations in Japanese population. in preparation) (Narahara et al., 2014) and Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.or) accessed on June 29, 2015.

Procedures

Peripheral blood samples were collected from the patients. Genomic DNA was extracted from the blood using standard protocols. All exons of *SCN1A* and *SCN2A* were polymerase chain reaction (PCR) amplified with flanking intronic primers and standard PCR conditions (Ohmori et al., 2002; Saitoh et al., 2012). PCR products