Rasmussen encephalitis associated with *SCN1A* mutation

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Abstract

Mutations in the *SCN1A* gene, encoding the neuronal voltage-gated sodium channel α1 subunit, cause SMEI, GEFS+, and related epileptic syndromes. We herein report the R1575C-*SCN1A* mutation identified in a patient with Rasmussen encephalitis. R1575C were constructed in a recombinant human SCN1A and then heterologously expressed in HEK293 cells along with the human β1 and β2 sodium channel accessory subunits. Whole-cell patch-clamp recording was used to define biophysical properties. The R1575C channels exhibited increased channel availability and an increased persistent sodium current in comparison to the wild-type. These defects of electrophysiological properties can result in neuronal hyperexitability. The seizure susceptibility allele may influence the pathogenesis of Rasmussen encephalitis in this case.

Introduction

Rasmussen encephalitis (RE) is an autoimmune-mediated brain disorder associated with intractable focal epilepsy, slowly progressive neurological deterioration and progressive unilateral hemispheric atrophy (Rasmussen et al., 1958). Serum samples of patients with RE contain antibodies to glutamate receptor GluR3, and immunization of animals with GluR3 induces a disorder resembling the human disease (Roger et al., 1994). Histopathological studies have shown an infiltration of T lymphocytes and microglia cells, astrocytosis and neuronal loss. A viral infection, humoral autoimmunity and T-cell mediated cytotoxicity have been postulated as the possible mechanisms of RE (Bien et al, 2005). Although the pathogenesis of RE has not yet been completely elucidated, it is widely accepted that environmental factors largely contribute to RE.

In contrast to RE, the inherited form of epilepsy is caused by genetic factors. Generalized epilepsy with febrile seizures plus (GEFS+) is a familial epilepsy syndrome characterized by heterogeneous phenotypes, including febrile seizures, febrile seizures plus, and mild to severe epilepsies (Scheffer and Berkovic., 1997). The *SCN1A* gene, which encodes the neuronal voltage-gated sodium channel $\alpha 1$ subunit, has been identified in patients with GEFS+, severe myoclonic epilepsy of infancy (SMEI, or Dravet syndrome) and related epileptic syndromes. Other undiscovered modifier genes

and environmental factors may lead to the wide spectrum of *SCN1A*-associated

epilepsies.

We herein report the *SCN1A*-R1575C mutation identified in a patient with

Rasmussen encephalitis. We found this mutation fortuitously in the process of the

SCN1A mutational screening of 98 patients having various types of epileptic syndromes.

The R1575C mutant channels transiently expressed in human embryonic kidney (HEK)

293 cells exhibited defects of the electrophysiological properties. Since *de novo*

mutations of the *SCN1A* gene have been reported in patients with alleged vaccine

encephalopathy (Berkovic et al., 2006), we should carefully assess this new aspect of

genetic-environmental interaction in an autoimmune-mediated brain disorder.

Materials and Methods

Patient A

The patient was a 10-year-old girl and her representative clinical data are shown in Fig. 1. Regarding the family history, a febrile seizure occurred in her paternal aunt and one of her maternal cousins, respectively (Fig. 1A). She was born after an uneventful 39-week-long pregnancy with a weight of 3422g.

She had febrile seizures at 1 year 1 month of age and 1 year 7 months of age. In the initial prodromal phase, her development and EEG were both age appropriate. Afebrile generalized tonic-clonic seizures (GTCS) began to occur weekly at 2 years of age. Her mental development delayed thereafter. Spikes were found in the left frontal region on EEG and valproate was started. She had varicella at 2 years 5 months of age. She has suffered from myoclonic seizures since 2 years 6 months of age. Her EEG showed many spike-and-wave complexes. A battery of examinations, including MRI, CT, single-photon emission computed tomography (SPECT) with ^{99m}Tc-ECD (ethyl cysteinate dimmer) (Fig.1 D-1), CSF, and analyses of chromosome, urinary organic acids and lysosomal enzymes, were all normal. The administration of clonazepam suppressed the myoclonic seizures and potassium bromide reduced the GTCS.

At 5 years of age, she had idiopathic thrombocytopenic purpura (ITP). She

frequently had focal motor seizures with asymmetric tonic posture. She had a mild gait abnormality with less movement in the right arm than in the left at around 5 years of age.

At 8 years of age, right hemiparesis was evident. EEG showed asymmetric slowing in the left hemisphere (Fig. 1B) and a cerebral atrophy of the left hemisphere was found in MRI (Fig. 1C). She was suspected to have RE, so autoantibodies against GluR2 and GluR3 in the serum were analyzed using ELISA. Autoantibodies against GluR3 were detected. Her condition has since deteriorated and her IQ (Tanaka-Binet) was 29 at 9 years of age. SPECT showed hypoperfusion in the left temporoparietal lobes (Fig. 1 D-2).

Molecular genetic analysis

SCN1A mutations were analyzed by the previously reported methods (Ohmori et al., 2002).

The study was approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences. Written informed consent was obtained from the parents.

Mutagenesis and heterologous expression of SCN1A

Mutagenesis of full-length human *SCN1A* cDNA was performed as described previously (Lossin et al., 2002). In all of the experiments, recombinant *SCN1A* was heterologously coexpressed with the human $\beta 1$ and $\beta 2$ accessory subunits in the HEK293 cells.

Electrophysiology and data analysis

Whole-cell voltage-clamp recordings were used to characterize the functional properties of the wild type (WT)-*SCN1A* and R1575C mutant sodium channel, as described previously (Ohmori et al., 2006). Sodium channel currents were recorded at room temperature, 24–72 h after transfection. Patch pipettes were pulled (1.3- 1.9MΩ) by multistage P-97 Flaming-Brown micropipette puller. The pipette solution consisted of (in m*M*): 110 CsF, 10 NaF, 20 CsCl, 2 EGTA, 10 HEPES, with a pH of 7.35 and osmolarity of 310 mOsmol/kg. The bath solution consisted of (in m*M*): 145 NaCl, 4 KCl, 1.8 CaCl$_2$, 1 MgCl$_2$, 10 HEPES, with a pH of 7.35 and osmolarity of 310 mOsmol/kg. Specific voltage-clamp protocols assessing channel activation, steady-state fast inactivation, recovery from fast inactivation and persistent current were used as described previously (Ohmori et al., 2006). The results are presented as the mean ±

SEM, and statistical comparisons were made in reference to WT-SCN1A by using the

unpaired Student's *t*-test. The data analysis was performed using the Clampfit 8.2

(Axon Instruments, Union City, CA, U.S.A.) and OriginPro 7.5 (OriginLab,

Northampton, MA, U.S.A.) software programs.

Results

SCN1A mutational analysis

The novel R1575C mutation was identified in patient A and her unaffected father

(Fig. 1E). No other family members agreed to undergo any genetic tests. We did not

find the R1575C mutation in the healthy individuals (n = 96).

Electrophysiological properties

We examined the R1575C mutation for functional defects by using a heterologous

expression system. Fig. 2A illustrates the representative whole-cell currents recorded

from the HEK293 cells expressing WT-*SCN1A*, or R1575C. Cells expressing the

R1575C mutant exhibited a voltage-dependent inward current which resembled the cells

transfected with WT channels. The inactivation proceeded in a rapid biexponential

manner, with no apparent difference between WT and R1575C (Fig. 2B). However, the

presence of a persistent sodium current in R1575C was observed. Fig. 2C illustrates the

representative tetrodotoxin (TTX)-subtracted current traces for both WT-*SCN1A* and

R1575C in response to a 100-ms voltage step to−10 mV from a holding potential of

−120 mV. The cells expressing R1575C exhibited a significantly greater persistent

current which was expressed as percentage of the peak current in comparison to

WT-*SCN1A* (Fig. 2D).

The current density, voltage-dependence channel activation, inactivation and

recovery from fast inactivation were examined (Fig. 3). R1575C showed no difference

for the current density, voltage-dependence channel activation and recovery from fast

inactivation in comparison to WT. R1575C displayed the most significant defect in fast

inactivation. R1575C exhibited a slight depolarizing shift in half-maximal steady-state

inactivation (Fig. 3C).

Discussion

The early clinical features of febrile and afebrile convulsions and family history are

likely to be caused by the *SCN1A* mutation. The later clinical features also met the

diagnostic criteria of RE (Bien et al., 2005). She had focal seizures, slowly progressive

right hemiparesis, mental deterioration, right hemispheric slowing with epileptiform

activity on EEG, and right hemispheric atrophy with a gray matter T2/FLAR hyperintense signal. There is the possibility that an association between the two conditions may be coincidental.

Regarding the etiology of RE, a direct viral infection, humoral autoimmunity and T-cell mediated cytotoxicity have been postulated as the possible mechanisms (Bien et al., 2005). The association of RE plus a low grade tumor, cortical dysplasia, tuberous sclerosis, or vascular abnormalities have also been described (Bien et al., 2005). More than 40% of patients with RE have prior infections, inflammatory episodes or vaccinations before the onset of epilepsy (Takahashi et al., 2006). Patient A had varicella and ITP around the periods of worsening epilepsy. However, we could not identify the exact influence of infectious diseases and ITP because she originally had epileptic seizures and the course of the neurological deterioration was slowly progressive.

The R1575C-*SCN1A* mutation in patient A was carried by her unaffected father. However, it was not detected in healthy individuals (n = 96). An electrophysiological study showed that the R1575C channels had increased channel availability and increased persistent sodium current, which can lead to neuronal hyperexcitability. Increased persistent current is a common functional abnormality in *SCN1A*-associated

epileptic syndromes   (Lossin et al., 2002, Ohmori et al., 2006). Taken together, these findings therefore suggest that R1575C may be a seizure susceptibility allele. The seizure susceptibility allele may influence the pathogenesis of Rasmussen encephalitis.

The spectrum of *SCN1A*-associated brain disorders has been expanding. The mildest form is simple febrile seizure (Mantegazza et al., 2005) and the most severe form is SMEI. *SCN1A* mutations have also been identified in familial hemiplegic migraines (Dichgans et al., 2005) and autism (Weiss et al., 2003). Moreover, differences in clinical severity among individuals carrying the same mutation are often observed in the GEFS+ family and the SMEI family. Factors that are believed to contribute to variable expressivity include intrinsic stochastic variability during the development of neuronal connectivity in the CNS, the accumulation of somatic mutations over a patient's lifetime, environmental insults, and differences in genetic backgrounds and modifier genes. Although the presence of such a susceptibility allele alone is insufficient to cause RE, it may exacerbate epileptic seizures when environmental factors are added. The combined effect of the *SCN1A* mutation, yet undiscovered susceptibility genes and the autoimmune-response caused by infection or vaccination should be carefully considered.

Acknowledgements

Disclosure of conflicts of interest

We have no conflicts of interest.

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Figure legends

Figure 1. Clinical data of patient A.

(**A**) The pedigree of patient A. (**B**) Awake EEG record at 8 years 6 months of age. The background activity is slow in the left hemisphere with irregular spike-and-wave complexes. (**C**) MRI showed a left hemispheric mild cerebral atrophy with a gray matter T2/FLAR hyperintense signal at 8 years 10 months of age (C-1, T1-weighted image; C-2, T2-weighted image, C-3, FLAIR). (**D**) SPECT was unremarkable at 2 years 10 months of age (D-1) and showed hypoperfusion in the left parietotemporal lobes at 9 years 8 months of age (D-2). (**E**) A mutation analysis of the SCN1A gene. Patient A and her father had the R1575C mutation. Numbering of amino acids is given in GenBank accession number AB093548.

Figure 2. Sodium currents were recorded from HEK293 cells expressing either WT-SCN1A or R1575C. (**A**) Representative whole-cell sodium currents. The currents were activated by voltage steps to between −80 and +60 mV in 20-mV increments from a holding potential of −120 mV. (**B**) Voltage dependence of fast inactivation time constants for WT-SCN1A and R1575C. The fast and slow time constants were plotted

versus voltage. The whole-cell current inactivation of R1575C is similar toWT-SCN1A.

(**C**) Representative tetrodotoxin-subtracted whole-cell sodium currents recorded from

HEK293 cells expressing WT-SCN1A or R1575C. Each current trace has been

normalized to its peak sodium current. (**D**) Average persistent sodium current expressed

as a percentage of the peak current for WT-SCN1A ($0.38\% \pm 0.10\%$; n = 7) and

R1575C ($2.12\% \pm 0.68\%$; n = 7; $p = 0.027$). R1575C exhibited a significantly increased

persistent current in comparison to WT-SCN1A ($p < 0.05$).

Figure 3. Electrophysical properties of R1575C. (**A**) Peak current density of whole-cell

currents elicited by test pulses to various potentials and normalized to cell capacitance.

Current density of R1575C is similar to WT-SCN1A. (**B**) Voltage dependence of

channel activation measured during voltage steps to between -80 and $+20$ mV from a

holding potential of -120 mV. Half-maximal activation occurred at -25.9 ± 1.5 mV with

a slope factor of -6.2 ± 0.3 (n=17) for WT-SCN1A and at -24.6 ± 1.1 mV with a slope

factor of -6.2 ± 0.2 (n=12) for R1575C. (**C**) Voltage dependence of fast inactivation

measured at -10 mV in response to a 100-ms prepulse voltage step to between -140 and

-10 mV from a holding potential of -120 mV. The membrane potentials for

half-maximal inactivation and slope factors were as follows: WT, -62.1 ± 1.3 mV and

-5.8 ± 0.2, n=15; R1575C, -58.5 ± 1.0 mV (*p*=0.043) and -6.2 ± 0.3, n=12. (**D**)

Time-dependent recovery from fast inactivation measured at −10 mV between 1 and

3,000 ms immediately after a 100-ms prepulse voltage step to −10mV from a holding

potential of −120 mV. The time constants and fractional amplitudes were as follows:

WT, τ_f =1.8 ± 0.1 ms (82% ± 1%), τ_s =40.3 ± 5.4 ms (18% ± 1%), n=14; R1575C,

τ_f =1.8 ± 0.2 ms (79% ± 2%), τ_s =40.8 ± 4.0 ms (21% ± 2%), n=13. There was no

statistically significant difference between WT and R1575C.

A

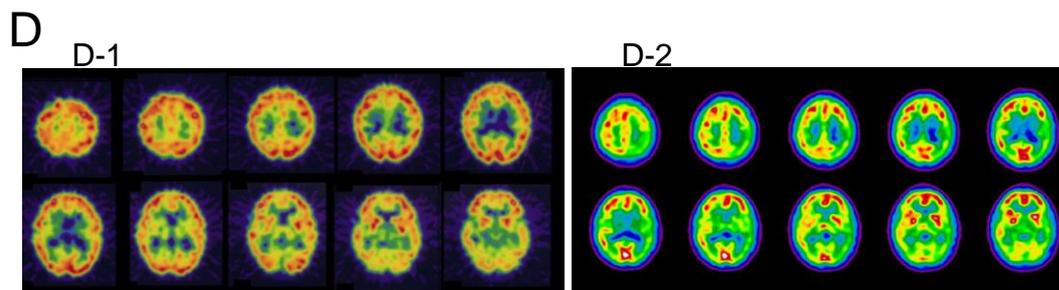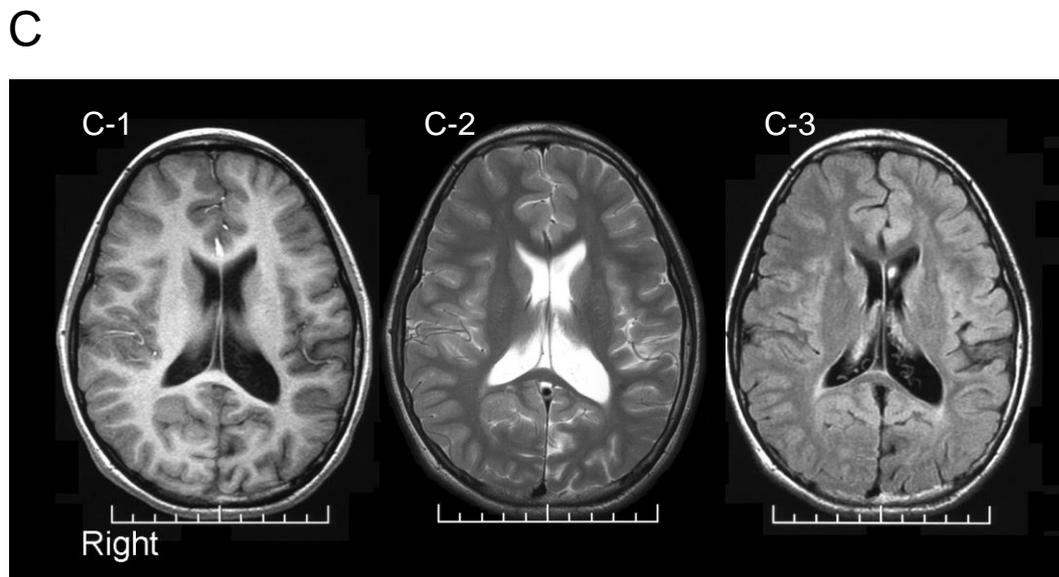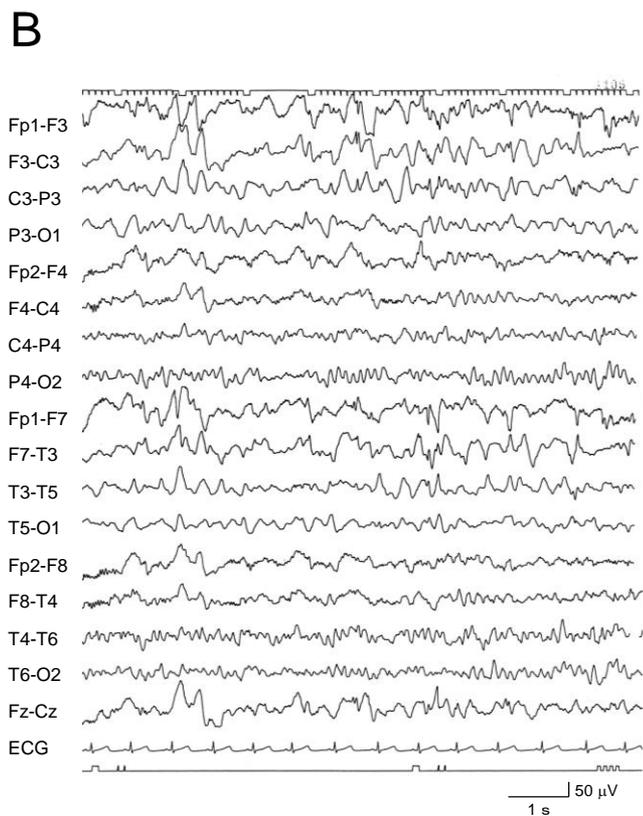● Rasmussen □ Febrile seizures * R1575C positive

C-1   C-2   C-3

Right

B

Fp1-F3
F3-C3
C3-P3
P3-O1
Fp2-F4
F4-C4
C4-P4
P4-O2
Fp1-F7
F7-T3
T3-T5
T5-O1
Fp2-F8
F8-T4
T4-T6
T6-O2
Fz-Cz
ECG

50 µV
1 s

D

D-1   D-2

E   I L S C I N L   I L S R I N L   I L S C I N L
ATT TTG T CA TGC AT C AAT CTG   ATT TTG T CA CGC AT C AAT CTG   ATT TTG T CA TGC AT C AAT CTG
          C   C   C

Father   Mother   Patient A
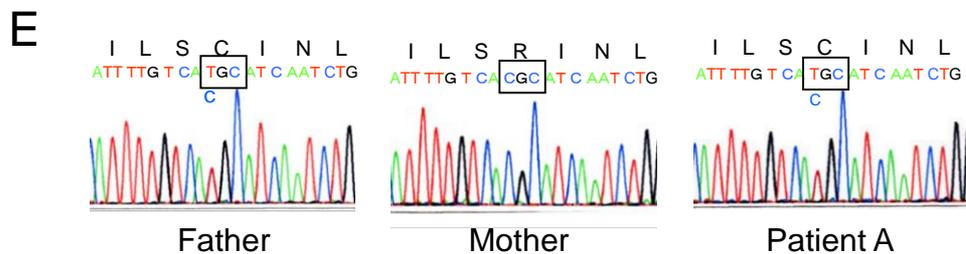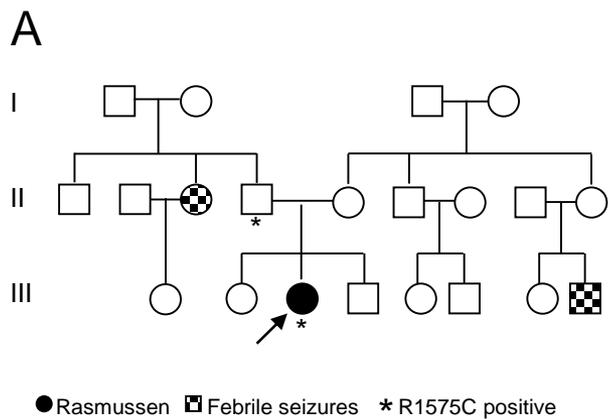
Figure 1.

Figure 2.

Figure 3.