Association between *SCN1A* polymorphism and carbamazepine-resistant epilepsy

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The *SCN1A* gene encodes the α subunit of the neuronal voltage-gated sodium channel, which is a target for carbamazepine and other antiepileptic drugs (AEDs).
- Recent studies have demonstrated that a common polymorphism of *SCN1A* IVS5-91 G > A was associated with carbamazepine and phenytoin use in daily practice.
- However, it has not been determined whether the polymorphism affects carbamazepine or other AED responsiveness.

WHAT THIS STUDY ADDS

- This study demonstrated a significant association between the *SCN1A* IVS5-91 AA genotype and carbamazepine-resistant epilepsy, while the AA genotype did not affect carbamazepine use.

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AIMS

To establish whether the *SCN1A* IVS5-91 G > A polymorphism of the *SCN1A* gene, which encodes the neuronal sodium channel α subunit, affects responsiveness to the antiepileptic drugs (AEDs) carbamazepine and/or phenytoin.

METHODS

SCN1A IVS5-91 G > A polymorphism was genotyped in 228 Japanese epileptic patients treated with AEDs. The association between AED responsiveness and the polymorphism was estimated by logistic regression analysis, adjusting for clinical factors affecting the outcome of AED therapy.

RESULTS

The frequency of the AA genotype was significantly higher in carbamazepine-resistant patients (odds ratio, 2.7; 95% confidence interval (CI), 1.1, 7.1) and was insignificantly higher in AED-resistant patients.

CONCLUSIONS

This is the first report demonstrating an association between the *SCN1A* polymorphism and carbamazepine-resistant epilepsy.

Introduction

Voltage-gated sodium channels are responsible for the initial depolarization of action potentials in brain neurons. Therefore, they are the target for widely used first-line anti-epileptic drugs (AEDs), including carbamazepine (CBZ) and phenytoin (PHT) [1, 2]. The *SCN1A* gene encodes the neuronal sodium channel α subunit and the genetic defects in

the coding sequence lead to generalized epilepsy with febrile seizures plus (GEFS+) and a range of childhood epileptic encephalopathies of varied severity, for example severe myoclonic epilepsy of infancy (SMEI) [3].

Recently, a common polymorphism *SCN1A* IVS5-91 G > A was shown to be significantly associated with maximum doses of both CBZ and PHT and with PHT serum concentrations at maintenance dose in English and

Chinese cohorts of patients [4, 5]. Therefore, this study was designed to verify directly whether or not the *SCN1A* IVS5-91 G>A polymorphism of a drug target, that is the pharmacodynamic variant, affects CBZ and/or PHT responsiveness.

Methods

The study included 228 Japanese epileptic patients treated with AEDs (129 males, mean age 19.9 ± 9.6 years, range 0.2–58.0 years) at Kumamoto Saishunso National Hospital after January 1996. Any patients with GEFS⁺ and SMEI were excluded from the study because these epilepsy syndromes are caused by genetic defects in the *SCN1A*. All patients and/or their parents gave their written consent to participate in the study. The protocol was approved by the Institutional Ethics Committees.

The type of seizure and epileptic syndrome was classified according to the guidelines of both the Japanese Society of Neurology (http://www.neurology-jp.org/guidelinem/neuro/tenkan/tenkan_index.html) and the National Institute for Health and Clinical Excellence (<http://www.nice.org.uk/nicemedia/pdf/CG020fullguideline.pdf>), and appropriate AEDs were chosen, as previously reported [6]. The patient was initially treated with a single AED, which was changed to another if the seizures remained uncontrolled or if the patient had any intolerable adverse drug reaction(s). A combination of AEDs was applied only to patients whose epilepsy remained uncontrolled despite attempts to treat with a different single AED. Any patients suspected of poor compliance were excluded from the study.

Based on the definition of a previous report [6], the seizure control was assessed at the last clinic visit under AED treatment. The patients were considered to be drug-responsive if they had not experienced any type of seizures for a minimum of 1 year after receiving AED(s). CBZ-responsive epilepsy was the drug-responsive epilepsy treated with a stable dose of CBZ. Drug-resistant epilepsy was defined as uncontrolled seizures over a year despite attempts to treat with three or more different AEDs. The maximum dose was defined as the highest dose during the study period. The maintenance dose was defined as the latest dose in the drug responsive patient.

Genomic DNA was extracted from whole blood and/or buccal cells using a protocol modified as previously reported [6]. The *SCN1A* IVS5-91 G>A genotypes were identified by a TaqMan polymerase chain reaction analysis as described previously [7].

Fisher's exact test and Student's *t*-test were used to compare the distributions of demographic characteristics. The association analyses were estimated by an odds ratio (OR) with a 95% confidence interval (95% CI) using a multiple logistic regression analysis, after adjusting for the aetiology, complications of mental retardation and AED

therapy at the last clinic visit. A *P* value < 0.05 was considered to be statistically significant. Statistical analyses were performed using the SPSS software package (version 15.0; SPSS Inc., IL, USA).

Results

The GG, GA, and AA genotypes were identified in 25, 108, and 95 individuals, respectively. The frequency for the A allele was 65.4%. The genotype distribution was consistent with the Hardy-Weinberg equilibrium (*P* = 0.79). No bias was found in the distributions of the demographic characteristics as shown in Table 1. Drug-resistant epilepsy was diagnosed in 104 (47.1%) patients and drug-responsive epilepsy was 117 (52.9%) (Table 2). Seven patients with uncontrolled seizures were unclassified because they had been treated by less than three AEDs. The frequency of the AA genotype was higher in AED-resistant epilepsy than in AED-responsive epilepsy, though the difference was not significant (adjusted OR, 1.8; 95% CI, 0.9, 3.7) (Table 2). However, the AA genotype was associated with a 2.7-fold increase in the risk for CBZ-resistant epilepsy (adjusted OR,

Table 1

Demographic characteristics of the patients

	<i>SCN1A</i> genotype		<i>P</i> value
	GG or GA (<i>n</i> = 133)	AA (<i>n</i> = 95)	
Sex			
Male	78 (58.6%)	51 (53.7%)	0.50
Female	55 (41.4%)	44 (46.3%)	
Age (years)	20.1 ± 10.5	19.6 ± 8.3	0.73
Body weight (kg)	48.4 ± 19.9	50.6 ± 19.2	0.42
Duration of AED therapy (years)	9.2 ± 4.4	9.5 ± 5.0	0.57
Mental retardation	81 (60.9%)	61 (64.2%)	0.68
Seizure type			
Partial	101 (77.1%)	70 (74.5%)	0.75
Generalized	30 (22.9%)	24 (25.5%)	
Aetiology			
Idiopathic	28 (21.1%)	17 (17.9%)	0.83
Symptomatic	46 (34.6%)	33 (34.7%)	
Cryptogenic	59 (44.4%)	45 (47.4%)	
History of AED therapy			
CBZ	114 (85.7%)	84 (88.4%)	0.70
Phenytoin	50 (37.6%)	32 (33.7%)	0.58
Valproic acid	95 (71.4%)	62 (65.3%)	0.38
Phenobarbital	74 (55.6%)	48 (50.5%)	0.50
Zonisamide	80 (60.2%)	62 (65.3%)	0.49
AED therapy at the last clinic visit			
Monotherapy	66 (49.6%)	51 (53.7%)	0.59
Polytherapy	67 (50.4%)	44 (46.3%)	0.59
CBZ monotherapy	28 (24.6%)	20 (23.8%)	1.00
polytherapy	38 (33.3%)	29 (34.5%)	0.77
withdrawn	48 (42.1%)	35 (41.7%)	1.00

Presented are mean ± SD or number of patients. AED, antiepileptic drug; CBZ, carbamazepine.

Table 2Association between the *SCN1A* genotypes and response to AEDs

	<i>SCN1A</i> genotype GG or GA	AA	OR (95% CI)	P value
Response to AEDs in total*				
Responsive epilepsy	73 (56.2%)	44 (48.4%)	1	0.12
Resistant epilepsy	57 (43.8%)	47 (51.6%)	1.8 (0.9, 3.7) [†]	
Response to carbamazepine				
Responsive epilepsy	38 (33.3%)	19 (22.6%)	1	0.04
Resistant epilepsy	76 (66.7%)	65 (77.4%)	2.7 (1.1, 7.1) [‡]	
among subjects without mental retardation				
Responsive epilepsy	25 (61.0%)	9 (30.0%)		0.02
Resistant epilepsy	16 (39.0%)	21 (70.0%)		
among subjects with idiopathic epilepsy				
Responsive epilepsy	16 (80.0%)	7 (50.0%)		0.14
Resistant epilepsy	4 (20.0%)	7 (50.0%)		
Carbamazepine dose§				
Maximum dose	114	84		
(mg day ⁻¹)	492.9 ± 316.6	502.4 ± 293.6		0.83
(mg kg ⁻¹ day ⁻¹)	13.9 ± 7.2	13.7 ± 6.5		0.81
Maintenance dose	38	19		
(mg day ⁻¹)	292.6 ± 190.3	304.2 ± 140.1		0.82
(mg kg ⁻¹ day ⁻¹)	6.2 ± 3.8	6.1 ± 2.7		0.94

*Seven patients with uncontrolled seizures who had been treated with less than three AEDs were excluded from the analysis. OR, odds ratio was adjusted by aetiology, complications of mental retardation and [†] monotherapy or polytherapy, or [‡] carbamazepine monotherapy, polytherapy or withdrawn at the last clinic visit. §P values were estimated by Student's t-test. AEDs, antiepileptic drugs; CI, confidence interval.

2.7; 95% CI, 1.1, 7.1). The maximum and maintenance doses of CBZ were comparable (Table 2); and the monotherapy : polytherapy ratio and the number of co-administered drugs in CBZ-responsive epilepsy did not differ between the genotypes (data not shown). In addition, the frequency of the AA genotype in epilepsy without mental retardation was significantly higher among CBZ-resistant subjects than CBZ-responsive subjects, and the frequency in idiopathic epilepsy was also higher among the CBZ-resistant subjects, but the difference was not significant (Table 2). The number of PHT-responsive patients was too small to analyze ($n = 4$) (data not shown).

Discussion

This study demonstrated a significant association between the *SCN1A* IVS5-91 AA genotype and CBZ-resistant epilepsy, while the AA genotype did not affect the CBZ maximum or maintenance doses. The result appeared to be functionally consistent with the previous findings [4, 5]: the average maximum doses for CBZ and PHT were the highest in the patients with the AA genotype and the serum PHT concentration at the maintenance dose was the highest again in the AA genotype, followed by the AG and GG genotypes. In this study, no association between the genotype and the maximum or maintenance dose was observed. The reason for the discrepancy between the present findings and those of previous studies may be due to the use of different dosing strategies. When one AED

proved to be ineffective, it was then changed to another AED rather than merely increasing the dosage. In addition, AEDs also tend to be administered at lower doses in Japan than in the UK. In fact, the range of CBZ maximum doses in this study was narrower than that in the previous study (70–1600 mg day⁻¹ vs. 200–> 2600 mg day⁻¹, respectively) and the average maximum dose was less than half (500 mg day⁻¹ vs. > 1000 mg day⁻¹) (Table 2) [4].

The frequency of the AA genotype was higher among CBZ-resistant patients with idiopathic epilepsy or those without complications, such as mental retardation. This finding is consistent with the hypothesis that a loss of sodium channel drug-sensitivity may underlie the development of CBZ-resistant epilepsy [8]. The polymorphism had an effect on the proportion of the adult and neonatal mRNA transcript forms in adults with and without epilepsy [7]. Individuals with the G allele had approximately 30–40% of *SCN1A* transcripts in the neonatal form, whereas those without had less than 1%. This functionally significant genetic polymorphism has been suggested to influence directly the therapeutic efficacy of AED treatment and to have other currently unknown phenotypic consequences [4, 5, 7].

This study has the following shortcomings: firstly, the number of subjects was too small to assess the effect of each genotype. Therefore, we compared the effects of the AA genotype vs. the GG and GA genotypes, which exhibited a close phenotype in proportion to the neonatal transcript form in human brain tissue [7]. In addition, the number of PHT-responsive cases was too small to

determine the association between the polymorphism and PHT-responsiveness. Secondly, many patients had mental retardation; therefore our results may not be generalized to other epileptic patients.

In conclusion, this study demonstrated a significant association between the *SCN1A* IVS5-91 AA genotype and CBZ-resistant epilepsy. These results confirmed previous findings from a pharmacodynamic point of view. However, the mechanism underlying the risk of the AA genotype for CBZ-resistant epilepsy still remains to be elucidated.

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REFERENCES

- 1 Remy S, Beck H. Molecular and cellular mechanisms of pharmacoresistance in epilepsy. *Brain* 2006; 129: 18–35.
- 2 Ragsdale DS, Avoli M. Sodium channels as molecular targets for antiepileptic drugs. *Brain Res* 1998; 26: 16–28.
- 3 Mulley JC, Scheffer IE, Petrou S, Dibbens LM, Berkovic SF, Harkin LA. *SCN1A* mutations and epilepsy. *Hum Mutat* 2005; 25: 535–42.
- 4 Tate SK, Depondt C, Sisodiya SM, Cavalleri GL, Schorge S, Soranzo N, Thom M, Sen A, Shorvon SD, Sander JW, Wood NW, Goldstein DB. Genetic predictors of the maximum doses patients receive during clinical use of the anti-epileptic drugs carbamazepine and phenytoin. *Proc Natl Acad Sci USA* 2005; 102: 5507–12.
- 5 Tate SK, Singh R, Hung CC, Tai JJ, Depondt C, Cavalleri GL, Sisodiya SM, Goldstein DB, Liou HH. A common polymorphism in the *SCN1A* gene associates with phenytoin serum levels at maintenance dose. *Pharmacogenet Genomics* 2006; 16: 721–26.
- 6 Seo T, Ishitsu T, Ueda N, Nakada N, Yurube K, Ueda K, Nakagawa K. *ABCB1* polymorphisms influence the response to antiepileptic drugs in Japanese epilepsy patients. *Pharmacogenomics* 2006; 7: 551–61.
- 7 Heinzen EL, Yoon W, Tate SK, Sen A, Wood NW, Sisodiya SM, Goldstein DB. *Nova2* interacts with a cis-acting polymorphism to influence the proportions of drug-responsive splice variants of *SCN1A*. *Am J Hum Genet* 2007; 80: 876–83.
- 8 Remy S, Gabriel S, Urban BW, Dietrich D, Lehmann TN, Elger CE, Heinemann U, Beck H. A novel mechanism underlying drug resistance in chronic epilepsy. *Ann Neurology* 2003; 53: 469–79.