

A PLETHORA OF *SCN1A* MUTATIONS: WHAT CAN THEY TELL US?

Effect of Localization of Missense Mutations in *SCN1A* on Epilepsy Phenotype Severity

Kanai K, Hirose S, Oguni H, Fukuma G, Shirasaka Y, Miyajima T, Wada K, Iwasa H, Yasumoto S, Matsuo M, Ito M, Mitsudome A, Kaneko S

Neurology 2004;63:329–334

BACKGROUND AND METHODS: Many missense mutations in the voltage-gated sodium channel subunit gene *SCN1A* were identified in patients with generalized epilepsy with febrile seizures plus (GEFS+) and severe myoclonic epilepsy of infancy (SMEI), although GEFS+ is distinct from SMEI in terms of clinical symptoms, severity, prognosis, and responses to antiepileptic drugs. The authors analyzed the localization of missense mutations in *SCN1A* identified in patients with GEFS+ and SMEI to clarify the phenotype-genotype relations. **RESULTS:** Mutations in SMEI occurred more frequently in the “pore” regions of *SCN1A* than did those in GEFS+. These SMEI mutations in the “pore” regions were more strongly asso-

ciated than mutations in other regions with the presence of ataxia and tendency to early onset of disease. The possibility of participation of ion selectivity dysfunction of the channel in the pathogenesis of SMEI was suggested by a mutation in the pore region (R946C) identified in a SMEI patient.

CONCLUSIONS: A significant phenotype-genotype relation existed in generalized epilepsy with febrile seizures plus and severe myoclonic epilepsy of infancy with *SCN1A* missense mutations. More severe sodium channel dysfunctions including abnormal ion selectivity that are caused by mutations in the pore regions may be involved in the pathogenesis of SMEI.

Noninactivating Voltage-gated Sodium Channels in Severe Myoclonic Epilepsy of Infancy

Rhodes TH, Lossin C, Vanoye CG, Wang DW, George AL Jr

Proc Natl Acad Sci U S A 2004;101:11147–11152

Mutations in *SCN1A*, the gene encoding the brain voltage-gated sodium channel α_1 subunit ($\text{Na}_v1.1$), are associated with at least two forms of epilepsy, generalized epilepsy with febrile seizures plus and severe myoclonic epilepsy of infancy (SMEI). We examined the functional properties of five SMEI mutations by using whole-cell patch-clamp analysis of heterologously expressed recombinant human *SCN1A*. Two mutations (F902C and G1674R) rendered *SCN1A* channels nonfunctional, and a third allele (G1749E) exhibited minimal functional alterations. However, two mutations within or near the S4 segment of the fourth repeat

domain (R1648C and F1661S) conferred significant impairments in fast inactivation, including persistent, noninactivating channel activity resembling the pattern of channel dysfunction observed for alleles associated with generalized epilepsy with febrile seizures plus. Our data provide evidence for a range of *SCN1A* functional abnormalities in SMEI, including gain-of-function defects that were not anticipated in this disorder. Our results further indicate that a complex relation exists between phenotype and aberrant sodium channel function in these inherited epilepsies.

COMMENTARY

More than 80 mutations have been described in the α_1 subunit of the voltage-gated sodium channel gene (*SCN1A*). The first mutations were associated with generalized epilepsy with febrile seizures plus, or GEFS+ (1). This inherited epilepsy syndrome has a broad spectrum of phenotypes, ranging from simple febrile seizures to severe myoclonic epilepsy of infancy (SMEI). In a single family with GEFS+, individuals

carrying the same *SCN1A* mutation can be asymptomatic or have severe epilepsy with major neurologic defects, suggesting that other modifying factors contribute to the severity of the disease. In addition to being part of the familial GEFS+ syndrome, SMEI can occur sporadically as a result of de novo mutations in *SCN1A* (2). With the large number of *SCN1A* mutations described, it is now possible to begin investigating

the relation between various sodium channel defects and disease severity.

Both SMEI and GEFS+ are associated with heterozygous *SCN1A* mutations, therefore one normal copy of the gene remains. Many of the SMEI mutations are predicted to result in a nonfunctional, truncated protein. Loss of one functional copy (haploinsufficiency) is sufficient to cause SMEI but has never been associated with GEFS+. However, missense mutations (single amino acid substitutions) in *SCN1A* have been associated with both SMEI and GEFS+. Several groups are now exploring the relation between the type of *SCN1A* mutation, the effect on sodium channel function, and the resulting epilepsy phenotype, in an attempt to answer the question of how *SCN1A* mutations cause epilepsy and whether specific mutations are associated with particular clinical features. Identifying genotype–phenotype relations will improve the understanding of normal sodium channel function and how disruption of this function causes epilepsy. Understanding the mechanisms involved also will aid in the development of new therapeutics. Once a suitable treatment for SMEI has been developed, it would be prudent to check all new febrile seizures cases for mutations in *SCN1A*. The ultimate goal is to be able to identify children that are likely to develop SMEI and begin treatment to prevent onset of this devastating disease. Two recent articles have contributed to this aim; Kanai et al. provide a detailed analysis of phenotype–genotype correlations, whereas Rhodes et al. investigated the biophysical properties of various *SCN1A* mutant channels. Both articles focus on missense mutations, which are associated with 100% of GEFS+ mutations and ~50% of SMEI mutations (3).

Kanai et al. compared the severity of epilepsy associated with >60 different missense mutations in *SCN1A*. The *SCN1A* protein contains four homologous domains (I–IV), and each domain contains six transmembrane segments (S1–S6). Kanai et al. divided the *SCN1A* mutations into separate categories based on the location of the mutation within the sodium channel, which included the voltage sensor (S4), the pore-forming region (S5–S6), other homologous domains, and regions other than the homologous domains (e.g., the intracellular N- and C-terminal domains). Patients were divided into three categories: GEFS+, SMEI, and borderline SMEI (SMEB). SMEB was defined as those patients who met most of the diagnostic criteria for SMEI but did not have both myoclonic and atypical absence seizures; the group included patients with intractable childhood epilepsy with tonic–clonic (ICEGTC) seizures. However, the authors found no difference in the location of *SCN1A* mutations between SMEI and SMEB patients, and therefore this broad phenotype will continue to be referred to simply as SMEI. It was discovered that SMEI patients had mutations more commonly in the pore-forming region of *SCN1A* (71%) than other homologous regions of the channel. Furthermore, SMEI pa-

tients with mutations in the pore region of *SCN1A* tended to have an earlier onset, with a higher prevalence of ataxia. The percentage of mutations in the same region in GEFS+ cases was significantly lower (33%). The authors concluded that missense mutations in the pore of the sodium channel are more frequently associated with SMEI than with GEFS+.

A plethora of sodium channel mutations have been described; however, functional analyses have been minimal. In a recent report, Rhodes et al. functionally characterized five *SCN1A* missense mutations associated with SMEI. Three of the mutations studied were located in the pore of the sodium channel, which, according to Kanai et al., is associated with a more severe phenotype. Two mutations in the S5 segment of the sodium channel pore had no measurable sodium current, demonstrating loss of function. A loss-of-function mutation in the S5 segment of the pore also has been associated with GEFS+ (4). The fact that similar mutations cause two different phenotypes implies that other environmental or genetic factors are associated with SMEI. Other groups also reported that SMEI-associated missense mutations in either the S5 or S6 segment of the sodium channel pore result in a loss of function (4,5). Combining these results with the findings of Kinai et al. (i.e., that SMEI mutations are more common in the pore region) adds support to the theory that most SMEI missense mutations result in loss of sodium channel function. However, most SMEI mutations reported by Kanai et al. were actually in the S5–S6 linker section of the pore region, and the one mutation in this region that was studied by Rhodes et al. had minimal effect on channel function. The only prominent defect exhibited by the SMEI mutation was reduced current density, suggesting reduced channel availability. Heterozygous loss-of-function mutations, such as truncations or the previously mentioned mutations in the S5 and S6 regions of the pore, also would result in reduced channel density, and this may be the common underlying effect of all SMEI mutations.

Of the other two *SCN1A* mutations analyzed by Rhodes et al., one was located in the S4 segment (R1648C), and one, between the S4 and S5 segments (F1661S). Both these mutations prolonged the normal fast inactivation time of the sodium channel and also reduced the time needed for recovery from inactivation. These alterations in inactivation would result in a gain of function, in which the channels are open for longer and can be reactivated more quickly, resulting in persistent sodium current. This finding is the first demonstration that SMEI mutations can result in a gain of function, suggesting that both loss and gain of sodium channel function can cause SMEI, adding to the complexity of possible phenotype–genotype correlations. However, Rhodes et al. also demonstrated additional functional defects in both F1661S and R1648C that further support the conclusion that SMEI is associated with reduced sodium channel availability.

TABLE1. Summary of *SCN1A* mutants for which functional data is available

Mutation	Location	Effect	Phenotype	Reference
D188V	S2-S3	Minimal effect	GEFS+	Cossette et al., 2003
T875M	S4	Gain of function	GEFS+	Lossin et al., 2002
R1648C	S4	Gain of function	SMEI + ataxia	Rhodes et al., 2004
R1648H	S4	Gain of function	GEFS+	Lossin et al., 2002
I1656M	S4	Gain of function	GEFS+	Lossin et al., 2003
R1657C	S4	Gain of function	GEFS+	Lossin et al., 2003
F1661S	S4-S5	Gain of function	SMEI	Rhodes et al., 2004
F902C	S5	Loss of function	SMEB	Rhodes et al., 2004
V1353L	S5	Loss of function	GEFS+	Lossin et al., 2003
G1674R	S5	Loss of function	SMEB + ataxia	Rhodes et al., 2004
G1749E	S5-S6	Minimal effect	SMEI + ataxia	Rhodes et al., 2004
G979R	S6	Loss of function	SMEB	Sugawara et al., 2003
N985I	S6	Loss of function	SMEI	Sugawara et al., 2003
L986F	S6	Loss of function	SMEI	Lossin et al., 2003
W1204R	II-III	Gain of function	GEFS+	Lossin et al., 2002
F1831S	C-ter	Reduced current	SMEI + ataxia	Sugawara et al., 2003

The Kanai et al. findings suggested that mutations in the pore region of *SCN1A* result in a more severe epilepsy phenotype. However, one can only observe the clinical characteristics and not the underlying physical cause because analysis of affected brain tissue is virtually impossible. Furthermore, the complexity of genetic background complicates the analysis, highlighting the importance of developing appropriate animal models, in which genetic background can be controlled. Currently, the only way to analyze the functional effect of *SCN1A* mutations is in cultured cells. Rhodes et al. characterized biophysical properties in mutant channels expressed in human embryonic kidney cells. Whereas this system has provided invaluable insight into sodium channel function, other functional consequences cannot be tested for in vitro. Ultimate demonstration of the affect of *SCN1A* mutations will require specific genetic manipulations in entire organisms. In the absence of such animal models, computer simulations have been used to determine the effect of different *SCN1A* mutations on neuron firing (6). By using a computational model based on the experimentally determined properties of GEFS+ mutant sodium channels, Spanpanato et al. (6) found that, despite the different effects on the threshold for firing a single action potential, all of the mutations resulted in an increased propensity to fire repetitive action potentials. These results suggested a common physiologic mechanism for epileptogenesis resulting from sodium channel mutations that cause GEFS+. Although the programs used are sophisticated, the results must be confirmed in a living system. Animal models will allow the study of the effect of *SCN1A* mutations on neuronal development, morphology, and connectivity and also will help answer questions such as, Which neurons, in which area of the brain, are most severely affected? Is there associated cell death? What is the effect of ge-

netic background and environmental factors, such as fever, on progression of the disease?

The overlapping phenotype and the common molecular basis for GEFS+ and SMEI suggest that they are part of a single syndrome with variable penetrance and variable modifiers, in which the manifestation of a particular epilepsy phenotype is the result of the complex interplay between sodium channel defects as well as other regulatory factors. Rhodes et al. speculated that the sodium channel defect creates the initial seizure predisposition, but concomitant excitotoxicity is the direct cause for other neurologic features of SMEI. Therefore *SCN1A* could be considered a susceptibility factor for both disorders, in which severity is modified by other environmental and genetic factors. Thorough investigation of genetic and environmental modifying factors is important to determine their influence on disease manifestation and progression. Phenotypic heterogeneity is increasingly recognized in all the sodium channel disorders. Mutations in the *SCN4A* gene have been identified in a group of related muscular disorders, including hyperkalemic periodic paralysis, paramyotonia congenita, potassium-aggravated myotonia, and hypokalemic periodic paralysis. Mutations in *SCN5A* result in multiple arrhythmic syndromes, including long-QT syndrome, Brugada syndrome, and an inherited cardiac conduction defect.

The two articles discussed provide evidence that missense mutations in *SCN1A* cause SMEI primarily because of a loss of function; however, other modifying factors are likely to be associated with severity of the disease. Insight into the relation between the location of *SCN1A* mutations and the severity of the phenotype will give insight into sodium channel function and epilepsy mechanisms and will, ultimately, allow the

clinical course of individual mutations to be predicted. Predicting the clinical outcome of particular mutations in *SCN1A* will likely require assessment of other environmental and genetic risk factors.

by Robyn Wallace, Ph.D.

References

1. Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, Brice A, LeGuern E, Moulard B, Chaigne D, Buresi C, Malafosse A. Mutations of *SCN1A*, encoding a neuronal sodium channel, in two families with GEFS+2. *Nat Genet* 2000;24:343–345.
2. Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. De novo mutations in the sodium-channel gene *SCN1A* cause severe myoclonic epilepsy of infancy. *Am J Hum Genet* 2001;68:1327–1332.
3. Ceulemans BP, Claes LR, Lagae LG. Clinical correlations of mutations in the *SCN1A* gene: from febrile seizures to severe myoclonic epilepsy in infancy. *Pediatr Neurol* 2004;30:236–243.
4. Lossin C, Rhodes TH, Desai RR, Vanoye CG, Wang D, Carniciu S, Devinsky O, George AL Jr. Epilepsy-associated dysfunction in the voltage-gated neuronal sodium channel *SCN1A*. *J Neurosci* 2003;23:11289–11295.
5. Sugawara T, Tsurubuchi Y, Fujiwara T, Mazaki-Miyazaki E, Nagata K, Montal M, Inoue Y, Yamakawa K. Nav1.1 channels with mutations of severe myoclonic epilepsy in infancy display attenuated currents. *Epilepsy Res* 2003;54:201–207.
6. Spanpanato J, Aradi I, Soltesz I, Goldin AL. Increased neuronal firing in computer simulations of sodium channel mutations that cause generalized epilepsy with febrile seizures plus. *J Neurophysiol* 2004;91:2040–2050.