BRAIN
LETTER TO THE EDITOR

Genotype-phenotype correlations in *SCN8A*-related epilepsy: a cohort study of Chinese children in southern China

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We read with great interest the article recently published in *Brain* by Johannesen and colleagues,[1] which revealed the clear genotype-phenotype correlations between the age at seizure onset, type of epilepsy and gain-of-function (GOF) or loss-of-function (LOF) effects of *SCN8A* variants. The authors collected the largest cohort of individuals with *SCN8A*-related epilepsy from a multi-country study and found that generalized epilepsy with absence seizures is the main epilepsy phenotype of LOF variant carriers and the extent of the electrophysiological dysfunction of the GOF variants is a main determinant of the severity of the clinical phenotype in focal epilepsies. Their pharmacological data indicated that sodium channel blockers (SCBs) present a treatment option in the *SCN8A*-related focal epilepsy with onset in the first year of life.[1]

We believe that this study constitutes to the understanding of *SCN8A*-related epilepsy. However, we would also like to discuss the similarities and discrepancies with respect to our results based on a cohort study of Chinese children and propose an interpretative linking on the findings of the study.

Specifically, we recruited 21 children (13 males and eight females) with *SCN8A de novo* missense variants from three hospitals in Southern China between January 2017 and February 2021 (Table 1); two of the patients were identical twins. All children experienced their first seizure during infancy with the average onset age of 3.9 ± 2.97 months and the maximum onset age of 9 months. Among the 21 cases, five experienced onset during the neonatal period. All 21 cases were *de novo* heterozygous mutations estimated as either pathogenic or likely pathogenic based on the American

College of Medical Genetics and Genomics guidelines,[2] and 14 sites have not been reported previously: c.2654T > C, p.I885T; c.5303A > G, p.N1768S; c.4378A > G, p.I1460V; c.4384G > A, p.V1462I; c.656T > C p.L219P; c.1243G > A, p.E415K; c.4814T > C, p.I1605T; c.3815T > A, p.V1272E; c.4798A > G, p.M1600V; c.2942G > C, p.S981T; c.2627G > A, p.G876D; C.4948G > T, p.A1650S; c.2944G > T, p.A982S; and c.2945C > T, p.A982V. Seven variants were previously confirmed as pathogenic: c.1099A > G, p.M367V;[3] C.667A > G, p.R223G;[4] c.2549G > A, p.R850E;[5] c.3953A > G, p.N1318S;[6] c.5614C > T, p.R1872W;[7] c.638T > C, p.L213P;[8] c.2300C > T, and p.T767I.[4] The domains in the voltage-gated sodium channel amino acid sequence were grouped according to approximate functional domains based on the method reported by Holland *et al.*[9]: the pore region was defined as segments S5, S5–S6, and S6, while the voltage sensor region was classified as S4 and its associated linkers of S3–S4 and S4–S5. Other transmembrane segments and their linking regions (TMOs) were grouped, and the intracellular loops linking domains I-III were also grouped together (Loops). The inactivation gate, N-terminus, and C-terminus were also grouped separately. The clinical data from all patients were also collected, focusing on the age of onset, the forms of seizures, the frequency of seizures, neurological development at onset, the effect of SCBs during follow-up, and neurologic and EEG evaluations during follow-up.

As a result, in our cohort, only five out of 21 cases had a good response to SCBs, with the frequencies of seizures significantly reduced up to 75% after treatment. All five patients had combined anti-seizure medications (ASMs) with valproate

Table 1 Clinical features of twenty-one cases with *SCN8A*-related epilepsy

No	Sex	Age (m)	Seizure	MRI	DEV	Diagnosis	Age (mo)	ASMs/ Therapy	Current dev. (DQ/IQ)	Variants	Location	Drug response	Effect of SCBs
1	Male	9	CGFS	Normal	Normal	DEE	26	VPA,LTG	48	c.2654T > C,p.I885T	Pore	DE	+++
2	Female	8	CGFS	Normal	Normal	BIFE	29	VPA	91	c.5303A > G, p.N1768S	C-terminus	DE	−
3	Male	2	GS	Normal	Normal	IE	36	VPA,OXC	65	c.4378A > G, p.I1460V	Pore	DE	+++
4	Male	2	CGFS	Normal	Normal	DEE	48	VPA,OXC, LCM,NZP	33	c.4384G > A, p.V1462I	Inactivation gate	DR	++
5	Male	3	CGFS	Normal	R	DEE	18	VPA,OXC	45	c.1099A > G,p.M367V	Pore	DE	+++
6	Female	7	GS	Normal	Normal	DEE	13	VPA,NZP, TPM,VGB/ ACTH	42	c.656T > C, p.L219P	VSR	DR	−
7	Female	3	CGFS	Normal	R	DEE	48	VPA,TPM, LCM	31	c.1243G > A, p.E415K	Loops	DR	++
8	Male	3	CGFS	Normal	Normal	DEE	12	OXC	61	c.4814T > C, p.I1605T	VSR	DR	++
9	Male	6	GS	Atrophy	R	DEE	36	VPA,LEV, LCM	<20	c.667A > G, p.R223G	VSR	DR	−
10	Male	3	GS	Atrophy	R	DEE	36	VPA,TPM, LCM	<20	c.2549G > A, p.R850E	VSR	DR	+
11	Male	0	CGFS	Normal	ID	DEE	11	OXC,TPM/ ACTH	<20	c.3815T > A, p.V1272E	TMOs	DR	+
12	Female	6	GS	Normal	Normal	GE	60	LEV,LTG	48	c.4798A > G, p.M1600V	TMOs	DE	+++
13	Female	2	GS	Normal	Normal	IE	60	VPA,LTG	34	c.3953A > G, p.N1318S	VSR	DE	+++
14	Female	8	CGFS	Normal	Normal	DEE	32	OXC,LTG, VPA,TPM	45	c.2942G > C, p.S981T	Loops	DR	++
15	Male	3	CGFS	Normal	Normal	DEE	20	LEV,OXC, LCM,VPA, NZP/KD	<20	c.5614C > T, p.R1872W	C-terminus	DR	+
16	Male	6	GS	Normal	Normal	DEE	21	VPA,LEV, PER/ACTH	30	c.638T > C, p.L213P	VSR	DR	−
17	Male	0	FS	Normal	ID	DEE	10	VPA,LTG,LEV	<20	c.2300C > T, p.T767I	TMOs	DR	+
18	Male	0	CGFS	Normal	ID	DEE	26	CBZ,CZP	<20	c.2944G > T, c.2945C > T, p.A982S(V)	Loops	DR	+
19	Male	0	CGFS	Normal	ID	DEE	22	CBZ,CZP	<20		Loops	DR	+
20	Male	0	CGFS	Normal	ID	DEE	4	PB,OXC,TPM, NZP	<20	c.2627G > A (p.G876D)	Pore	DR	+
21	Female	7	GS	Normal	Normal	GE	96	LEV	40	c.4948G > T, p.A1650S	VSR	DE	−

+ = somewhat response, ++ = partial response, +++ = good response, − = no response.

ACTH = adrenocorticotropic hormone; ASMs = anti-seizure medicines; BIFE = benign familial infantile epilepsy; CGFS = combined generalized and focal seizures; CBZ = carbamazepine; CZP = clonazepam; DE = drug effective; DEE = developmetal and epileptic encephalopathy; Dev. = development; DQ = developmental quotient; DR = drug refractory; FS = focal seizures; GE = generalized epilepsy, frequently with absence seizures; GS = generalized seizures; ID = inapplicable data (not easy to evaluate because occurred in the neonate period); IE = intermediate epilepsy; IQ = intellectual quotient; KD = ketogenic diet; LCM = lacosamide; LEV = levetiracetam; LTG = lamotrigine; mo = months; No = patient number; NZP = nitrodiazepam; OXC = oxcarbazepine; PB = phenobarbital; PER = perampanel; R = retardation; SCBs = sodium channel blockers; TPM = topiramate; TMOs = other transmembrane segment and linking regions; VPA = valproate; VGB = vigabatrin; VSR = voltage sensor region.

(VPA) plus lamotrigine (LTG) for two cases, levetiracetam and LTG for one case, and VPA plus oxcarbazepine for the remaining two cases. Second, four of 21 cases had only a partial response to SCBs. Specifically, the frequencies of seizures of the four cases were reduced to some extent (25–50%) by a variety of high-doses SCBs given. Third, 7 of 21 patients had only some response to SCBs, i.e. the administration of SCBs could not reduce the frequencies of seizures, but the SCBs could not be stopped during treatment, because if reduced, status epilepticus would occur. Finally, the other five remaining patients had a negative response to SCBs, as non-SCBs had controlled the seizures or SCBs had deteriorated their seizures (Table 2).

All cases in our study were grouped into four clinical phenotypes including benign familial infantile epilepsy (BFIE), intermediate epilepsy (IE), developmental and epileptic encephalopathy (DEE) and generalized epilepsy (GE), frequently with absence seizures (Table 2). Most of patients in our study belonged to the DEE group, and the patients with DEE were classified by the EEG findings, e.g. diffuse slow waves or hypsarrhythmia, with moderate to severe developmental delay/intellectual disability. The findings of our study showed that the clinical phenotypes significantly correlated with the effect of SCBs (Fisher = 13.198, $P = 0.016$, $r = 0.646$). For example, one girl belonged to the BFIE group, having self-limiting seizures controlled by VPA, with normal cognitive development; two children belonged to the IE group, with a better response to SCBs than the other phenotype groups; two children belonged to the GE group, one with seizures controlled by VPA + LTG and the other one with seizures controlled by levetiracetam. Interestingly, the study by Johannesen et al.[1] revealed that the patients with BFIE or IE showed a mild GOF, whereas the patients with GE had the LOF mutation of *SCN8A*. Similarly, our data supported the above findings.

However, some differences based on the outcomes of our cohort were as follows. The first discrepancy was regarding the outcomes of a subgroup of DEE patients. Johannesen and colleagues revealed that missense variants in most patients with DEE showed a strong GOF and only 3/34 patients with LOF exhibited DEE. Most patients with DEE revealed frequent resistance to ASMs.[1] In our Chinese

Table 2 Relationship between the effect of SCBs and clinical characteristics in *SCN8A*-related epilepsy

		Effect of SCBs				Fisher	r	P
		+++	++	+	None			
Age of onset	Newborn	0	0	5	0	18.952	0.733	**<0.001**
	<6 months	3	3	2	0			
	>6 months	2	1	0	5			
Forms of seizures	Only focal seizures	0	0	1	0	13.163	0.632	**0.010**
	Only generalized seizures	2	0	1	5			
	Generalized seizures+focal seizures	3	4	5	0			
Distribution of missense variants	Pore	3	0	1	0	7.659	0.517	0.054
	The other	2	4	6	5			
Distribution of missense variants	Voltage sensor region	1	1	1	4	17.186	0.671	**0.046**
	Inactivation gate + C-terminus + loops	0	3	3	1			
	Pore	3	0	1	0			
	TMOs	1	0	2	0			
Clinical phenotype	BIFE	0	0	0	1	10.847	0.628	0.063
	IE	2	0	0	0			
	DEE	2	4	7	3			
	GE	1	0	0	1			
Total		5	4	7	5			

BIFE = benign familial infantile epilepsy; DEE = developmental and epileptic encephalopathy; GE = generalized epilepsy, frequently with absence seizures; IE = intermediate epilepsy; SCBs = sodium channel blockers; TMOs = other transmembrane segment and linking regions.

cohort, 16 out of 21 cases belonged to the DEE group, and all mutation were missense variants. The response to SCBs was not particularly satisfactory in most patients with DEE in our cohort. Thirteen out of 16 patients with DEE in our study had different degrees of response to SCBs (Table 1). In particular, three out of 13 cases (21.4%) showed spasms as the only phenotype with onset at 6 months of age with severe developmental delay/intellectual disability and hypsarrhythmia of EEG. Unfortunately, they showed a negative response to SCBs but some response to non-SCBs, including vigabatrin, levetiracetam, adrenocorticotropic hormone or perampanel. That non-SCBs controlled the seizures or SCBs deteriorated the seizures may implicate the prompt function in those variants being associated with LOF. The function of one previously published variant, i.e. p.(Arg223Gly), is controversial.[1,4] The case with the p.(Arg223Gly) variant seemed to have the clinical characteristics of 'LOF' from previous publications.[4]

Consequently, we summarize the following common characteristics based on the outcomes of 21 cases of Chinese patients with *SCN8A*-related epilepsy: (i) in many cases from our cohort, even if the children showed some response to SCBs, the frequencies of seizures could not be completely controlled by the SCBs alone; instead, combination therapy with other non-SCBs was often necessary for them; and (ii) patients with only general seizures (GE, or DEE with only epileptic spasms) after 6 months exhibited a negative response to SCBs. Therefore, clinical characteristics, including age of onset, seizure type and clinical phenotype together, will indicate the response to SCBs, helping us to select ASMs more accurately.

Second, neuroelectrophysiological methods are generally used to evaluate whether the function of a *SCN8A* missense mutation is GOF or LOF.[9,10] Also, Johannesen and colleagues[1] examined the functions of seven missense mutations. It was found that the functions corresponded to the clinical spectrum. They suggested that IE and BFIE patients had a mild GOF, while most DEE patients had a strong GOF; GE patients had a LOF, with the majority of LOF variants found in the pore region. In our cases, however, all four cases with variants in the pore area had focal seizures, with two cases from the IE group and one patient with DEE showing a good response to SCBs combined with VPA. Seizures in three cases were controlled well, and the treatment effects were better than the other cases with non-pore variants. Consequently, this indicated that the good responses trend to SCBs were in the cases with the variants in the pore area (Fisher = 7.659, *P* = 0.054, *r* = 0.517) (Table 2). According to the genotype-phenotype correlations by Johannesen and colleagues,[1] IE patients indicated a mild GOF, and the function of missense variations in the pore area need to be studied in the future. Therefore, we considered that variants in the pore area might be a good indication for the selection of ASMs.

Finally, different functional domains had different roles.[9] In our cohort, grouped by the different function domains (voltage sensor region, pore, inactivation gate + C-terminus + Loops, TMOs), the responses to SCBs were significantly different (Fisher = 17.186, *P* = 0.046, *r* = 0.671) (Table 2). This showed a possible relationship between genotype and treatment effects.

In conclusion, SCBs are the first choice of therapy for *SCN8A* epilepsy; however, responses to SCBs are closely related to clinical phenotype, genotype and the function of the *SCN8A* missense variation. Further studies are required to explore the Nav 1.6 function changes of epilepsy-related *SCN8A* missense variants and potential therapy for patients with *SCN8A*-related DEE.

Data availability

Data are available from the corresponding author on reasonable request.

Competing interests

The authors report no competing interests.

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