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SCN1A Gene Mutation and Adaptive Functioning in 18 Vietnamese Children with Dravet Syndrome

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Background and Purpose Dravet syndrome is a rare and severe type of epilepsy in infants. The heterogeneity in the overall intellectual disability that these patients suffer from has been attributed to differences in genetic background and epilepsy severity.

Methods Eighteen Vietnamese children diagnosed with Dravet syndrome were included in this study. *SCN1A* variants were screened by direct sequencing and multiplex ligation-dependent probe amplification. Adaptive functioning was assessed in all patients using the Vietnamese version of the Vineland Adaptive Behavior Scales, and the results were analyzed relative to the *SCN1A* variants and epilepsy severity.

Results We identified 13 pathogenic or likely pathogenic variants, including 6 that have not been reported previously. We found no correlations between the presence or type of *SCN1A* variants and the level of adaptive functioning impairment or severity of epilepsy. Only two of nine patients aged at least 5 years had an adaptive functioning score higher than 50. Both of these patients had a low frequency of convulsive seizures and no history of status epilepticus or prolonged seizures. The remaining seven had very low adaptive functioning scores (39 or less) despite the variability in the severity of their epilepsy confirming the involvement of factors other than the severity of epilepsy in determining the developmental outcome.

Conclusions Our study expands the spectrum of known *SCN1A* variants and confirms the current understanding of the role of the genetic background and epilepsy severity in determining the developmental outcome of Dravet syndrome patients.

Key Words dravet syndrome, Vietnamese, adaptive functioning, *SCN1A*.

INTRODUCTION

Dravet syndrome (DS) is a rare and catastrophic type of epilepsy in infants presenting in the first year of life with febrile or afebrile, generalized or unilateral, and clonic or tonic-clonic seizures that are often prolonged in children with previously normal development. Other seizure types, including myoclonic jerks and focal and atypical absence seizures, generally appear at between 1 and 4 years of age. The epilepsy is often refractory to standard antiepileptic medication, and from their second year of life the affected children often develop cognitive, behavioral, and motor impairments. The outcome of DS patients is poor, usually with intellectual disability and ongoing seizures.[1]

Since Claes et al.[2] reported in 2001 that *SCN1A* mutations caused DS, it has been found that 70–80% of DS cases are caused by mutations in *SCN1A*. These are mostly sequence mutations, with also some copy-number variants.[3] Although other genes have been recently found to be related to DS, including *GABRG2*, *SCN1B*, *GABRA1*, *STXBP1*, *PCDH19*, and *CHD2*,[4,5] *SCN1A* is still the most clinically relevant gene in DS, with more than 1,000 *SCN1A* variants having been identified.[6] However, inconsistent correlations between specific

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SCN1A mutations (presence and type) and phenotype severity (epilepsy and intellectual disability) have been reported,[7-11] and further data collection seems necessary to clarify this complex genotype-phenotype relationship. In addition, although DS is often considered an "epileptic encephalopathy" in which developmental delay is the result of severe epilepsy, there are preliminary data suggesting that the cognitive course may worsen independently from the epilepsy, thereby questioning whether *SCN1A* intrinsic alterations play a role in determining the psychomotor delay.[12]

Adaptive functioning refers to the ability to independently perform age-appropriate tasks of daily living, and represents the real-world application of intellectual abilities. Adaptive functioning is an important outcome metric of intellectual disability but has seldom been examined in children with DS. This study was the first to investigate *SCN1A* variants in Vietnamese patients and examine their adaptive functioning relative to the severity of their epilepsy and their genetic background.

METHODS

Subjects

This study was approved by the Institutional Review Board of Children Hospital 2 (reference no. CS/N2/15/01HT). The study subjects were 18 DS patients, comprising 13 males and 5 females. Written informed consent was obtained from the parents of all of the children involved in the study. The diagnostic criteria for DS included the following: normal early development, generalized or unilateral clonic seizures or myoclonic seizures beginning in the first year of life, sensitivity to fever, normal interictal EEG and normal MRI findings at onset, and the presence of afebrile seizures. The diagnosis of DS was further confirmed by the emergence of other progressive symptoms, such as slowing or arrest of cognitive development after 2 years of age, ataxic gait, pyramidal signs, persistence of clonic seizures, and continued sensitivity to fever. When other seizure types (including myoclonias) were present, typical DS was distinguished from mild or incomplete DS (IDS), i.e., borderline DS without myoclonias, and intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC), in which patients have only one type of seizure.[1,13] The epilepsy history of each patient was reviewed with particular attention to the age at seizure onset, the frequency and duration of the convulsive seizures, and the administration of antiepileptic drugs. The term "status epilepticus" (SE) was reserved for clonic seizures that continued for more than 30 minutes, while prolonged seizures were defined as seizures that lasted more than 5 minutes.

SCN1A variant analysis

Molecular analysis was performed on genomic DNA extracted from blood using standard procedures. All coding exons and flanking introns of *SCN1A* were sequenced directly from PCR products using primer pairs designed from the sequence of *SCN1A* on human chromosome 2 (GenBank accession number: NG_011906). Sequence variations were analyzed by comparison with the wild-type sequence (transcript reference NM_001165963.1). For detecting large deletions or duplications, multiplex ligation-dependent probe amplification (MLPA) analysis was performed using the SALSA MLPA Kit P137-025R (MRC Holland, Amsterdam, Netherlands) in accordance with the manufacturer's instructions. The MLPA result was then confirmed by long-range PCR and gap PCR. When an alteration was found, genetic testing of the parents was requested to search for inherited variants. To confirm the biological relationship of the probands and their parents, parentage testing was performed using the PowerPlex® Fusion System (Promega, Madison, WI, USA) in accordance with the manufacturer's instructions. For patients with a positive family history, genetic tests were also performed in other available family members.

All of the candidate variants were examined in the 50 normal control alleles and searched in global human variant databases, including 1000G (http://www.1000genomes.org) and ExAC (http://exac.broadinstitute.org/). Missense substitutions were carefully analyzed with three software programs: PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), PROVEAN (http://provean.jcvi.org/index.php), and VEP (http://grch37.ensembl.org/Homo_sapiens/Tools/VEP). The novelty of the variants was determined by searching the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) and the SCN1A mutation database (http://www.gzneurosci.com/scn1adatabase/index.php). All identified variants were interpreted and classified according to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines.[14] Variants that were single-nucleotide polymorphisms were excluded from this study.

Adaptive functioning and behavior evaluation

An adaptive functioning evaluation was performed for each patient during his or her last visit by a single psychologist using the Vietnamese version of the Vineland Adaptive Behavior Scales (VVABS). This version was translated from the 1984 version of the Vineland Adaptive Behavior Scales (VABS) and adapted to be more relevant to the Vietnamese culture.[15] The semistructured interviews addressed the parents' assessments and provided composite scores across the following four domains: communication, daily living skills, socialization, and motor skills. Behavioral problems were noted by clinical ob-

servations and the interviews with parents.

RESULTS

Patients and their epilepsy

Among the 18 children included in this study, 7 could be considered typical DS cases, 10 had IDS due to the lack of myoclonias, and 1 had ICEGTC. A positive family history for seizures was reported in four patients (22%): patient 1 (male), patient 2 (female), patient 4 (male), and patient 16 (male).

The clinical characteristics and epilepsy severity of the patients are presented in Table 1. At the last follow-up the patients were aged from 1 year and 9 months to 8 years and 8 months. All of the patients had their first seizures at an age of 7±1 month (mean±standard error; range=3–10 months). A convulsive status that continued for more than 30 minutes was reported in eight patients (44%). Repetitive seizures, which were defined as seizures occurring in groups over periods of several hours or days, were common during febrile illness episodes and were reported in nine patients (50%). In most cases of repetitive seizures it was not clear if the patient regained consciousness between seizures, and so we did not categorize repetitive seizures as SE. There was a trend toward the epilepsy improving after 4 years, and there were five cases with nearly remitted seizures for at least 19 months prior to the last follow-up. A history of misdiagnosis and mistreatment with lamotrigine and oxcarbazepine was common, especially in older patients. Clobazam and stiripentol are not yet officially available in Vietnam. Only 1 of the 18 patients in our cohort had tried clobazam (patient 12). This patient had experienced approximately three seizures per month until the age of 4 years despite receiving treatment with valproic acid, levetiracetam, and topiramate. At 4 years of age he was switched to clobazam in combination with low-dose topiramate, and this significantly decreased his seizure frequency to several febrile seizures per year for nearly 2 years until the last follow-up. His average seizure duration also decreased from several minutes to less than 1 minute.

SCN1A variant analysis

Of the 18 patients examined, 13 (72%) carried *SCN1A* variants: 12 were detected by direct sequencing, and 1 large deletion of approximately 170 bp encompassing 64 bp of exon 7 was detected by MLPA and confirmed by long-range PCR and gap PCR (Table 2). Four of the variants detected by direct sequencing were truncation variants (three nonsense and one small deletion), and eight were missense variants. Six of the eight missense variants were located in the ion-pore region (S5, S6, or S5–S6 linker), and one (c.602C>A) could cause a donor loss in the splice-site region. None of these 13 variants were found in the 50 normal control alleles or reported in the global human variant databases, including 1000G and ExAC.

The PolyPhen-2, VEP, and PROVEAN software programs predicted that all eight missense variant would be highly damaging or deleterious. Multiple sequence alignments performed using PolyPhen-2 showed that all of the substitutions involved highly conserved amino acid residues. This analysis also showed that a novel variant (c.602C>A) that occurs at the first base in the exon region of the intron-exon boundary could lead to a truncation due to its possible effect on the splicing of the messenger ribonucleic acid. Six of the 13 detected variants have not been reported previously. The parental DNAs of 10 of the variant-positive cases were available for variant confirmation and parentage testing- and revealed that all of the variants occurred *de novo*. Of four patients with a positive family history, no *SCN1A* variant was identified in patient 1 and so genetic testing of the other family members of this patient was not performed. The genetic testing of the older sister of patient 4, who had a history of febrile seizure, and his unaffected parents revealed that three of them did not carry the variant found in the patient. Genetic testing was not performed on the family members of patients 2 and 16 due to them either being unavailable or unwilling to undergo testing. Using the guidelines developed by the ACMG for the interpretation of sequence variants, 11 of 13 variants were classified as "pathogenic" variants and the remaining 2 were classified as "likely pathogenic" variants.[14]

Adaptive functioning evaluation of the patients and correlation analysis

All of the children who were aged 3 years or older exhibited a marked slowing of psychomotor development, which was accompanied by behavior disorders. Hyperactivity and opposing and provocative behavior were obvious in all but two of the patients. Although most of the patients were not definitively diagnosed with autism, they had common autistic features, including poor eye contact, poor relational capacities, and difficulty following social rules. They also had restricted interests, poor danger awareness, and a poor ability to express emotions. During the last examination, all of the patients were assessed using the VVABS semistructured interview. Their adaptive functioning development showed a steep decline from 1 to 5 years of age, after which it appeared to stabilize (Fig. 1). In patients aged 5 years or younger, the total VVABS score was 65±5 (n=9, range=42–87), and only two of them (patients 8 and 9) had a VVABS score of <50. In contrast, the total VVABS score in patients older than 5 years was 32±4 (n=9, range=19–53), and only two of them (patients 12 and 16) had a VVABS score of >50 (Table 3). These

Table 1. Summary of clinical characteristics of 18 patients

Patient, gender	Age of last exam (y:m)	Age of seizure onset (months)	Type of DS	Number of SE	Number of cluster seizures	Frequency of convulsive seizure and age of changing (y:m)	Seizures often prolonged (>5 mins)	Total AED received	AED at last exam
1, M	1:9	4	IDS	3	2	(++)	(+)	VPA, CZP	VPA, CZP
2, F	2:3	4	IDS	7	6	(++)	(+)	VPA	VPA
3, M	3:0	4	DS	6	0	(++)	(+)	VPA	VPA
4, M	3:1	5	IDS	18	0	(++)	(+)	VPA, TPM	VPA, TPM
5, F	3:3	8	IDS	0	4	(++)	(+)	VPA, LEV, LTG	VPA, LEV
6, M	3:5	6	IDS	0	3	(++) $\underset{3:0}{\rightarrow}$ (+++)	(-)	VPA, CZP, LTG	VPA, CZP
7, M	3:7	9	IDS	0	0	(++)	(-)	VPA	VPA
8, M	3:11	4.5	DS	19	3	(++)	(+)	VPA, OXC, TPM, LEV, CZP	VPA, TPM, CZP
9, M	4:7	8	IDS	0	0	(++)	(-)	VPA, LTG, LEV	VPA, LEV
10, M	5:6	10	DS	0	0	(++) $\underset{3:10}{\rightarrow}$ (+)	(-)	VPA, OXC	(-)
11, F	5:7	10	IDS	0	0	(++) $\underset{4:0}{\rightarrow}$ (+)	(-)	VPA	VPA
12, M	5:8	8	DS	0	4	(++) $\underset{4:0}{\rightarrow}$ (+)	(-)	VPA, OXC, LEV, TPM, CLB	TPM, CLB
13, M	6:2	8	DS	0	0	(++) $\underset{1:6}{\rightarrow}$ (+) $\underset{3:0}{\rightarrow}$ (++++) $\underset{5:6}{\rightarrow}$ (+++)	(-)	VPA, OXC, CZP, TPM, LEV	VPA, LEV, TPM
14, M	7:1	7	ICEGTC	2	0	(++) $\underset{3:0}{\rightarrow}$ (+++) $\underset{5:11}{\rightarrow}$ (++)	(-)	VPA, PB, CZP	VPA, CZP
15, F	7:4	3.5	IDS	5	0	(++++) $\underset{0:8}{\rightarrow}$ (+++) $\underset{1:6}{\rightarrow}$ (++)	(+)	VPA, PB, TPM, LEV	VPA, PB
16, M	8:4	6	IDS	0	>10	(++) $\underset{5:6}{\rightarrow}$ (+)	(-)	VPA, OXC, LEV, TPM	TPM
17, M	8:4	4	DS	0	>10	(++++)	(-)	VPA, OXC, LTG, CBZ, PB, TPM	VPA, TPM
18, F	8:8	8	DS	1	>10	(++) $\underset{1:1}{\rightarrow}$ (+++) $\underset{5:0}{\rightarrow}$ (+)	(-)	VPA, TPM, LEV, CBZ	LEV, CBZ

(+): <4 seizures/year, (++): 1–4 seizures/month, (+++): 5–10 seizures/month, (++++): >10 seizures/month. AED: antiepileptic drugs, CBZ: carbamazepine, CLB: clobazam, CZP: clonozepam, F: female, LEV: levetiracetam, LTG: lamotrigine, M: male, mins: minutes, OXC: oxcarbazepine, PB: phenobarbital, VGB: vigabatrin, VPA: valporic acid, y:m: year:month.

Table 2. Summary of SCN1A variants found in 18 patients

Patient, gender	Exon	Type of variants	cDNA	Protein	Subunit location	Inheritance	Previous report	ACMG-based classification and supporting evidence
1, M	Negative							
2, F	21	Missense	c.4088T>A	p.I1363N	DIIIS5	N/A	DS	Pathogenic (PS1, PM1, PM2, PM6, PP2, PP3, PP4)
3, M	4	Missense	c.602C>A	p.A201E	DIS3	*De novo*	Novel	Likely pathogenic (PS2, PM2, PP2, PP3, PP4)
4, M	15	Missense	c.2792G>A	p.R931H	DIIS5-S6	*De novo*	DS	Pathogenic (PS1, PS2, PM1, PM2, PP2, PP3, PP4)
5, F	21	Missense	c.4246G>C	p.D1416H	DIIIS5-S6	N/A	DS, Unknown	Likely pathogenic (PM1, PM2, PM6, PP2, PP3, PP4)
6, M	22	Missense	c.4313T>C	p.M1438T	DIIIS5-S6	*De novo*	Novel	Pathogenic (PS2, PM1, PM2, PP2, PP3, PP4)
7, M	11	Missense	c.1876A>G	p.S626G	DI-DII	*De novo*	DS, CGE (FS)	Pathogenic (PS1, PS2, PM2, PP2, PP3, PP4)
8, M	24	Truncation	c.4573C>T	p.R1525X	L3	*De novo*	DS	Pathogenic (PS1, PVS1, PS2, PM2, PP4)
9, M	Negative							
10, M	Negative							
11, F	15	Missense	c.2906T>C	p.L969P	DIIS6	*De novo*	Novel	Pathogenic (PS2, PM1, PM2, PP2, PP3, PP4)
12, M	7	Missense	c.1007G>A	p.C336Y	DIS5-S6	*De novo*	DS	Pathogenic (PS1, PS2, PM1, PM2, PP2, PP3, PP4)
13, M	Negative							
14, M	12	Truncation	c.2134C>T	p.R712X	DI-DII	*De novo*	DS	Pathogenic (PVS1, PS1, PS2, PM2, PP4)
15, F	24	Truncation	c.4503delA	p.T1501fs	DIII-DIV	*De novo*	Novel	Pathogenic (PVS1, PS2, PM2, PP2, PP4)
16, M	13	Truncation	c.2259T>G	p.Y753X	DI-DII	N/A	Novel	Pathogenic (PVS1, PM2, PM6, PP4)
17, M	7	Large deletion	Exon 7 deletion		DIS5-S6	*De novo*	Novel	Pathogenic (PVS1, PS2, PM2, PP4)
18, F	Negative							

ACMG: American College of Medical Genetics and Genomics, cDNA: complementary deoxyribonucleic acid, CGE: cryptogenic generalized epilepsy, DS: Dravet syndrome, F: female, FS: febrile seizures, M: male.

two patients, who had the most favorable adaptive functioning outcome, had a low frequency of convulsive seizures and no history of SE or prolonged seizures.

The small number of included patients and their age heterogeneity meant that statistical analysis could not be performed. However, it is likely that there are no clear relationships between the genetic background (presence and type of *SCN1A* variants) and adaptive functioning level. Patients of a similar age with null variants (truncations or large deletions), missense variants, or who do not carry *SCN1A* variants could have different levels of adaptive functioning impairment. Beyond the age of 5 years, patients with the worst adaptive functioning levels (VVABS score <30) carried either null variants or missense variants or did not carry *SCN1A* variants. Patients with the most favorable outcomes (VVABS score >50) also carried either null variants or missense variants. A similar analysis also showed no correlation between the genetic background and epilepsy severity (including type of DS, SE, or duration and frequency of convulsive seizures).

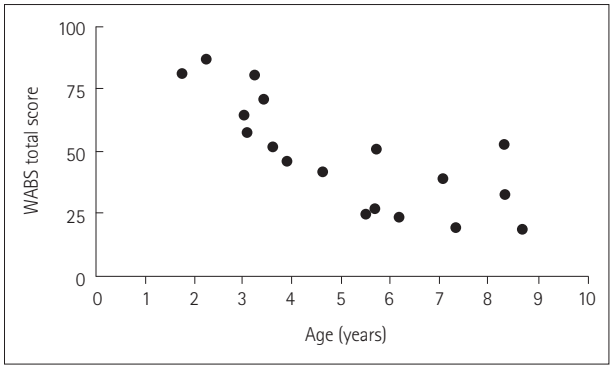

Fig. 1. Adaptive functioning as assessed using the Vietnamese version of the Vineland Adaptive Behavior Scales (VABS) in all 18 included patients during their last visit.

A particularly interesting finding from analyzing the correlation between epilepsy and the adaptive functioning level is that the two patients older than 5 years with the most favorable adaptive functioning (patients 12 and 16) had only mild epilepsy, while all of the remaining patients in that age group had a low adaptive functioning level, although with some variability in the severity of their epilepsy. In particular, there were two patients with very low adaptive functioning scores who had relatively mild epilepsy (patients 10 and 11). Both of these patients had no history of SE or prolonged seizures and had a low frequency of convulsive seizures.

DISCUSSION

The presence of myoclonic jerks has been considered a highly characteristic feature of DS, but their absence does not exclude a clinical diagnosis of DS.[16] Only 7 of the 18 patients (39%) in this study had myoclonias and could be considered as having complete/typical DS. This proportion is reasonably consistent with the findings of previous studies.[13,17] An improvement in epilepsy was observed in most of the patients

Table 3. Adaptive functioning scores using Vietnamese version of the Vineland Adaptive Behavior Scales (VABS)

Patient, age (year:month)	Communication	Daily living skill	Socialization	Motor skills	Total
1 (1:9)	90	62	86	86	81
2 (2:3)	100	78	89	81	87
3 (3:0)	44	64	58	92	65
4 (3:1)	49	57	57	70	58
5 (3:3)	92	90	74	67	81
6 (3:5)	66	59	73	85	71
7 (3:7)	42	53	54	60	52
8 (3:11)	43	45	45	49	46
9 (4:7)	32	47	34	55	42
10 (5:6)	6	26	27	42	25
11 (5:8)	19	32	23	35	27
12 (5:8)	52	56	41	53	51
13 (6:2)	22	26	24	24	24
14 (7:1)	40	45	34	38	39
15 (7:4)	11	27	13	30	20
16 (8:4)	71	47	40	Nd	53
17 (8:4)	22	34	45	31	33
18 (8:8)	14	22	11	29	19
Mean	45	48	46	55	49
Standard error	7	4	6	5	5

after approximately 4 years. In five patients, the seizures mostly remitted (to less than four per year) for nearly 2 years. It is interesting to note that four of these five patients had never experienced SE, and the fifth had suffered only one previous episode of SE. Four of the five patients also had a history of a lower seizure frequency (from one to four seizures per month) during the worsening stage of the disease (i.e., while aged 1–5 years). The presence of a correlation between a history of SE and seizure frequency outcome during adolescence and adulthood has also been suggested by Kobayashi et al.[18] and Akiyama et al.[19]

While DS patients typically have medically refractory epilepsy and they often require polytherapy, careful pharmacological management is believed to improve their clinical picture and quality of life. Current first-line agents include valproate and clobazam, and there are also supportive data for topiramate, levetiracetam, and stiripentol. Among these medications, clobazam and stiripentol are not officially available in Vietnam yet. Clobazam was administered to only one patient (patient 12) after he became refractory to valproic acid, oxcarbazepine, levetiracetam, and topiramate, and his seizures subsequently decreased significantly. This response supports the efficacy of clobazam in DS treatment and the necessity for patients in developing countries such as Vietnam to receive new medications when their current treatments fail.

Most recent studies have detected *SCN1A* mutations in patients with DS at rates of 70–80%. Missense mutations and truncation mutations (referring to both frame-shift and nonsense mutations) account for approximately 90% of the detected changes, with the remaining mutation types including splice-site mutations and large deletions or duplications.[5] In the present study, 13 variants (72%) were identified in 18 patients, comprising 8 missense variants, 4 small truncating variants, and 1 large deletion. Therefore, the proportion of *SCN1A* variants and the distribution of variant types in the Vietnamese DS patients in this study appeared to be consistent with those in other studied populations. In addition, variant-site analysis and parental confirmations also confirmed that there are no clear-cut mutation hotspots for *SCN1A* and that most of the disease-associated alterations are *de novo* in origin. Among the missense variants, those affecting the voltage region (region S4) and/or pore-forming region (region S5–6) have been found to appear at a high frequency in DS patients.[7,20-22] Although we found no variants in region S4, six of the eight missense variants were found in the pore-forming region, confirming a predominant localization of missense variants in that region. Distinct from truncation and splice-site variants, the effects of missense variants are more complex and their association with the phenotypic expression of DS is more controversial. Therefore, all eight missense variants were carefully analyzed using three annotation programs (PolyPhen-2, VEP, and PROVEAN) to predict the effect of the amino acid substitutions on protein function. All of the analyses showed a significant consistency between the prediction of protein function damage and the clinical expression of DS in the patients.

As widely reported in the literature, the psychomotor development of children with DS follows the characteristic pattern of a steep decline between 1 and approximately 5 years of age, followed by stabilization.[9,11,23,24] Most studies have assessed patients using developmental quotient (DQ) or intelligence quotient (IQ) scales.[9,11,23,24] Adaptive functioning has seldom been evaluated, despite a intellectual disability diagnosis requiring the assessment of both cognitive capacity (i.e., IQ) and adaptive functioning. The present study used the VVABS—which is very popular in legal, clinical, and research contexts—to assess the adaptive functioning of all 18 patients in our cohort. All of our patients who were 3 years of age or older showed an impairment in adaptive functioning in all four test areas. The development of adaptive functioning in our cohort continued to decline until the age of 5 years, after which it remained stable. Villeneuve et al.[25] recently used VABS to study the adaptive functioning of 21 DS patients aged 6–10 years. Their adaptive functioning scores appeared to be higher (VABS score=50±3, n=20, range=28–76) than those in our cohort. Variability in developmental scores has also been found in other studies that have used DQ/IQ scales to assess patients.[9,11,23,24] This wide range of developmental outcomes may be explained by the variability of the phenotypes, antiepileptic drug treatment, familial environment, and educational intervention. As mentioned above, many of the patients in our cohort, especially the older patients, had been misdiagnosed and inappropriately treated previously. In addition, most of them stayed at home and did not receive any special treatments such as physiotherapy or speech therapy, nor did they participate in any other type of special education program.

The overall analysis of the present cohort did not reveal any correlations between the genetic background (presence and type of *SCN1A* variant) and phenotype severity. Indeed, the degree to which individuals are affected by an *SCN1A* mutation is difficult to predict. Missense mutations can alter protein function in various ways, from very minor changes in protein function to complete abolition. As mentioned above, mutations affecting the voltage region (region S4) and/or pore-forming region (region S5–6) were found frequently in DS patients. Missense mutations in these key functional regions of *SCN1A* can be as deleterious as a truncating mutation, whereas truncating mutations do not always

lead to the most severe phenotypes. Milder phenotypes (other than DS) have been sporadically reported to be associated with *SCN1A* truncation mutations. There are rare familial DS cases of an *SCN1A* truncation mutation being transmitted by an asymptomatic or mildly symptomatic parent. These cases highlight the phenotypic importance of nongenotypic effects, such as other genes and environmental factors.[26,27] Similarly, for patients without identified *SCN1A* mutations, the degree of phenotype severity is difficult to predict due to the various possible genetic and other influences. Most previous studies have failed to find any significant genotype or phenotype correlations in the context of *SCN1A* mutations and DS.[9-12,21]

DS has been referred to as an epileptic encephalopathy, a condition in which developmental deterioration or delay results from severe epilepsy.[23,28,29] Like other authors,[9,23,24] we found that the adaptive functioning levels declined until the age of 5 years in our cohort, after which they remained approximately stable. This pattern parallels the following two-stage evolution of epilepsy: more active in the first few years followed by improvement after 5 years of age. We also observed that the two patients aged 5 or older with the most favorable adaptive functioning levels (VVABS scores of 51 and 53) both had a lower frequency of convulsive seizures and had never experienced SE or prolonged convulsive seizures. On the other hand, the patients with a high frequency of seizures all had lower VVABS scores of 19–39. These observations confirm that epilepsy is a contributing factor to the neurocognitive outcome. Correlations between the cognitive and/or behavioral impairments and the frequency of convulsive seizures have been reported previously.[10,23,24] Interestingly, two of our patients had very low adaptive functioning scores despite the relative mildness of their epilepsy (patients 10 and 11), which indicates that epileptic activity is not the only cause of neurocognitive impairment in DS. Recent studies using the DS mouse model generated by knocking out *SCN1A* (*SCN1A*$^{+/-}$) have demonstrated the involvement of different neuronal networks across the brain and support channelopathy being the cause of DS.[29] Down-regulation of Nav1.1 did not cause seizures but lead to difficulties with spatial recognition, demonstrating that channel dysfunction rather than the epileptic activity itself contributed to the cognitive and behavioral impairments.[30] In a recent prospective neuropsychological evaluation of 67 patients with DS, Nabbout et al.[31] also found no correlations between DQ/IQ and most of the epilepsy variables that they evaluated. Those authors concluded that encephalopathy in children with DS is not a pure consequence of epilepsy. Together these findings indicate the importance of recognizing that encephalopathy in DS may be a product of an underlying cause, which is predominantly *SCN1A* mutations, the result of an epileptic process, or a combination of both of these factors.

In summary, this is the first study of DS in a Vietnamese population. The epilepsy course, *SCN1A*-mutation findings, and genotype-phenotype correlations in the present cohort were largely consistent with the findings of previous studies involving other populations. The lower adaptive functioning levels that we observed probably reflect suboptimal diagnoses, treatment, and educational support. Our analysis of the correlation between epilepsy and adaptive functioning confirmed the recent suggestions that encephalopathy in DS is caused by both the epileptic process and other underlying factors.

Conflicts of Interest

The authors have no financial conflicts of interest.

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