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

Thi Thu Hang Do, Diem My Vu, Thi Thuy Kieu Huynh, Thi Khanh Van Le, Eun-Hwa Sohn, Thieu Mai Thao Le, Huu Hao Ha, Chi Bao Bui

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.4 However, inconsistent correlations between specific genetic background and epilepsy severity indicate that other factors in addition to the presence of a SCN1A variant should be considered in determining the outcome of these patients.

These three authors contributed equally to this work as first authors.
SCN1A mutations (presence and type) and phenotype severity (epilepsy and intellectual disability) have been reported7,11 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.13,13 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.gene-neurosci.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 (VABS). 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.

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                           | [++] $\rightarrow$ [+] $\rightarrow$ [+++ $\rightarrow$ 3:0] | (-)                               | 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                           | [++] $\rightarrow$ [+] $\rightarrow$ [3:10]           | (-)                               | VPA, OXC          | (-)              |
| 11, F           | 5:7                    | 10                            | IDS        | 0            | 0                           | [++] $\rightarrow$ [+] $\rightarrow$ [4:0]           | (-)                               | VPA               | VPA             |
| 12, M           | 5:8                    | 8                             | DS         | 0            | 4                           | [++] $\rightarrow$ [+] $\rightarrow$ [4:0]           | (-)                               | VPA, OXC, LEV, TPM, CLB | TPM, CLB       |
| 13, M           | 6:2                    | 8                             | DS         | 0            | 0                           | [++] $\rightarrow$ [+] $\rightarrow$ [+++ $\rightarrow$ 3:0] $\rightarrow$ [+++ $\rightarrow$ 5:6] | (-)                               | VPA, OXC, CZP, TPM, LEV | VPA, LEV, TPM |
| 14, M           | 7:1                    | 7                             | ICEGTC     | 2            | 0                           | [++] $\rightarrow$ [+++ $\rightarrow$ 3:0] $\rightarrow$ [+++ $\rightarrow$ 5:11] | (-)                               | VPA, PB, CZP      | VPA, CZP         |
| 15, F           | 7:4                    | 3.5                           | IDS        | 5            | 0                           | [+++ $\rightarrow$ [+++ $\rightarrow$ [+] $\rightarrow$ [+] $\rightarrow$ 0:8] $\rightarrow$ [+] $\rightarrow$ [+] $\rightarrow$ 1:6 | (+)                               | VPA, PB, TPM, LEV | VPA, PB         |
| 16, M           | 8:4                    | 6                             | IDS        | 0            | >10                         | [++] $\rightarrow$ [+] $\rightarrow$ [5:6]           | (-)                               | 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                         | [++] $\rightarrow$ [+++ $\rightarrow$ [+] $\rightarrow$ [+] $\rightarrow$ 1:1 $\rightarrow$ [+] $\rightarrow$ [+] $\rightarrow$ 5:0 | (-)                               | 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: clonazepam, 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            | 1    | Negative         |      |         |                  |             |                 |                                                  |
| 2, F            | 21   | Missense         | c.4088T>A | p.I1363N | DIII5            | 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  | DIII5-S6         | De novo     | DS              | Pathogenic (PS1, PS2, PM1, PM2, PM6, PP2, PP3, PP4) |
| 5, F            | 21   | Missense         | c.4246G>C | p.D1416H | DIII5-S6         | N/A         | DS, Unknown     | Likely pathogenic (PM1, PM2, PM6, PP2, PP3, PP4) |
| 6, M            | 22   | Missense         | c.4313T>C | p.M1438T | DIII5-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            | 7    | Missense         | c.1876A>G | p.S626G  | DI-DII           | De novo     | DS              | Pathogenic (PS1, PS2, PM1, PM2, PP2, PP3, PP4)   |
| 10, M           |      |                  |      |         |                  |             |                 |                                                  |
| 11, F           | 15   | Missense         | c.2906T>C | p.L969P  | DIIIS6           | 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           |      |                  |      |         |                  |             |                 |                                                  |
| 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 | DIIIS6-S6 |             | De novo     | Novel           | Pathogenic (PVS1, PS2, PM2, PP4)                |
| 18, F           |      |                  |      |         |                  |             |                 |                                                  |

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).

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. 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. 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 (VVABS)

| 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    |

**Fig. 1.** Adaptive functioning as assessed using the Vietnamese version of the Vineland Adaptive Behavior Scales (VVABS) in all 18 included patients during their last visit.
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 the history of SE and seizure frequency outcome during adolescence and adulthood has also been suggested by Kobayashi et al.\textsuperscript{18} and Akiyama et al.\textsuperscript{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.\textsuperscript{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 \textit{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.\textsuperscript{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.\textsuperscript{9,11,23,24} Most studies have assessed patients using developmental quotient (DQ) or intelligence quotient (IQ) scales.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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. 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.

DS has been referred to as an epileptic encephalopathy, a condition in which developmental deterioration or delay results from severe epilepsy. Like other authors, 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. 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. 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. In a recent prospective neuropsychological evaluation of 67 patients with DS, Nabbout et al. 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.

Acknowledgements
This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106-YS.05-2013.05.

The authors thank Dr. Anh Tuan Nguyen, (Department of Diagnostic Imaging, Children Hospital 1, Ho Chi Minh City, Vietnam) for interpreting the MRI images and Dr. Pierre Jallon (Children Hospital 2, Ho Chi Minh City) for assistance with the preparation of the manuscript.

REFERENCES
1. Dravet C. The core Dravet syndrome phenotype. Epilepsia 2011;52 Suppl 2:S–9.
2. Claes L, Del-Fuvero 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. Scheffer IE. Diagnosis and long-term course of Dravet syndrome. Eur J Paediatr Neurol 2012;16 Suppl 1:S3-S8.
4. Carvill GL, Weckhuysen S, McMahon JM, Hartmann C, Møller RS, Hjalgrim H, et al. GABRA1 and STXBP1: novel genetic causes of Dravet syndrome. Neurology 2014;82:1245-1253.
5. Suls A, Jaehn JA, Kecskés Á, Weber Y, Weckhuysen S, Craiu DC, et al. De novo loss-of-function mutations in CHD2 cause a fever-sensitive myoclonic epileptic encephalopathy sharing features with Dravet syndrome. Am J Hum Genet 2013;93:967-975.
6. Parihar R, Ganesh S. The SCN1A gene variants and epileptic encephalopathies. J Hum Genet 2013;58:573-580.
7. Lim BC, Hwang H, Chae JH, Choi JE, Hwang YS, Kang SH, et al. SCN1A mutational analysis in Korean patients with Dravet syndrome. Seizure 2011;20:789-794.
8. Kwong AK, Fung CW, Chan SY, Wong VC. Identification of SCN1A and PCDH19 mutations in Chinese children with Dravet syndrome. PLoS One 2012;7:e41802.
9. Chieffo D, Battaglia D, Lettori D, Del Re M, Brogna C, Dravet C, et al. Neuropsychological development in children with Dravet syndrome. Epilepsy Res 2011;95:86-93.
10. Brunklaus A, Ellis R, Reavey E, Forbes GH, Zuberi SM, et al. Prognostic, clinical and demographic features in SCN1A mutation-positive Dravet syndrome. Brain 2012;135( Pt 8):2329-2336.
11. Ragona F, Granata T, Dalla Bernardina B, Offredi F, Darra F, Battaglia D, et al. Cognitive development in Dravet syndrome: a retrospective, multicenter study of 26 patients. Epilepsia 2011;52:386-392.
12. Brunklaus A, Zuberi SM. Dravet syndrome--from epileptic enceph-
Study of Dravet Syndrome in 18 Vietnamese Children

...pallot to channelopathy. *Epilepsia* 2014;55:979–984.
13. Guerrini R, Oguni H. Borderline Dravet syndrome: a useful diagnostic category? *Epilepsia* 2011;52 Suppl 2:10–12.
14. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405–424.
15. Goldberg MR, Dill CA, Shin JY, Nguyen VN. Reliability and validity of the Vietnamese Vineland Adaptive Behavior Scales with preschool-age children. *Res Dev Disabil* 2009;30:592–602.
16. Depienne C, Trouillard O, Saint-Martin C, Gourfinkel-An I, Bouteiller D, Carpenter W, et al. Spectrum of SCN1A gene mutations associated with Dravet syndrome: analysis of 333 patients. *J Med Genet* 2009;46:183–191.
17. Oguni H, Hayashi K, Awaya Y, Fukuyama Y, Osawa M. Severe myoclonic epilepsy in infants—a review based on the Tokyo Women's Medical University series of 84 cases. *Brain Dev* 2001;23:736–748.
18. Kobayashi K, Ohmori I, Ouchida M, Ohatsu Y. Dravet syndrome with an exceptionally good seizure outcome in two adolescents. *Epileptic Disord* 2011;13:340–344.
19. Akiyama M, Kobayashi K, Yoshinaga H, Ohatsu Y. A long-term follow-up study of Dravet syndrome up to adulthood. *Epilepsia* 2010;51:1043–1052.
20. Kanai K, Hirose S, Oguni H, Fukuma G, Shirasaka Y, Miyajima T, et al. Effect of localization of missense mutations in SCN1A on epilepsy phenotype severity. *Neurology* 2004;63:329–334.
21. Zuberi SM, Brunklas A, Birch R, Reavey E, Duncan J, Forbes GH. Genotype-phenotype associations in SCN1A-related epilepsies. *Neurology* 2011;76:594–600.
22. Marini C, Mei D, Temudo T, Ferrari AR, Buti D, Dravet C, et al. Idiopathic epilepsies with seizures precipitated by fever and SCN1A abnormalities. *Epilepsia* 2007;48:1678–1685.
23. Wolff M, Cassé-Perrot C, Dravet C. Severe myoclonic epilepsy of infants (Dravet syndrome): natural history and neuropsychological findings. *Epilepsia* 2006;47 Suppl 2:45–48.
24. Ragona E, Brazzo D, De Giorgi L, Morbi M, Frieri E, Teutonico F, et al. Dravet syndrome: early clinical manifestations and cognitive outcome in 37 Italian patients. *Brain Dev* 2010;32:71–77.
25. Villeneuve N, Laguitton V, Viellard M, Lépine A, Chabrol B, Dravet C, et al. Cognitive and adaptive evaluation of 21 consecutive patients with Dravet syndrome. *Epilepsy Behav* 2014;31:143–148.
26. Yu MJ, Shi YW, Gao MM, Deng WY, Liu XR, Chen L, et al. Milder phenotype with SCN1A truncation mutation other than SMEI. *Seizure* 2010;19:443–445.
27. Gennaro E, Veggiotti P, Malacarne M, Madia F, Cecconi M, Cardinalli S, et al. Familial severe myoclonic epilepsy of infancy: truncation of Nav1.1 and genetic heterogeneity. *Epileptic Disord* 2003;5:21–25.
28. Dravet C. How Dravet syndrome became a model for studying childhood genetic epilepsies. *Brain* 2012;135(Pt 8):2309–2311.
29. Catanzaro CB, Liu JY, Liagkouras J, Gibbons VS, Labrum RW, Ellis R, et al. Dravet syndrome as epileptic encephalopathy: evidence from long-term course and neuropathology. *Brain* 2011;134(Pt 10):2982–3010.
30. Bender AC, Natola H, Ndong C, Holmes GL, Scott RC, Lenck-Santiini PP. Focal Scn1a knockdown induces cognitive impairment without seizures. *Neurobiol Dis* 2013;54:297–307.
31. Nabbout R, Chemaly N, Chipaux M, Barcia G, Bouix C, Dubouch C, et al. Encephalopathy in children with Dravet syndrome is not a pure consequence of epilepsy. *Orphanet J Rare Dis* 2013;8:176.