＜用法・用量＞

10%グリセロール 5～10 ml/kg/回を 1 日 3～4 回，30 分～1 時間で点滴静注する。

＜副作用・注意点＞

原因不明の代謝性アシドーシスや低血糖，高乳酸血症，けいれん重積，意識障害が認められる症例では，代謝異常症（先天性のグリセリン・果糖代謝異常症，シトルリン血症，ミトコンドリア異常症など）の疑いがもたれるので，その場合はグリセロールを投与せずマンニトールを選択する[4]。また，脂肪酸代謝異常の推定される Reye 症候群でも投与を避ける[5]。糖代謝に干渉するので高血糖をひき起こす可能性があり，血糖のモニターをする[5]。

2）ステロイド薬

a）デキサメサゾン（販売名：デカドロンほか）

＜作用機序＞

副腎皮質ホルモンによる抗炎症作用，損傷脳毛細管の透過性亢進の防止，脳血液関門の安定化と修復作用，脳循環代謝の改善，脳脊髄液の産生の抑制などの作用により血管性浮腫に対して有効である。細胞性浮腫には無効とされている[2,3]。

＜用法・用量＞

デキサメサゾンを初回 0.2 mg/kg/回を静注し，以後，0.1 mg/kg/回を 1 日 4 回静注する。

＜副作用・注意点＞

消化性潰瘍の合併予防に H_2 ブロッカーなどの抗潰瘍薬を併用する。そのほか高血圧や高血糖，易感染性に注意する。

b）メチルプレドニゾロン（販売名：ソル・メドロールほか）

＜作用機序＞

メチルプレドニゾロンの中枢神経系への移行は良好で，中枢神経系内の高サイトカイン状態や高サイトカイン血症の抑制に有効と考えられる。また，脳浮腫を軽減する効果もある。インフルエンザ脳症の全国調査の解析から，早期（脳症発症 1～2 日目）にメチルプレドニゾロンパルス療法を行った症例で予後が比較的良好であったというデータが得られている[6]。

＜用法・用量＞

メチルプレドニゾロン 30 mg/kg/回（最大量 1 日 1 g）を 2 時間かけて点滴静注する。原則として 3 日間投与し，1 週間ごとに 2～3 クール行う。

＜副作用・注意点＞

ステロイド薬により凝固が亢進するため，血栓形成の予防として，パルス療法終了翌日までヘパリン 100～150 IU/kg/日，持続点滴による抗凝固療法を併用する。

3）静注バルビタール薬

抗けいれん療法だけでなく，鎮静による脳保護療法として用いられるが，通常人工呼吸管理下に投与する。なお，頻用されていたペントバルビタール（販売名：ネンブタール）は 2007 年をもって製造・販売が中止された。

a）チオペンタールナトリウム（販売名：ラボナール）

＜作用機序＞

即効性がある静脈麻酔薬である。脳代謝の低下や脳血管を収縮させ脳血流が低下することにより，頭蓋内圧を低下させる。そのほか細胞膜安定化作用も有する。

＜用法・用量＞

初回 0.5 mg/kg をゆっくり静注し，以後 2～5 mg/kg/時で持続点滴する（最大 10 mg/kg/時まで）。10 mg/ml になるように調整し（チオペンタールナトリウム 1A〔500 mg〕20 ml＋注射用蒸留水 30 ml），シリンジポンプで持続点滴すると管理しやすい。

＜副作用・注意点＞

呼吸抑制，血圧低下，腸蠕動抑制には注意を要する。

輸液や他の薬剤との混合により白色の沈殿を生じルートが閉塞するため，単独ルートから投与する。本剤は強アルカリ（pH 10～11）のため，静脈外漏出による組織壊死や静脈炎を生じるため，中心静脈からの投与が望ましい。そのほか Na を多く含むため高 Na 血症に注意する。

4）脳保護薬

a）エダラボン（販売名：ラジカット）

＜作用機序＞

エダラボンは，脳血管や神経細胞の障害因子であるフリーラジカルを消去することにより脳保護作用を示す。本剤は発症後 24 時間以内の急性期脳梗塞に伴う神経徴候，日常生活動作障害，機能障害の改善に効果を示すとされ，小児においても脳梗塞やインフルエンザ脳症，急性脳症などの小児急性神経疾患の治療に本剤を試みた症例報告が散見されるようになった。

＜用法・用量＞

本剤は現時点で小児では保険適用外であり，小児に対する用法・用量は決定されていない。小児に対し投与した報告では，エダラボン 0.5～1.5 mg/kg/日を生理食塩水に溶解し，1 日 2 回の点滴静注，7～14 日間投与していた施設が多かったとされている[7]。

＜副作用・注意点＞

小児例における副作用発現率は 5.04%（6 例/119 例）で，肝機能異常が 2.52%（3 例）で最も多かったが，小児特有の副作用はみられなかったと報告されている[7]。

糖を含む輸液と混合すると，本剤の濃度低下をきたすことがあるため，生理食塩水で希釈する。

4．外科的治療

保存的療法で頭蓋内圧の制御が困難な場合，外科的治療を選択することになる。その適応については小児科医のみならず，脳神経外科医との十分な連携が不可欠である。

1）基礎疾患の治療

2）外減圧術

3）内減圧術

4）脳室ドレナージ

Key Points

❶ 基礎疾患の治療と並行し，迅速に対応することが重要である。

❷ 原因となる疾患が判明している場合，その疾患に対する治療が最優先される。

❸ 呼吸，循環，体温，栄養管理などの全身管理が重要である。

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ORIGINAL ARTICLE

Autosomal dominant nocturnal frontal lobe epilepsy: a genotypic comparative study of Japanese and Korean families carrying the *CHRNA4* Ser284Leu mutation

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Autosomal dominant nocturnal frontal lobe epilepsy is a familial partial epilepsy syndrome and the first human idiopathic epilepsy known to be related to specific gene defects. Clinically available molecular genetic testing reveals mutations in three genes, *CHRNA4*, *CHRNB2* and *CHRNA2*. Mutations in *CHRNA4* have been found in families from different countries; the Ser280Phe in an Australian, Spanish, Norwegian and Scottish families, and the Ser284Leu in a Japanese, Korean, Polish and Lebanese families. Clear evidence for founder effect was not reported among them, including a haplotype study carried out on the Australian and Norwegian families. Japanese and Koreans, because of their geographical closeness and historical interactions, show greater genetic similarities than do the populations of other countries where the mutation is found. Haplotype analysis in the two previously reported families showed, however, independent occurrence of the Ser284Leu mutation. The affected nucleotide was highly conserved and associated with a CpG hypermutable site, while other *CHRNA4* mutations were not in mutation hot spots. Association with a CpG site accounts for independent occurrence of the Ser284Leu mutation.
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Keywords: acetylcholine receptor; autosomal dominant nocturnal frontal lobe epilepsy; epilepsy; founder effect; mutation

INTRODUCTION

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE; MIM 118504) is a familial partial epilepsy syndrome characterised by clusters of brief frontal lobe motor seizures during drowsiness or sleep.[1,2] Seizures—often misdiagnosed as other nocturnal motor activities such as parasomnia or night terror[1–4]—vary from simple arousals to hyperkinetic activity with tonic or dystonic features. Onset usually occurs during the first two decades (mean age 10 years), but later onset has also been reported.[5]

ADNFLE is the first human idiopathic epilepsy known to be related to specific gene defects.[6] Clinically available molecular genetic testing reveals mutations in three genes encoding the α4, β2 and α2 subunits of the neuronal nicotinic acetylcholine receptor (*CHRNA4*, *CHRNB2* and *CHRNA2*, respectively).[7–9] Overall mutations are found in less than 20% of individuals with ADNFLE/NFLE phenotypes, suggesting their genetic heterogeneity.[10] Approximately 10–20% of patients have a positive family history and fewer than 5% a negative one.[11]

Among the four mutations in *CHRNA4* (Ser280Phe, Ser284Leu, Leu291dup and Thr293Ile), Ser280Phe and Ser284Leu have been identified in several unrelated families (mutation names may be different from those of previous papers; we use NP_000735.1): the Ser280Phe in an Australian, Spanish, Norwegian and Scottish families,[12–15] and the Ser284Leu in a Japanese, Korean, Polish and Lebanese families.[16–19] It has been assumed that founder effect is not relevant to ADNFLE, because most of the families studied come from different countries,[20] and a previous haplotype study of the Australian and Norwegian family[14] showed no genetic connection.

Japanese and Koreans, because of their geographical closeness and historical interactions, show greater genetic similarities than do the populations of other countries where the mutation is found; indeed, previous studies between Japanese and Koreans have shown a closer genetic similarities than that between other east Asians.[21,22] Founder effects between the two countries have also been reported in several autosomal recessive diseases,[23,24] however, not in autosomal dominant ones. For ADNFLE patients, however, propagating their pathological genes is easier than it is for individuals with other more visible epilepsy syndromes. They have no seizures during the daytime, show normal neurological examinations and have a normal life expectancy and reproductive capacity. In addition, family members or the affected individual himself may believe that his symptoms are not an indication of illness and thus may not seek medical attention (or, knowing himself to be ill, he may deliberately refuse to reveal his condition to others).[25] To determine whether founder effect is present

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in ADNFLE, we compare haplotype structures in the two previously reported Japanese and Korean families.

MATERIALS AND METHODS

We reviewed the medical histories and electroencephalogram findings of a three-generational family from Japan and one from Korea (pedigrees in Figure 1).

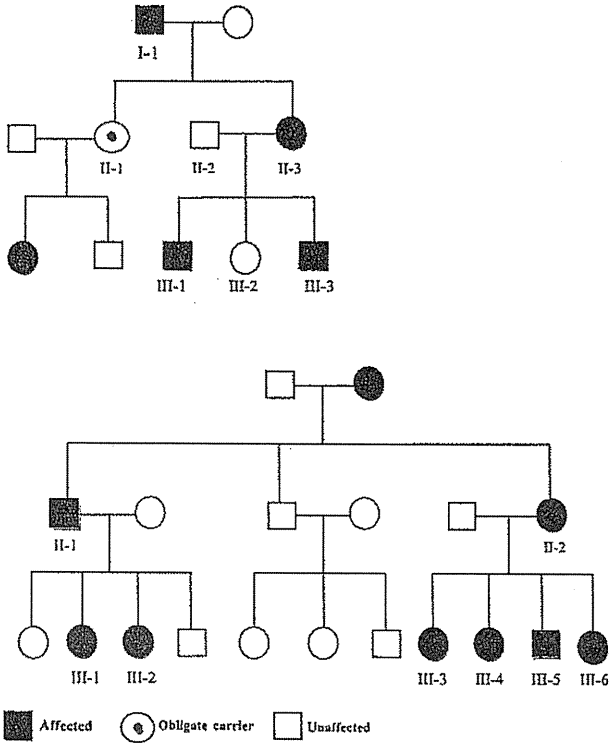

Figure 1 Pedigrees of a Japanese (the upper) and Korean (the lower) ADNFLE family. Only members-assigned numbers were evaluated.

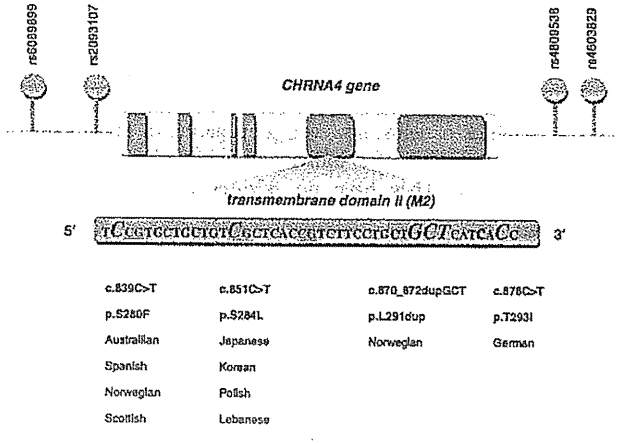

Figure 2 Schematic representation of SNPs around the *CHRNA4* gene and overview of all CHRNA4 mutations identified in ADNFLE. All the mutations are located in the second transmembrane domain (M2). Affected nucleotides are in larger italic fonts and CpG dinucleotide sites are underlined.

Samples were collected from the two families and DNA was extracted from whole blood using standard protocols. A genetic analysis was carried out for seven members of the Japanese and eight of the Korean family. Among them, four of the Japanese had ADNFLE, as did eight of the Koreans. Four single nucleotide polymorphisms (SNPs) around the *CHRNA4* were selected from a database of Japanese single nucleotide polymorphisms (JSNPS: http://snp.ims.u-tokyo.ac.jp/index_ja.html). To identify SNP-based haplotypes, four SNPs including rs 6089899, rs2093107, rs4809538 and rs4603829 (Figure 2) were typed by fluorescent sequencing on an automated DNA sequencer. Sequencing was carried out in both directions using four primer pairs. For comparison, the genotype frequencies of HapMap-JPT are listed in Table 1a.

We next examined the evolutionary conservation and phenotypic effect of Ser280Phe and Ser284Leu, which have shown recurrent occurrence in *CHRNA4*. The evolutionary conservation was estimated using phyloP score, available from UCSC Genome Browser (UCSC Genome Bioinformatics: http://genome.ucsc.edu/). The phenotypic effect of mutant protein was predicted using SIFT score (http://sift.jcvi.org/). In the phyloP, the conserved nucleotides are assigned positive scores, and the fast-evolving ones negative scores; the SIFT score ranges from 0 to 1. Deleterious amino-acid substitutions are assigned the scores of <0.05; tolerable substitutions are $\geqslant 0.05$.

We also checked hypermutable sites in *CHRNA4*. In the hypermutable sites analysis, we focused on CpG hypermutability. CpG is known as one of the major causes of codon substitution in mammalian genes, and is used to refer to cytosine followed by guanine in the Watson–Crick pair of a cytosine and guanine. The coding sequence of *CHRNA4* was analysed and the adjacent nucleotides were taken into account to distinguish CpG hypermutations from non-CpG transitions.

RESULTS

As shown in the pedigrees (Figure 1), there were five affected members and an obligated carrier in the Japanese family and nine affected members in the Korean one. Mutation in the two pedigrees showed an autosomal dominant mode with incomplete penetrance. All the affected individuals had a seizure semiology consistent with frontal lobe epilepsy and carried the Ser284Leu mutation in the *CHRNA4*. Both families had similar clinical manifestations, and their electroencephalogram findings were consistent with those of ADNFLE. They had brief motor seizures during sleep and no auras were reported. They shared features such as drug resistance and mental retardation, which are uncommon findings in ADNFLE. Still, SNP-based haplotypes were different in each family (Table 1b).

Ser280Phe and Ser284Leu showed higher phyloP scores (5.88 and 5.80, respectively) than the other nucleotides in *CHRNA4* (mean, 0.08). SIFT scores were lesser than 0.05 (0.00, each). CpG dinucleotide sites are shown in Figure 2. Nine CpG dinucleotide sites were detected in the coding sequence of *CHRNA4*, three of them in the second transmembrane domain (M2). Among the four *CHRNA4* mutations, only Ser284Leu mutation is associated with a CpG hypermutable site.

DISCUSSION

All *CHRNA4* mutations identified so far in ADNFLE have been located only in the M2 region, which has one of the most important functional roles in the neuronal nicotinic acetylcholine receptor. This receptor has a pentameric structure comprised of various combinations of alpha and beta subunits encoded by *CHRNA4* and *CHRNB2*. The overall tertiary structure of nicotinic acetylcholine receptor subunits is similar; each subunit comprises four segments (M1 to M4) of the transmembrane domain and the N and C termini on the extracellular side of the membrane. M2 lines the central pore of the receptor and determines the ion selectivity of the receptor.[26]

Table 1 Genotype frequency (a) and SNP-based haplotypes in the two families (b)

RefSNP	*HGVS names*	*Genotype frequency (HapMap-JPT)*		
(a)				
rs6089899	NT_011333.6:g.735939G>A	AA	AG	GG
		0.13	0.40	0.47
rs2093107	NG_011931.1:g.3754T>C NT_011333.6:g.730573A>G	AA	AG	GG
		0.11	0.53	0.36
rs4809538	NT_011333.6:g.706807G>A	AA	AG	GG
		0.11	0.47	0.42
rs4603829	NT_011333.6:g.705523C>T	CC	CT	TT
		0.13	0.53	0.33

	rs 6089899	*rs 2093107*	*rs 4809538*	*rs 4603829*
(b)				
Japanese family	**G**	**G**	**G**	**T**
I-1	G/G	G/G	G/G	T/T
II-1	G/G	G/G	A/G	C/T
II-2	G/G	G/G	G/G	T/T
II-3	ND	G/G	ND	T/T
III-1	A/G	G/G	G/G	T/T
III-2	A/A	G/G	G/G	T/T
III-3	ND	G/G	G/G	T/T
Korean family	**G**	**A**	**A**	**C**
II-1	A/G	G/A	ND	C/T
II-2	G/G	A/A	A/A	C/C
III-1	G/G	G/A	A/G	C/T
III-2	G/G	G/A	A/G	C/T
III-3	G/G	A/A	A/A	C/C
III-4	G/G	A/A	A/A	C/C
III-5	G/G	A/A	A/A	C/C
III-6	G/G	A/A	A/A	C/C

Abbreviations: HGVS, Human Genome Variation Society (http://www.hgvs.org/); SNP, single nucleotide polymorphism.
Bold letters represent the most common allele of each SNPs.

The results obtained here do not support the hypothesis that the Ser284Leu mutations originated with a common founder. Association with a CpG site accounts for the independent occurrence of the Ser284Leu mutation. The other three mutations, however, are not associated with hypermutable sites such as homonucleotide runs, direct and inverted repeats and CpG dinucleotide sites.[27] Thus, the repetitive occurrence of Ser280Phe is not easily understood.

To explain it, we need to take into account ADNFLE development. One possible explanation is that ADNFLE is caused by mutations on a few functionally important sites within the M2.[14] Together with S280F, *in vitro* expression studies indicate that all CHRNA4 mutations increase receptor sensitivity to acethylcholine, suggesting gain of function.[28,29] In our results, both S280F and S284L showed high phyloP scores and low SIFT scores, indicating that the affected nucleotides are highly conserved and that their amino-acid substitutions will be deleterious.

Another explanation is that rare mutations have strong phenotypic effects in complex disorders.[30] A recent report uncovered significant excess of rare variants in neurological disorders, providing a 'rare allele-major effect model', and suggesting that the rare variants or *de novo* mutations in neurologically expressed genes are more likely to accumulate.

This is the first report comparing the haplotype structure of Japanese and Korean ADNFLE families. Further functional studies will be required to ascertain the ADNFLE's pathophysiology.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FULL-LENGTH ORIGINAL RESEARCH

Retrospective multiinstitutional study of the prevalence of early death in Dravet syndrome

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Summary

Purpose: A questionnaire survey was conducted in Japan to investigate the causes and prevalence of death related to Dravet syndrome.

Methods: A questionnaire was delivered to 246 hospitals at which physicians were treating childhood epilepsy to gain information about the total number of patients with Dravet syndrome and their prevalence of early death.

Key Findings: Responses to the survey were collected from 91 hospitals, and a total of 63 of 623 patients with Dravet syndrome died. Data from 59 of these patients were analyzed. The patients' ages at death ranged from 13 months to 24 years and 11 months, with a median age of 6 years and 8 months. The analysis showed that the risk of mortality remained high up to approximately 12 years of age. The causes of mortality included sudden death in 31 patients (53%), acute encephalopathy with status epilepticus (SE) in 21 patients (36%), drowning in 6 patients (10%), and acute hepatopathy in one patient (1%). The incidence of sudden death reached a first peak at 1–3 years of age and reached a second peak at 18 years and older. In contrast, the incidence of acute encephalopathy with SE reached a sharp peak at 6 years of age. Seven of 10 patients who underwent an *SCN1A* mutation analysis exhibited positive mutations without a specific mutation site.

Significance: In the present study, the prevalence of Dravet syndrome–related mortality was 10.1%. The incidence of sudden death and acute encephalopathy with SE was the highest in infancy (1–3 years) and at early school ages (with a peak at 6 years), respectively. After approximately 12 years of age, the risk of mortality declined sharply. Neither the treatment nor the number of seizures was associated with any cause of mortality. In addition, it is difficult to predict which factors lead to a fatal outcome.

KEY WORDS: Dravet syndrome, Severe myoclonic epilepsy in infants, Mortality, Sudden death, Acute encephalopathy.

Dravet syndrome is one of the most malignant epileptic syndromes among the various types of childhood epilepsy (Oguni et al., 2001; Dravet et al., 2005). Recent advances in molecular biology have demonstrated that *SCN1A* mutations cause this rare but catastrophic epilepsy and have increased our understanding of its pathogenesis (Claes et al., 2001). This disorder exhibits specific clinical features: Seizures are easily provoked by a rise in the body temperature; various types of seizures are combined with one another; and strong photosensitivities and pattern sensitivities are involved. Furthermore, sudden death from unknown causes and mortality or serious sequelae associated with lethal status epilepticus (SE) have been reported, accounting for a percentage that is constant from author to author (Oguni et al., 2001; Dravet et al., 2005). The number of patients diagnosed with Dravet syndrome has increased throughout the world (Dravet et al., 2005); the *SCN1A* mutation test facilitates a definitive diagnosis in the early stage of the condition, and characteristic clinical features have been widely recognized among pediatric neurologists. In contrast, the seizures and mental prognosis do not seem to improve despite various treatment trials that have been conducted. In addition, unexpected death during the treatment course for this catastrophic disorder may have influenced the reliance of patients' families on physicians (So et al., 2009). Until now, no systematic study has been conducted to clarify the incidence of unexpected death or the prognostic factors associated with this mortality. Because Dravet syndrome is a rare type of epileptic syndrome, an analysis involving a number of patients in a single hospital is difficult, necessitating a nationwide survey. In this study, we conducted a nationwide questionnaire survey regarding Dravet syndrome–related mortality in Japan and investigated the

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causes of mortality, the clinical characteristics at the time of death, and the risk factors related to mortality. The results may help provide information that prevents mortality for the families of children with this disorder and for health care professionals.

Methods

In July 2009, a questionnaire was delivered to the epilepsy training hospitals that were authorized by the Japanese Society of Epilepsy, to hospitals/institutions that were authorized to train specialists from the Society of Pediatric Neurology, and to university hospitals (total: 246 hospitals). This survey allowed us to collect information about the total number of past and present patients with Dravet syndrome, the number of patients who died, and the number of patients with serious sequelae. A secondary questionnaire was sent by mail to the 26 hospitals that reported mortality cases to obtain information on the following items for each patient: (1) gender; (2) age at the onset of epilepsy (in months); (3) clinical type (typical or borderline groups) (Fujiwara et al., 2003; Oguni et al., 2005); (4) presence or absence of an *SCN1A* gene test and its results; (5) age at death; (6) Causes of mortality; (7) presence or absence of risk factors at death, such as fever/infection, bathing, seizures, and the child's state (sleep or awake); (8) frequency of seizures and of SE before death; (9) treatment regimen; (10) neurological condition; (11) electroencephalographic and neuroimaging findings; and (12) autopsy findings.

The time from the onset of epilepsy to death and the age at death were very close because of the early onset of the epilepsy; therefore, we compared the age at death between the typical and borderline groups and among the causes of death.

Prior to this study, the protocol was approved by the Tokyo Women's Medical University Ethics Review Board and by the Dravet Syndrome Prognosis Survey/Study Group Ethics Review Board.

Statistical analyses

Statistical analyses were performed using SPSS 15.0J (SPSS Japan, Tokyo, Japan) for Windows. The chi-square test, *t*-test, and Mann-Whitney *U*-test were employed to compare the results between two variables. A comparison among more than three variables was performed using the chi-square test with cross tabulation. The Bonferroni correction was added to the statistics when multiple statistical comparisons were performed between several groups. A p-value of <0.05 was regarded as significant.

Results

Subjects

Responses were collected from 147 of the 246 hospitals (response rate: 59.8%). In 91 of the 147 hospitals, a total of 623 patients with Dravet syndrome were treated (median: two patients/hospital; range 1–109/hospital). In addition, data on 63 patients who died were collected from 26 hospitals (438 patients). Of these patients, the data from 59, excluding 4 for whom the information at the time of death was insufficient, were analyzed.

Clinical characteristics of the patients who died

In the 59 patients included in the analyses, the male-to-female ratio was 26:33. The ages of the patients at the onset of epilepsy ranged from 2–10 months, with a mean of 5.1 months. Of the 59 patients included in the analyses, 20 patients comprised the borderline group without myoclonic or atypical absence seizures, and 39 patients comprised the typical group (Table 1). No significant differences were observed between the two groups for the age at the onset of epilepsy, the age at death, the causes of mortality, the frequency of seizures before death, or the number of antiepileptic drugs ($p > 0.05$). In the typical group, the frequency of seizures before death was slightly higher than that in the borderline group, and the rates of mental retardation were slightly higher; however, there were no significant differences in the rates of severe mental retardation between the two groups ($p > 0.05$). The number of antiepileptic drugs, the frequency of seizures, and the grade of mental retardation were unclear or not described for three patients, three patients, and one patient, respectively.

Mortality

For the 26 hospitals that reported at least one patient who died for this nationwide survey, mortality accounted for 14.4% of their patients with Dravet syndrome (63 of 438 patients). When using the 91 hospitals (623 patients) as a denominator population, the prevalence of death was 10.1%.

Causes of mortality and age distribution

The patients' ages at the time of death ranged from 13 months to 24 years and 11 months, with a median age of 6 years and 8 months. The ages were distributed most frequently between 13 months and approximately 12 years and were rarely older in both the typical and borderline groups (Fig. 1). The distribution of the time between the two groups did not differ significantly ($p = 2.88 > 0.05$).

The causes of mortality were largely classified into three groups: sudden death (n = 31, 53%), acute encephalopathy with SE (n = 21, 36%), and drowning (n = 6, 10%). The remaining one patient died of fulminant hepatitis B (1%). When reviewing the age distribution with respect to the causes of mortality, two characteristic patterns were observed (Fig. 2). Briefly, the prevalence of sudden death reached a first peak at 1–3 years of age and a second small peak at 18 years and older. In contrast, the prevalence of acute encephalopathy with SE was prevalent between

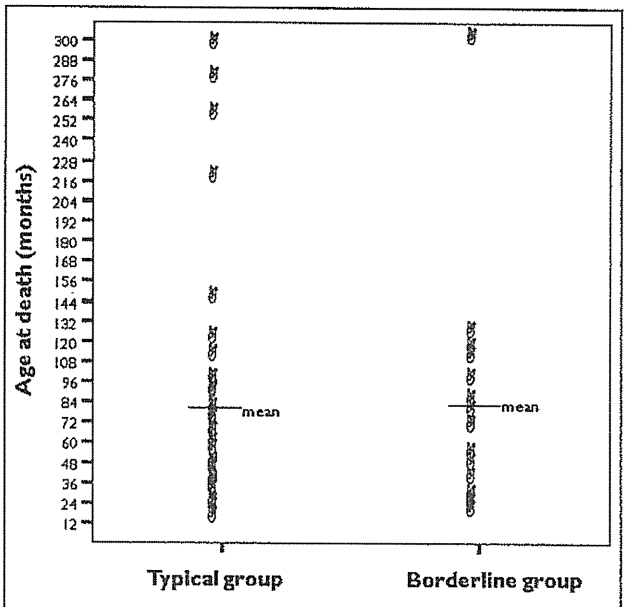

Figure 1.
Dot plots showing the distribution of age at death in the typical and borderline groups (n = 59). The age at death appeared prevalent between 13 and 140 months of age and sparse thereafter in both typical and borderline groups. No significant difference was observed between these two groups ($p > 0.05$).
Epilepsia © ILAE

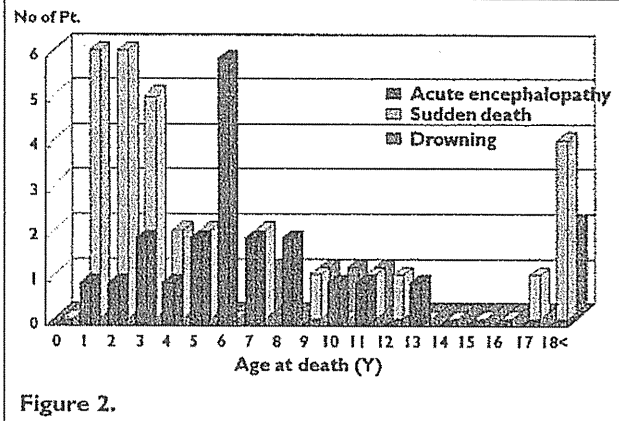

Figure 2.
Distribution of ages at death with respect to the causes of mortality. The incidence of sudden death reached a first peak at 1–3 years old and a second small peak at 18 years and older. In contrast, the acute encephalopathy-related mortality rate reached a sharp peak at 6 years old. All patients were 7 years old or older in the drowning group.
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approximately 3 and 8 years of age with a sharp peak at age 6.

Causes of mortality and clinical features

The causes of mortality were associated with fever and the age at death (Table 2). In the sudden death group, the median age at death was 43 months, which was lower than that (72 months) in the acute encephalopathy group. These two groups exhibited characteristic age distributions with different peaks ($p < 0.05$), that is, the sudden death group was more likely to die at an age <47 months and after 168 months, whereas the acute encephalopathy group was more likely to experience death between 48 and 167 months (Table 2). In 81% of the patients who died of acute encephalopathy with SE, fever was noted at the time of death. In addition, fever was observed in 26% of the patients who died suddenly ($p < 0.05$). Twenty (65%) and eight (26%) of the 31 patients in the sudden death group were found to have died during sleep (or in the early morning) and during the daytime, respectively. For the remaining three patients, information about the exact time at their deaths was not available.

In 6 of the 31 patients in the sudden death group, rigid limbs and trace amounts of vomit suggested that epileptic seizures or suffocation was involved in their deaths. However, an autopsy was not performed for any of these six patients who showed the rigid limbs and trace amounts of vomit; therefore, the cause of death was not specified. In 14 patients (67%) in the acute encephalopathy group, systemic involvements, such as multiple organ failure and disseminated intravascular coagulation (DIC), became evident during or after the successful treatment of SE. Therefore, the clinical response and features of SE differed from those that the children had repeatedly experienced before. This lethal febrile SE developed suddenly at a peak age of 6 years, when the seizure or SE frequency was abated, and led to coma and multiple organ failure despite vigorous treatment. The interval of time from the onset of SE until death was 24 h or less in five patients (24%), 1 week or less in six patients (29%), more than 1 week in six patients (29%), and not known for the remaining four patients (18%).

An autopsy was performed for only 6 (10%) of the 59 patients: one who died suddenly, 4 who died of acute encephalopathy with SE, and one who died of fulminant hepatitis B. For these six patients, the causes of mortality were identified as Reye syndrome for two patients and fulminant hepatitis for one patient. However, for the remaining three patients, no cause of mortality was identified, despite the autopsy.

In the six patients who died from drowning, accidents occurred while bathing at home or in the hospital. All patients were 7 years old or older, including two patients older than 18 years of age; as a result, these patients were permitted to bathe alone.

SCN1A mutation analysis

An *SCN1A* gene test was performed for only 10 of the 59 patients. Gene mutations were detected for 7 of these 10 patients. The mutation sites were scattered in the *SCN1A* gene tests that were previously reported; as a result, no

Table 1. Clinical manifestations and phenotype

	Typical (N = 39)	Borderline (N = 20)	All cases (N = 59)	p-Value*
Gender (M/F)	17/22	9/11	26/33	3.99
Age at onset (mo.)	4.9 ± 1.7	5.5 ± 2.0	5.1 ± 1.8	2.04
Age at death (mo.)	80.7 ± 73.1	79.5 ± 70.5	80.4 ± 71.6	2.88
Cause of death Sudden/status/drowning	21/15/3	10/6/3	31/21/6	2.80
Seizure frequency Daily/weekly/monthly	13/16/8	2/7/10	15/23/18	0.567
Number of AED polytherapy (<4)/2–3	15/21	6/12	23/33	4.788
Mental retardation Severe/moderate/mild	18/14/6	4/9/7	22/23/13	0.980

M, male; F, female, mo., months; AEDs, antiepileptic drugs.
*The Bonferroni collection was added to p-values.

Table 2. Comparison between those with sudden death and acute encephalopathy

	Sudden death (N = 31)	Acute encephalopathy (N = 21)	p-Value*
Gender (M/F)	18/13	5/16	0.42
Phenotype (typical/borderline)	21/10	15/6	8.0
Age at death (mo.) ≤47 or ≤168/48–167	22/9	5/16	0.01*
Fever at death (%)	25.8	81.0	0.00*
Seizure frequency Daily/weekly/monthly	8/11/7	7/5/9	3.69
Mental retardation Severe/moderate/mild	12/10/9	7/9/4	2.16
AED polytherapy <4 (%)	32.3	38.1	4.86
Epileptic EEG abnormality (%)	56.7	42.1	4.50
Neuroimaging abnormality (%)	29.0	33.3	5.13

M, male; F, female, mo., months; AED, antiepileptic drug.
*The Bonferroni collection was added to p values.

mutation site that was characteristic of mortality was detected (Depienne et al., 2009; Lossin, 2009).

Discussion

In this nationwide survey, data were collected for 63 patients with Dravet syndrome who died, and data from 59 of these patients were used for the analyses. The result showed that the risk of mortality remained high up to the age of approximately 12 years of age, regardless of the clinical type, and sharply declined thereafter. The causes of mortality were classified into three types: sudden death, acute encephalopathy with SE, and accidents (mostly drowning). In particular, sudden death and acute encephalopathy with SE accounted for 53% and 31% of the causes of death, respectively.

The incidence of sudden death reached a first peak at 1–3 years of age and reached a second small peak at 18 years and older. Sudden unexpected death in epilepsy (SUDEP) has been reported to account for approximately 2–18% of all epilepsy-related deaths. Therefore, the incidence of SUDEP in this disorder is high (Gaitatzis & Sander, 2004; Tomson et al., 2008). During infancy, patients with Dravet syndrome experience recurrent febrile/afebrile SE despite vigorous antiepileptic drug (AED) treatments (Claes et al., 2001; Dravet et al., 2005). In the present study, neither the number of AEDs nor the frequency of the seizures was abnormally high immediately before death; however, no control group was established. The epileptic seizures associated with Dravet syndrome are presumably generated by epileptogenic pyramidal neurons because of an *SCN1A* mutation–mediated dysfunction of inhibitory interneurons (Yu et al., 2006; Ogiwara et al., 2007). The involvement of this channelopathy in epilepsy suggests that cardiac arrhythmia is a complication that is involved in the episodes of sudden death. Both arrhythmia and respiratory hypoventilation have been considered to be causes of SUDEP (Gaitatzis & Sander, 2004; Tomson et al., 2008). Most patients with Dravet syndrome who died suddenly were found in bed early in the morning or after sleeping in the afternoon. This result is consistent with common-type SUDEP. Neither electrocardiographic abnormalities nor heart/respiratory dysfunction has been reported in any children with this disorder. Unfortunately, no study has demonstrated any other arrhythmia-associated gene mutations in patients with Dravet syndrome. More work is needed to clarify whether the *SCN1A* mutation site is associated with sudden death in Dravet syndrome.

The mortality rate resulting from acute encephalopathy with SE reached a sharp peak at 6 years old (prevalent between 4 and 8 years of age). In these cases, coma or multiple organ failure led to a fatal outcome despite seizure control. Recently, catastrophic SE that led to severe neurologic sequelae has been reported in infants with Dravet syndrome (Chipaux et al., 2010; Takayanagi et al., 2010). The catastrophic SE did not seem to be related to a delay in seizure treatment or to insufficient treatment. This SE was always associated with fever and was resistant to conventional SE

treatment, requiring high doses of barbiturates or short-acting barbiturates to control the SE, which may have contributed to cerebral damage resulting from a reduction of cerebral blood flow (Chipaux et al., 2010). Although barbiturates are often chosen to treat refractory SE in the intensive care unit (ICU) setting, it may be beneficial to consider an alternative treatment such as propofol or a combination of hypothermia therapies in these cases (Munakata et al., 2000). The frequency of SE or of prolonged seizures decreases markedly in children with Dravet syndrome who are older than 4 years of age. Therefore, mortality related to acute encephalopathy with SE at this age was an unexpected event for the families of these patients and for the health care professionals (Oguni et al., 2001; Dravet et al., 2005). In Japan, fulminant acute encephalopathy associated with SE in children has recently been identified as a complication of an influenza infection. The individual genetic factors that contribute to the susceptibility to acute encephalopathy may suggest an important role in its pathogenesis (Mizuguchi et al., 2007). For patients with Dravet syndrome, *SCN1A* mutations are related to seizures that are markedly sensitive to elevated temperature. In an *SCN1A*-knockout mouse model, a rise in body temperature markedly decreased the threshold of the seizures; therefore, the complication of acute encephalopathy with SE may be associated with *SCN1A* mutations (Oakley et al., 2009). A previous study indicated that Dravet syndrome was present in most patients who had been diagnosed with vaccine encephalopathy (Berkovic et al., 2006). This disorder frequently causes acute encephalopathy; however, the peak incidence of fatal acute encephalopathy with SE at approximately 6 years of age should be clarified.

All of the patients who had accidental deaths drowned while bathing. Drowning-related mortality is avoidable in patients with Dravet syndrome and in patients with other types of epilepsy (Gaitatzis & Sander, 2004). Because seizures that are hypersensitive to elevated body temperature continue through adulthood in most patients with this disorder, the Japanese-style bathing that raises body temperature is a potential risk factor (Oguni et al., 2001). Therefore, it is necessary to train caregivers to be vigilant when the patients take a bath.

The prevalence of mortality in patients with Dravet syndrome has been shown to range from 5–20%, which is markedly higher than in patients with other types of epilepsy (Oguni et al., 2001; Dravet et al., 2005). In the present study, the statistical analyses involving the 91 hospitals showed a mortality rate of 10.1%. The data from the 26 hospitals that reported mortality cases indicated that the mortality rate was 14.4%. However, a limitation of this study was that the survey period differed among the hospitals, leading to difficulty in accurately evaluating the population (as a denominator). In addition, the lack of detailed information on the population did not permit us to create a survival curve. Other limitations of this study included a 60% response rate to the questionnaire, retrospective case ascertainment, a very low autopsy rate, and a low incidence of *SCN1A* mutation analyses, all of which lowered the validity of this study. However, even if these limitations are considered, the prevalence of early death would be estimated to be 10–15%, which is still markedly higher than the rate in patients with other types of epilepsies.

The mortality rate in childhood epilepsy has been estimated to be 3–7 times higher than that in the general population (Berg et al., 2004; Autry et al., 2010). The risk factors for mortality were considered to be symptomatic etiology, epileptic encephalopathy, especially West and Lennox-Gastaut syndromes, and severe comorbid neurologic disorders. The death rate for the epileptic syndromes was highest for symptomatic generalized epilepsy, which was 15–16% and was almost equivalent to that of Dravet syndrome. However, the causes of death for generalized epilepsy were markedly different from those for Dravet syndrome and were mostly related to the complications of severe neurologic deficits (infections and accidents, among others), not to the occurrences of seizures or sudden death.

In conclusion, this study identifies the high-risk age periods with respect to the specific causes of mortality; however, no other prognostic factors, including *SCN1A* mutations, could be discerned. Since the 1980s, there has been a strong medical/social interest in SUDEP in patients with patients (Nilsson et al., 1999; Gaitatzis & Sander, 2004; Tomson et al., 2008). According to a report that was published by a collaborative special committee of the American Society of Epilepsy and the Foundation of Epilepsy, future endeavors should emphasize the importance of talking with patients' families about SUDEP, facilitating physicians' and community members' understanding of SUDEP, and planning nationwide/international prospective studies (So et al., 2009). It is necessary to provide the information obtained in this nationwide survey regarding the causes of mortality and the high-risk age periods to the hospitals that are involved in the treatment of this disorder and to the patients' families, despite objections that have been raised concerning the difficulty of SUDEP prediction and families' anxiety levels. A worldwide multiinstitutional study needs to be performed to identify the risk factors at a molecular level and to prevent catastrophic events associated with this syndrome.

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Disclosure

There are no conflicts of interest related to this manuscript. We confirm that we have read the Journal's position regarding issues pertaining to ethical publication and affirm that this report is consistent with those guidelines.

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Appendix

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Case report

A boy with a severe phenotype of succinic semialdehyde dehydrogenase deficiency

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Abstract

Succinic semialdehyde dehydrogenase (SSADH) deficiency is a rare autosomal recessive disorder affecting γ-aminobutyric acid degradation. We describe here a boy with a severe phenotype of SSADH deficiency. He was referred because of a developmental delay at 4 months of age. At the age of 8 months, severe seizures developed. The diagnosis of SSADH deficiency was confirmed by an increase in 4-hydroxybutyric acid and heteroallelic mutation in the *ALDH5A1* gene. His seizures were successfully treated with high-dose phenobarbital, and the electroencephalogram (EEG) abnormalities were ameliorated. However, the patient showed a degenerative clinical course with severe neurological deficits. A magnetic resonance imaging (MRI) scan revealed abnormal high intensities in the putamina and caudate nuclei on T2-weighted images, followed by marked atrophic changes. The clinical manifestation of our patient indicates the wide variety of SSADH deficiency phenotypes.

1. Introduction

Succinic semialdehyde dehydrogenase (SSADH) deficiency is a rare autosomal recessive disorder affecting the degradation of γ-aminobutyric acid (GABA). SSADH works with GABA transaminase to converts GABA to succinate. In the absence of SSADH, GABA is not broken down into succinic acid, but is converted into γ-hydroxybutyric acid (GHB). It is unclear whether elevated GABA, GHB, or another neurometabolic change accounts for the phenotype, but the primary metabolic abnormality is an excessive concentration of GHB in the physiological fluids, with elevations up to 800-fold in the plasma and 1200-fold in the cerebrospinal fluid [1]. The SSADH gene (*ALDH5A1*) has been mapped to chromosome 6p22. More than 350 patients with SSADH deficiency have been identified worldwide. In Japan, Ishiguro et al. [2] reported the first patient in 2001, and since then, only a few patients have been reported.

SSADH deficiency often presents in childhood with non-specific clinical manifestations. The most common neurological symptoms include language delay, ataxia, hypotonia, and mental retardation. Seizures are less common, occurring in about half of all patients [3]. Although SSADH deficiency does not generally manifest as a degenerative condition, developmental regres-

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sion has been occasionally reported. Moreover, some patients may have a more fulminant clinical course, with the early onset of clinical symptoms, seizures, choreoathetosis, myoclonus, and optic atrophy, resulting in death in infancy [3].

We describe here a boy with SSADH deficiency who had a severe phenotype with severe seizures, marked neuroimaging abnormalities, and a degenerative clinical course. His seizures were successfully treated using high-dose phenobarbital (PB). We also describe the magnetic resonance imaging (MRI) and amplitude-integrated electroencephalogram (aEEG) findings for this patient.

2. Patient report

A 4-month-old boy was referred to our hospital for an evaluation of developmental delay. He was the third child of non-consanguineous healthy parents. His elder sisters were healthy and had achieved normal development. On the first presentation, the patient's visual tracking and head control were insufficient. Neurological examination revealed generalized hypotonia with normal deep tendon reflexes. No external anomaly was observed. Blood tests including blood gas analysis, blood chemistry, thyroid function, lactic and pyruvic acids, and karyotypic chromosome analysis were unremarkable. No abnormal findings were seen in the cranial MRI or EEG.

The patient had had repetitive aspiration pneumonia since the age of 7 months. He was diagnosed as having gastro-esophageal reflux on the basis of 24-h pH monitoring and a barium esophagram. He required an esophagoduodenal tube to prevent aspiration pneumonia. A deterioration in psychomotor development was noted when the patient was 8 months old, along with the development of intractable seizures. The diagnosis of SSADH deficiency was made on the basis of urine organic acid analysis using gas chromatography–mass spectrometry. A marked increase in 4-hydroxybutyric acid, which is crucial for the diagnosis of SSADH deficiency, was found. Analysis of the *ALDH5A1* gene encoding SSADH revealed compound heterozygous mutations with c. 366 del G (W112fsX112) and c.1294 A > C (M432L).

The patient's seizures were characterized by loss of consciousness followed by clonic convulsion of the left upper and lower extremities lasting for several minutes. Carbamazepine, zonisamide, and vitamin B6 were ineffective in controlling the seizures. Although continuous infusion of midazolam was effective, the seizures recurred. High-dose PB treatment was started at 10 months of age. PB in a dose of 20 mg/kg/day was given rectally for the first two days and then orally in a dose of 10 mg/kg/day from the third day. The patient's seizures were controlled after the initiation of high-dose PB treatment, and the midazolam infusion was discontinued. The EEG conducted prior to the administration of high-dose PB showed markedly disorganized background activity and frequent high-voltage slow waves, sharp waves, and spikes (Fig. 1). However, after the high-dose PB treatment, the frequency and voltage of the sharp waves and spikes were significantly reduced, and no high-voltage slow waves were observed (Fig. 1).

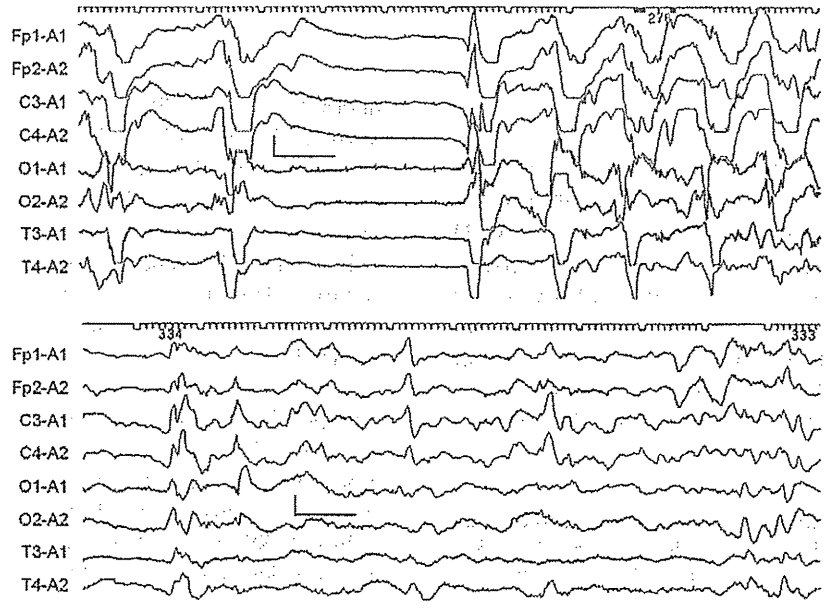

Fig. 1. Conventional EEG. Top: EEG before high-dose phenobarbital treatment. Markedly disorganized background EEG activity and frequent appearance of high-voltage slow waves, sharp waves, and spikes were observed. Bottom: EEG after high-dose phenobarbital treatment. The frequency and voltage of the sharp waves and spikes were markedly reduced. High-voltage slow waves were not present. Calibration, 100 μV, 1 s.

Single-channel aEEG (CFM-6000, Olympic Medical, Seattle, WA, USA) was performed for 353 h to monitor the efficacy of antiepileptic drugs. We used the Fp1–Fp2 rather than the P3–P4 derivation usually used in neonates because the patient's hair interfered with stable long-term electrode placement in the parietal area. The upper border of the aEEG trace was continuously greater than 50 μV before the administration of PB (Fig. 2). The raw EEG data showed frequent high-voltage sharp waves and spikes, and seizures were also visible on the aEEG (Fig. 2). In this patient, the seizures observed on the aEEG were always accompanied by clinical manifestations such as convulsive movement. Administration of high-dose PB decreased the upper border of the aEEG trace to 25–50 μV. No seizures were observed on the EEG after the initiation of high-dose PB.

The head MRI at 4 months of age was unremarkable (Fig. 3). However, at 8 months of age, the MRI scan revealed abnormally high intensities in the caudate nuclei and putamina associated with mild enlargement of the extra-axial space on T2-weighted images (Fig. 3). The MRI at 17 months of age revealed severe atrophy of the caudate nuclei, high intensities in the

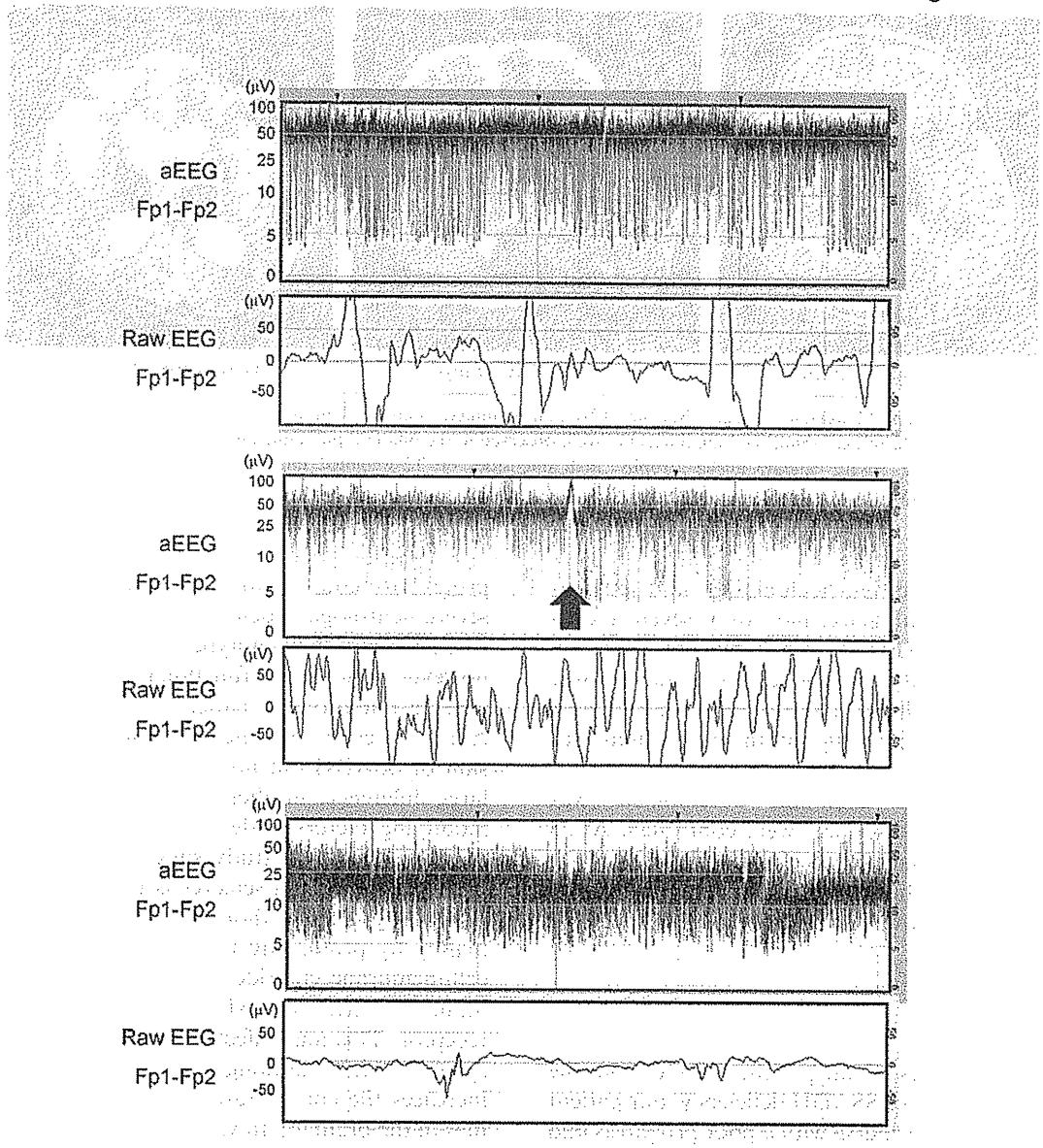

Fig. 2. Amplitude-integrated EEG. Top: Amplitude-integrated EEG (aEEG) before high-dose phenobarbital treatment. The upper border of the aEEG trace was constantly beyond 50 μV. This indicates that high amplitude EEG activities were present throughout aEEG recording. The raw EEG also showed frequent appearance of high-voltage sharp waves and spikes. Middle: A seizure detected on the aEEG. A transient rise in the lower border suggested ictal EEG changes (arrow). Ictal EEG changes were confirmed by the raw EEG showing repetitive and rhythmic sharp waves during the same period. Bottom: The aEEG after high-dose phenobarbital treatment. The upper border of the aEEG trace was 25–50 μV. This indicated the reduction in amplitude of background EEG activities. The raw EEG also demonstrated sporadic appearance of paroxysmal discharges with reduced amplitude.

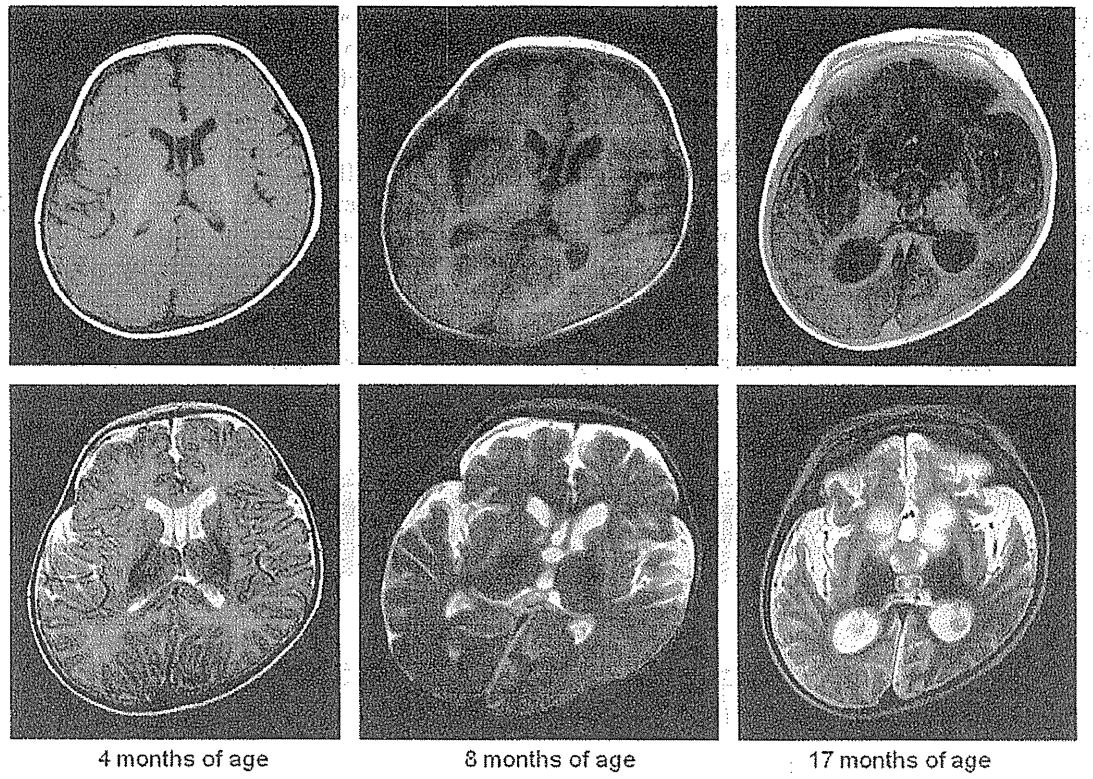

Figure 3. MRI findings. Top: T1-weighted images, Bottom: T2-weighted images. Left: at 4 months of age, no remarkable abnormalities were observed. Middle: at 8 months of age, abnormal high intensities were observed in the bilateral putamina and caudate nuclei on T2-weighted images. Right: at 17 months of age, severe caudate nuclei atrophy, high intensities on T2-weighted images in the bilateral putamina, and marked ventriculomegaly and enlargement of extra-axial space were seen.

putamina, and marked ventriculomegaly and enlargement of the extra-axial space, indicating severe reduction of brain volume (Fig. 3). No significant alteration in the signal intensity of the globus pallidus was observed during the clinical course, and no atrophy or change in the signal intensity of the cerebellum was found.

A regression in psychomotor development was observed even after the seizures were controlled. At 23 months of age, the patient remained bed-ridden, with poor eye fixation and head control and remarkable generalized hypotonia, however, seizures did no occur for several months.

3. Discussion

The clinical features of our patient differed from the typical manifestations of SSADH deficiency: our patient showed a degenerative course with a poor prognosis and had MRI lesions in the putamina and caudate nuclei, together with severe atrophy of the cerebrum and severe EEG abnormalities.

SSADH deficiency is typically a slowly progressive or static encephalopathy with late infantile to early childhood onset [4]. In contrast, our patient had an obvious progressive course with mid-infantile onset resulting in severe neurological deficits with marked brain atrophy. The difference in phenotype may be related to genotype; however, following a functional analysis of 27 disease-causing mutations in patients with SSADH deficiency, Akaboshi et al. [5] concluded that the residual expression of SSADH did not significantly contribute to the large differences in phenotype and suggested that other modifying factors underlie the disease pathology. The results of our case study suggest that the effectiveness of high-dose PB on seizures is related to the difference in phenotype. PB is thought to act as an anticonvulsant mainly by prolonging the opening of the postsynaptic cell-membrane chloride channel [6]. PB potentiates the inhibitory effect of GABA by binding to the GABA-A receptor. This may affect the turnover of GABA in the synaptic cleft, although it is unclear whether PB increases the concentration of GABA and its metabolites in the brain [6]. In view of the rapid cessation of seizures, we conclude that the PB treatment was beneficial to our patient.

The characteristic neuroimaging abnormalities in patients with SSADH deficiency include increased signal intensity in the globus pallidus, subcortical white matter, cerebellar dentate nucleus, and brainstem on T2-

weighted images [7]. Other neuroimaging abnormalities are cerebral atrophy, delayed myelination, and a pattern of dentate-pallidal hyperintensity [8,9]. The putamina and caudate nuclei were predominantly involved in our patient, whereas the globus pallidus and cerebellar dentate nuclei were almost completely spared. In our patient, homogeneous hyperintensity was observed in the putamina at 8 and 17 months of age. The caudate nuclei showed increased T2 signals at 8 months of age and marked atrophy at 17 months of age. To our knowledge, no reports have described this pattern of lesions on MRI scans in patients with SSADH deficiency. Pearl et al. [10] reported that MRI scans showed predominant changes in the thalamus and basal ganglia of epileptic infants treated with a relatively high dose of vigabatrin, an irreversible GABA transaminase inhibitor, suggesting that this treatment may also affect brain lesions in children with SSADH deficiency.

Epilepsy is present in nearly half of the patients with SSADH deficiency [4]. EEG abnormalities in patients with SSADH deficiency include generalized and focal epileptiform discharges, photosensitivity, background slowing, and sleep spindle asynchrony [4]. Compared with children who have a typical SSADH deficiency, our patient had more frequent seizures and more severe abnormalities in background EEG activity, with frequent epileptiform discharges. No standard therapy for SSADH deficiency exists, although vigabatrin is considered to be a logical choice because it inhibits the conversion of GABA–GHB [11]. However, the laboratory and clinical reports of the effect of vigabatrin have been inconsistent [12]. Moreover, vigabatrin is not currently available in Japan; thus, we administered PB to control severe seizures in our patient. Although PB effectively stopped the seizures in our patient, its effects on the clinical course of SSADH are unclear. Further studies are necessary to clarify the optimal treatment for SSADH deficiency.

The continuous EEG monitoring of our patient using aEEG was useful because aEEG monitoring can show seizure burden objectively and quantitatively. Precise evaluation of the seizure burden is difficult using direct observation because the clinical symptoms of seizures in children can be subtle and may be overlooked. Ishikawa et al. performed aEEG monitoring combined with conventional EEG during clusters of seizures in a child with frontal lobe epilepsy [13]. A total of 197 seizures were recorded during the first 24 h and the decrease and disappearance of seizures were confirmed using aEEG monitoring. Stewart et al. evaluated the diagnostic accuracy of color density spectral array and aEEG for seizure identification in the intensive care unit [14]. The median sensitivity for seizure identification was 83.3% using color density spectral array and 81.5% using aEEG. These results suggest usefulness of aEEG for seizure identification. In addition, aEEG monitoring showed the changes in background EEG activity, revealing the efficacy of the antiepileptic treatment in our patient. The lower border of the aEEG trace was markedly reduced after administration of high-dose PB, indicating that the EEG abnormalities were ameliorated. These findings suggest that aEEG is useful for monitoring the effect of treatment in children with frequent seizures and/or severe EEG abnormalities as well as in children with acute encephalopathy [15].

In summary, the present paper presents the case of a boy with a severe phenotype of SSADH deficiency characterized by obviously progressive clinical course with severe neurological deficits. His clinical features included a degenerative course, characteristic MRI lesions in the putamina and caudate nuclei, which has not been previously reported in patients with SSADH, and seizures that were controlled by PB administration. The clinical manifestations of our patient indicate the wide variety of SSADH deficiency phenotypes.

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Research Report

The developmental changes of $Na_v1.1$ and $Na_v1.2$ expression in the human hippocampus and temporal lobe

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ABSTRACT

Alterations of the genes encoding α1 and α2 subunits of voltage-gated sodium channels (SCN1A, SCN2A) have been reported as causes of various types of epilepsy, most of which occur during the first year of life; as yet, however, the detailed mechanisms are unclear. We suppose that developmental changes of SCN1A and SCN2A in the human brain, which are unknown yet, may play an important role. So here, we studied the developmental changes of their corresponding proteins ($Na_v1.1$ and $Na_v1.2$) in the human hippocampus and temporal lobe in 28 autopsy cases, which age from 13 weeks of gestation (GW) to 63 years of age (Y). Using comparative microscopic immunohistochemical (IHC) analysis, we found that $Na_v1.1$ and $Na_v1.2$ immunoreactivity first appeared at 19GW, simultaneously in the hippocampus and the white matter of temporal lobe. In nearly all age groups, $Na_v1.1$ immunoreactivity was weak and relatively homogeneous. In general, $Na_v1.1$ immunoreactive (IR) neurons and neurites increased during the late fetal and postnatal periods, reached their peaks 7–9 months after birth (M), then decreased and remained stable at a relatively low level during childhood and adulthood. On the other hand, $Na_v1.2$ immunoreactivity was strong and heterogeneous. In the hippocampus, $Na_v1.2$ IR neurons increased gradually during the late fetal period, reached their peaks at 7–9M, sustained this high level during childhood, and then decreased slightly at adulthood. In the temporal lobe, $Na_v1.2$ IR neurons reached a high level during the late fetal period, and maintained that level during subsequent developmental stages; $Na_v1.2$ IR neurites also increased to a relatively high level during the late fetal period and continued to increase up to and during adulthood. Using double-staining IHC, we found that $Na_v1.1$ and $Na_v1.2$ had a relatively high colocalization rate with parvalbumin and showed distinct developmental changes. These findings extend our previous understanding of sodium channels and may help us discover the pathomechanisms of sodium channel-related age-dependent epilepsy.

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1. Introduction

Voltage-gated sodium channels play an important role in neuronal excitability and are significant therapeutic targets in epilepsy, pain and local anesthesia, and are currently under investigation for stroke, bipolar disorder, and other disorders (Clare et al., 2000). Our previous and other related studies have proven that abnormalities of the genes encoding α1 and α2 subunits of voltage-gated sodium channels (*SCN1A*, *SCN2A*) are associated with a variety of epilepsies: Dravet syndrome; intractable childhood epilepsy with generalized tonic–clonic seizures (ICEGTC); generalized epilepsy with febrile seizures plus (GEFS+); and some other rare early onset epileptic encephalopathies, most of which usually occur in the first year of life (Fukuma et al., 2004; Kumakura et al., 2009; Shi et al., 2009; Sugawara et al., 2001; Wang et al., 2008). Still, how these sodium channels cause these age-dependent onset epilepsies is unclear. In animal brains, there have been many reports of the expression and developmental changes of *SCN1A* and *SCN2A*'s corresponding proteins ($Na_v1.1$ and $Na_v1.2$), and these have indicated some possible correlation between the expression, developmental changes, and onset of epileptic symptoms (Liao et al., 2010; Ogiwara et al., 2007; Vacher et al., 2008). For the human brain, however, there are few studies and these contain only adults and do not include developmental changes (Lu et al., 1992; Whitaker et al., 2000, 2001a, 2001b).

Recent mouse model studies show that the impairment of interneuron sodium channel activity contributes to seizure generation (Martin et al., 2010; Oakley et al., 2009; Ogiwara et al., 2007; Tang et al., 2009; Yu et al., 2006). Among those, Ogiwara shows that "$Na_v1.1$ is clustered predominantly at the axon initial segments of parvalbumin-positive interneurons" (Ogiwara et al., 2007), indicating that such a colocalization pattern may play an important role in the pathogenesis of epilepsy. There are reports of similar colocalization pattern in rats (Kaneko and Watanabe, 2007; Van Wart et al., 2007). This pattern, however, has not been reported in the human brain. Such information, including the developmental changes, are important not only for a basic understanding of $Na_v1.1$ and $Na_v1.2$'s functions in the human CNS but also for further insight into their roles in different human disease states, especially in age-dependent epilepsies.

The hippocampus and the temporal lobe are considered as the most important regions for seizure generation and are the focus of current epilepsy research. In this report, using comparative microscopic IHC analysis, we studied the developmental changes of $Na_v1.1$ and $Na_v1.2$ expression, and their colocalization with parvalbumin in the human hippocampus and temporal lobe.

2. Results

2.1. *Mono-staining IHC*

2.1.1. *Distribution and developmental changes of $Na_v1.1$ immunoreactivity*

$Na_v1.1$ IR neurons first appeared at 19GW, simultaneously in the hippocampus and the white matter of the temporal lobe. The immunoreactivity was found solely in the nucleus of neurons, and avoided from the cytoplasm and the neuropil. At 22GW, few short IR neurites first appeared in the Cornu Ammonis of the hippocampus, connecting with IR neuronal somata. At 27GW, such neurites could be found in each sub-region of the hippocampus and the temporal lobe. Simultaneously, the IR segment of neurites was longer and more delicate than before. Since then, $Na_v1.1$ IR signals were detected in both neuronal somata and neurites in various shapes, while nuclei avoided. After that, there were no obvious changes in cellular distribution.

In the hippocampus, $Na_v1.1$ IR neurons appeared simultaneously in the Cornu Ammonis (CA) and the Dentate Gyrus (DG), increased gradually and reached their peaks 7–9 months after birth (M), since then decreased and remained relatively low level during childhood and adulthood (Fig. 1). The pyramidal neurons of CA showed denser immunoreactivity than the granular neurons of DG, and showed relatively obvious developmental changes. The $Na_v1.1$ IR neurites scattered in the hippocampus, in various lengths and directions, and showed developmental changes similar to the neurons.

In the white matter of the temporal lobe, $Na_v1.1$ IR neurons first appeared at 19GW, and then remained at this level during the subsequent developmental stages. Those IR neurons were in various shapes, such as oval, pyramidal and spindle shapes, and showed obvious immunoreactivity in their neurites (Fig. 2). In the cortex of the temporal lobe, $Na_v1.1$ IR neurons appeared in each layer by 27GW, increased gradually during late fetal period and reached their peaks at 7–9M, then decreased and sustained a relatively low level during childhood and adulthood. In nearly all age groups, the granular neurons in layer IV showed much denser immunoreactivity than other layers. In the cortex, $Na_v1.1$ IR neurites showed nearly similar developmental changes to the neurons, without obvious decrease (Table 1). These neurites were sparse, delicate and in various lengths, scattered in each sub-region of the temporal lobe. There was no obvious immunoreactivity in neuropil (the background was clean) or fiber tracts.

2.1.2. *Distribution and developmental changes of $Na_v1.2$ immunoreactivity*

$Na_v1.2$ IR neurons first appeared at 19GW, simultaneously in the hippocampus and the temporal lobe. The IR signals were mainly in the nuclei of neurons, and mildly in the neuropil. At 27GW, $Na_v1.2$ IR neurons appeared in each sub-region of the hippocampus and the temporal lobe. Although the neuropil immunoreactivity was strong, we could recognize the dense IR neuronal somata and neurites, while the nuclei usually avoided. After that, there were no obvious changes in cellular distribution.

In the hippocampus, $Na_v1.2$ IR neurons and neurites first appeared at 19GW, simultaneously in the CA and DG, increased gradually with gestational and postnatal age, peaked at 7–9M and sustained such level during childhood. Then the IR neurons slightly decreased to relatively low adult level; the IR neurites sustained that high level during adulthood (Fig. 1). The pyramidal neurons in CA showed much denser immunoreactivity than the granular neurons in DG; while the neuropil in CA showed much weaker immunoreactivity than those in DG.

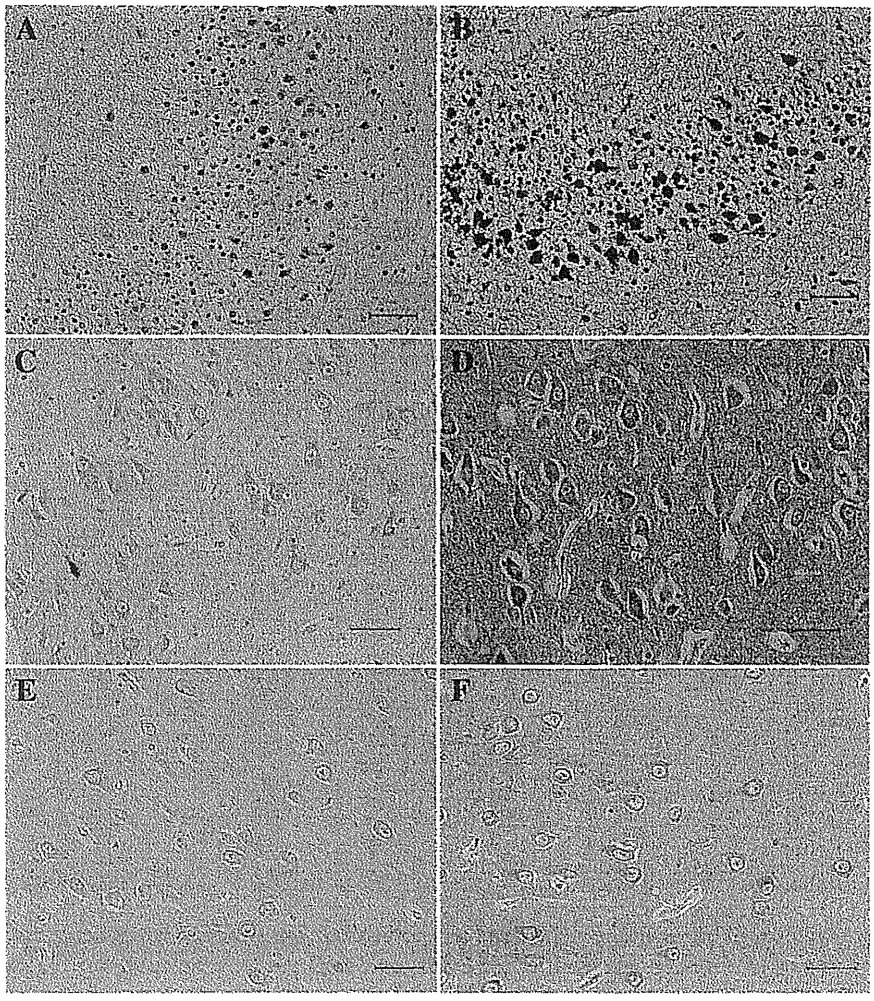

Fig. 1 – Developmental changes of $Na_v1.1$ and $Na_v1.2$ in the hippocampus. A and B from one 22GW fetus case. C and D from one 7M infant case. E and F from one adult case. Left column, $Na_v1.1$ immunoreactivity; right column, $Na_v1.2$ immunoreactivity. Bar, 50 μm. $Na_v1.1$ immunoreactivity is weak, increases with fetal age, reaches the peak at 7M and then decreases to relatively low adult level. $Na_v1.2$ immunoreactivity is strong, especially in the neuropil. It increases with age and only decreases slightly during adulthood.

In the temporal lobe, $Na_v1.2$ IR neurons first appeared simultaneously in the cortex and the white matter at midgestation, increased with fetal age, reached their peaks before birth for nearly all sub-regions and remained at that level. Nearly in all age groups, the pyramidal neurons in layer III and layer V showed much denser immunoreactivity than the granular neurons in layer II and layer IV. Interestingly, the IR pyramidal neurons were continuous from the CA of the hippocampus to layer V of the temporal lobe. $Na_v1.2$ IR neurites increased gradually during the entire human developmental stages, reached their peaks at different stages for different layers respectively, and remained at those levels since then.

2.2. *Double-staining IHC*

2.2.1. *Distribution and developmental changes of $Na_v1.1$ and parvalbumin double-staining*

$Na_v1.1$ and parvalbumin double-stained neurons were first detected since 22GW, simultaneously in the hippocampus and white matter of the temporal lobe. In the hippocampus, the most double-stained neurons were in the CA area, the colocalization rate was 49.8% (105/211), and relatively obvious in the CA3 sub-region. In the white matter of the temporal lobe, the colocalization rate was 65.6% (21/32). Before 27GW, in the double-stained neurons, $Na_v1.1$ IR signals were denser in the periphery while parvalbumin was denser in the central part of the neurons. Before birth, double stained neurons could be detected in the cortex of the temporal lobe. By 2M, the double stained neurons turned into a relatively homogenous "purple color", representing that the immunoreactive signals mixed more uniformly. In the CA area of the hippocampus, the colocalization rate increased gradually, and the CA1 sub-region became more obvious than the CA3. At 7M, the colocalization rate in the CA area of the hippocampus increased to 80.3% (61/76). In the white matter of the temporal lobe, the colocalization rate decreased and couldn't detect double-stained neurons after 2M. In the cortex of the temporal lobe, the double-stained neurons were predominant in layer IV—only few double-stained neurons scattered in other layers,