Aims and scope
Annals of Child Neurology is an interdisciplinary peer-reviewed biomedical journal publishing articles in the fields of child neurology, pediatric neurosurgery, pediatric neuroradiology, child psychiatry, pediatric neuropsychology, developmental and behavior pediatrics, pediatric neuroscience, and developmental neurobiology. The aims of Annals of Child Neurology are to contribute to the advancements in the fields of pediatric neurology through the scientific reviews and interchange of all of pediatric neurology. It aims to reflect the latest clinical, translational, and basic research trends from worldwide valuable achievements. In addition, genome research, epidemiology, public education and clinical practice guidelines in each country are welcomed for publication.

Focusing on the needs of neurologic patients from birth to age 18 years, Annals of Child Neurology covers topics ranging from assessment of new and changing therapies and procedures; diagnosis, evaluation, and management of neurologic, neuropsychiatric, and neurodevelopmental disorders; and pathophysiology of central nervous system diseases.

Area of specific interest include the following: behavioral neurology & neuropsychiatry, clinical neurophysiology, epilepsy, headache medicine, neurocritical care, neurodevelopmental disabilities, neurogenetics, neuromaging, neuromuscular medicine, neuroimmunology and inflammation, neuro-oncology, sleep medicine, vascular neurology, as well as other diseases affecting the developing nervous system.

Acceptance for publication of submitted manuscript is determined by the editors and peer reviewers, who are experts in their specific fields of pediatric neurology. The journal includes the following sections: original articles, reviews, letters to the editor. The editorial board invites articles from international studies or clinical, translational, and basic research groups. Supplements can be published when they are required.

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Once-Daily Extended-Release Levetiracetam Improves Medication Compliance in Adolescent Epilepsy Patients

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Purpose: Since pharmacologic agents are the mainstay of epilepsy treatment, drug compliance is one of the most important factors in seizure control. Once-daily levetiracetam (LEV) has been proven to have the same efficacy as that of an immediate-release (IR) formulation. A reduced number of doses may improve drug compliance and patient satisfaction. The aim of this study was to assess drug compliance and patient satisfaction when changing from IR to an extended-release (ER) formulation.

Methods: Adolescent patients diagnosed with epilepsy who were taking LEV from 2018 to 2020 were included in this study. Compliance charts were reviewed retrospectively. We compared the frequency of seizure occurrence with the frequency of skipping doses and adverse effects before and after changing formulations. Changes in subjective compliance and satisfaction were also investigated.

Results: Among 585 patients taking LEV, 44 were included in this study. The average age of the included patients was 16.4 ± 2.0 years. There was no significant change in the average seizure frequency (P=0.491) after switching formulations. Objective compliance based on chart records significantly improved after switching formulations (P=0.021). Additionally, 26 of 44 patients mentioned how they felt about switching formulations, of whom 25 (96.2%) were satisfied with the ER formulation. Thirteen of 24 patients (54.2%) reported better compliance.

Conclusion: Our study shows that the efficacy of LEV ER was similar to that of the IR formulation. The reduced number of medication doses improved patient satisfaction and medication compliance. LEV ER may be preferable in adolescent epilepsy patients.

Keywords: Compliance; Delayed-action preparations; Levetiracetam; Epilepsy

Introduction

Since pharmacologic agents are the mainstay of epilepsy treatment, drug compliance is one of the most important factors in seizure control. Outcomes of missing antiepileptic drug (AED) can be associated with higher seizure frequency, increased likelihood hospital admission, epilepsy-related mortality, and higher cost of healthcare [1,2]. Some factors influencing compliance in epilepsy patients are age, understanding importance of taking medication, fear of side effects, feelings of stigma, and number of drugs [3]. Dosing frequency can also affect drug compliance [4-6]. Cramer et al. [7] reported patients having seizures after missed doses were associat-
ed with the frequency of seizure medication doses \((P=0.04)\) and the number of seizure medication tablets/capsules \((P=0.01)\).

Levetiracetam (LEV) has been widely used in the treatment of epilepsy because of its broad-spectrum efficacy, unique mechanism of action, and fewer adverse effects \([8-10]\). Extended-release (ER) LEV has been proven to have the same efficacy as that of an immediate-release (IR) formulation \([11]\). A pharmacokinetic study also showed that LEV ER was bioequivalent to LEV IR \([12]\). Several studies reported that LEV ER was effective for treating focal seizures \([13,14]\). The safety and adverse effect profile is also tolerable \([15]\). Previous studies showed that children and adolescents are more likely to have poor drug compliance \([16]\), and a reduced number of doses may improve drug compliance and satisfaction in adolescent patients. However, there is no study exploring how LEV ER affects medication compliance. The aim of this study was to assess drug compliance and patient satisfaction after changing medication formulations from IR to ER.

**Materials and Methods**

1. **Subjects**

Subjects who visited the Department of Pediatrics at Chungnam National University Hospital, were diagnosed with epilepsy, and had switched formulation LEV IR to ER between 2018 and 2020 were enrolled in the study. Epilepsy was diagnosed according to the clinical definition of the International League Against Epilepsy as follows: (1) at least two unprovoked (or reflex) seizures occurring > 24 hours apart; (2) one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years; and (3) diagnosis of an epilepsy syndrome \([17]\).

Among 62 patients who fit the inclusion criteria, 18 patients were excluded. Exclusion criteria were as follows: (1) older than 20 years of age; (2) less than one month using LEV medication (either IR and ER each); and (3) the addition or removal of other medication during the transition time (Fig. 1).

2. **Method**

We reviewed medical records retrospectively. Clinical characteristics of patients, such as age, sex, epilepsy type, frequency of seizure and missing dose, dose of LEV, number of AED, etiology of epilepsy, and comorbidities were included. We defined transition time as 3 months before and after changing formulation, compared the frequency of seizure occurrence with the frequency of skipping medicine before and after switching. And we reviewed occurrence of adverse events after switching formulation. All the patients who had seizure without any provocative factors such as fever, sleep deprivation and drug omit were received escalated dose of 250 mg of LEV during both periods. Additionally, alterations in subjective compliance and satisfaction were investigated.

3. **Statistical analysis**

The Wilcoxon signed-rank test was performed using SPSS version 20.0 (IBM Co., Armonk, NY, USA). We compared the seizure frequency and compliance between prior to changing formulation and after changing formulation, analyzed the data, and considered \(P\) values < 0.05 as significant for all comparisons and analysis. Since two of the enrolled patients had insufficient time of 3 months of IR and ER medication duration, these patients were excluded from comparison.

4. **Ethics**

This study was reviewed and approved by the Institutional Review Board of the Chungnam National University Hospital (CNUHIRB 2020-04-173). Due to its retrospective nature, the study was exempt from requiring informed consent from the participants.

**Results**

1. **Clinical characteristics**

A total of 44 patients were enrolled in this study. The average age of the included patients was 16.4 ± 2.0 years (range, 13 to 20), and 22 were males (50%). Out of the 44 patients, 30 patients (68.2%) had focal epilepsy and 14 (31.8%) had generalized epilepsy. The average dose of LEV was 1,068.2 ± 382.6 mg (range, 500 to 2,000). The duration of patients taking LEV medication before switching formulation was 827.2 ± 852.2 days (range, 42 to 3,678). Most of the patients (84.1%) were on LEV monotherapy. Six out of 44 patients (13.6%) were taking two AEDs and one patient (2.3%) was taking three AEDs. The majority of patients had no comorbidities (68.2%) and had epilepsy with an unknown etiology (79.5%). Out of the 44 patients, four (9.2%) had adverse effects, such as drowsiness and dyspepsia. Among the four with adverse effects, two pa-
tients had serious adverse events of suicidal ideas and visual hallucinations. All the adverse events, except suicidal idea was occurred within 3 months of LEV ER period. Suicidal idea was occurred about 1 year after switching formulation. They were switched back to the previous formulation and their status returned to normal (Table 1).

2. Comparison of seizure frequency and compliance
The average seizure frequencies during the period of IR and ER medications were $0.24 \pm 0.73$ and $0.19 \pm 0.49$ times per month, respectively. Twenty-two of 42 patients (52.4%) were seizure-free during the transition time. There was no significant difference in the average seizure frequency ($P = 0.491$) before and after switching the formulation. A total of six seizures were related with missing a dose, which were excluded from this comparison. Average missing doses during IR and ER were $0.46 \pm 0.90$ and $0.15 \pm 0.39$ times per month, respectively. Twenty-four of 42 patients (57.1%) never missed a dose during the transition time. Compliance was significantly improved after switching formulation from IR to ER ($P = 0.021$) (Table 2 and Fig. 2).

3. Difference of seizure frequency and compliance according to clinical characteristics
Differences in seizure frequency did not depend on sex, age, drug dose, epilepsy type, number of AEDs, IR duration, or presence of comorbidity. Enhancement of compliance was more prominent in the patients with older age ($P = 0.047$), generalized epilepsy ($P = 0.027$), higher dose ($P = 0.041$), and longer IR duration ($P = 0.034$), especially in patients without any comorbidities ($P = 0.009$) (Table 3).

### Table 1. Clinical characteristics (n=44)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>$16.4 \pm 2.0$ (13–20)</td>
</tr>
<tr>
<td>Male sex</td>
<td>22 (50.0)</td>
</tr>
<tr>
<td>Epilepsy type</td>
<td></td>
</tr>
<tr>
<td>Focal</td>
<td>30 (68.2)</td>
</tr>
<tr>
<td>Generalized</td>
<td>14 (31.8)</td>
</tr>
<tr>
<td>Dose (mg/day)</td>
<td>$1,068.2 \pm 382.6$ (500–2,000)</td>
</tr>
<tr>
<td>Duration of IR medication (day)</td>
<td>$827.2 \pm 852.2$ (42–3,678)</td>
</tr>
<tr>
<td>Number of AEDs</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>37 (84.1)</td>
</tr>
<tr>
<td>2</td>
<td>6 (13.6)</td>
</tr>
<tr>
<td>3</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>35 (79.5)</td>
</tr>
<tr>
<td>Structural</td>
<td>5 (11.4)</td>
</tr>
<tr>
<td>Genetic</td>
<td>3 (6.8)</td>
</tr>
<tr>
<td>Infectious</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>30 (68.2)</td>
</tr>
<tr>
<td>Migraine</td>
<td>5 (11.4)</td>
</tr>
<tr>
<td>Psychological disorder</td>
<td>4 (9.1)</td>
</tr>
<tr>
<td>Intellectual disability</td>
<td>4 (9.1)</td>
</tr>
<tr>
<td>Cerebral palsy</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Adverse event</td>
<td>4 (9.1)</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation (range) or number (%).

### Table 2. Comparison of the frequency of seizures and missed doses between IR and ER

<table>
<thead>
<tr>
<th>Variable</th>
<th>IR</th>
<th>ER</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seizure frequency (n = 42)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>32</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Less than one (/mo)</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>One or more than one (/mo)</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Seizure-free for both periods</td>
<td>22 (52.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average seizure frequency (/mo)$^a$</td>
<td>$0.24 \pm 0.73$ (0–4)</td>
<td>$0.19 \pm 0.49$ (0–2)</td>
<td>0.491</td>
</tr>
<tr>
<td>Total no. of seizure events</td>
<td>39</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Missing dose-related seizures</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>No. of missed doses (n = 42)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>27</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Less than one (/mo)</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>One or more than one (/mo)</td>
<td>11</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Never missed for both periods</td>
<td>24 (57.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average no. of missed doses (/mo)</td>
<td>$0.46 \pm 0.90$ (0–4)</td>
<td>$0.15 \pm 0.39$ (0–1.5)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Values are presented as number (%) or mean±standard deviation (range). IR, immediate-release formulation; ER, extended-release formulation. $^a$n = 36, six patients had seizures related with missing a dose.
Subjective compliance and satisfaction
Twenty-six of 44 patients disclosed how they felt about switching. Thirteen of 24 patients (54.2%) expressed better compliance, and 25 (96.2%) of 26 patients were satisfied with the ER formulation (Table 4). The most common reason for missing a dose was forgetfulness (13 of 13, 100%).

Discussion
Since phenytoin ER was introduced to the market in 1976, many

Table 4. Patients’ feelings about compliance and satisfaction

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satisfaction (n = 26)</td>
<td></td>
</tr>
<tr>
<td>Satisfied</td>
<td>25 (96.2)</td>
</tr>
<tr>
<td>Same as before</td>
<td>1 (3.8)</td>
</tr>
<tr>
<td>Unsatisfied</td>
<td>0</td>
</tr>
<tr>
<td>Compliance (n = 24)</td>
<td></td>
</tr>
<tr>
<td>Better compliance</td>
<td>13 (54.2)</td>
</tr>
<tr>
<td>Same as before</td>
<td>11 (45.8)</td>
</tr>
<tr>
<td>Worse than before</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Comparison of seizure frequency and compliance between IR and ER according to clinical characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Average seizure frequency (/mo)</th>
<th>Average number of missed doses (/mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>IR</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>0.20 ± 0.47</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
<td>0.28 ± 0.91</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 16</td>
<td>15</td>
<td>0.20 ± 0.48</td>
</tr>
<tr>
<td>&gt; 16</td>
<td>21</td>
<td>0.27 ± 0.87</td>
</tr>
<tr>
<td>Epilepsy type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal</td>
<td>26</td>
<td>0.33 ± 0.84</td>
</tr>
<tr>
<td>Generalized†</td>
<td>10</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Dose (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 1000</td>
<td>23</td>
<td>0.07 ± 0.22</td>
</tr>
<tr>
<td>&gt; 1,000†</td>
<td>13</td>
<td>0.54 ± 1.14</td>
</tr>
<tr>
<td>Duration of IR (day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 365</td>
<td>9</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>&gt; 365†</td>
<td>27</td>
<td>0.32 ± 0.83</td>
</tr>
<tr>
<td>No. of AEDs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEV monotherapy</td>
<td>31</td>
<td>0.12 ± 0.35</td>
</tr>
<tr>
<td>≥ 2</td>
<td>5</td>
<td>1.00 ± 1.70</td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without comorbidity†</td>
<td>22</td>
<td>0.38 ± 0.91</td>
</tr>
<tr>
<td>With comorbidity</td>
<td>14</td>
<td>0.02 ± 0.09</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation.
IR, immediate-release formulation; ER, extended-release formulation; AED, antiepileptic drug; LEV, levetiracetam.
†P<0.05 in the comparison of missed dose frequency.
other AED ERs, such as carbamazepine, oxcarbazepine, topiramate, valproate, lamotrigine, and LEV, have been marketed and used widely [10]. ER formulations have potential advantages compared to IR drugs. They may have a higher compliance, better efficacy, less adverse events, and, as a consequence, reduced healthcare costs [18]. Higher compliance might be achieved by reducing dosing frequency. In a meta-analysis of results from six studies, once-daily dosing had significantly better compliance than more than once-daily dosing (odds ratio, 3.50; 95% confidence interval, 1.73 to 7.08, P < 0.001) [4]. This is also true for epilepsy patients. Doughty et al. [19] found that switching from valproate IR to ER results in improvement of compliance, reduction of seizure frequency, and less reported adverse effects. Similar results were reported in patients taking carbamazepine ER [20]. Regarding LEV ER, Wu et al. [11] reported significant improvements in quality of life by using the European Quality of Life-5 Dimensions questionnaire. Our study showed significant improvements (P = 0.021) in compliance when patients were taking LEV ER medication, which is compatible with previous studies. Additionally, we investigated how patients felt about switching, and most patients were satisfied with LEV ER. However, about half of the patients felt their compliance was unchanged. This may be due to the considerable number of patients (10 of 24, 41.7%) who did not miss doses prior to switching formulations. Higher satisfaction may lead to better compliance.

Enhanced control of seizures and less adverse effect can be achieved by a relatively stable concentration of ER drugs. Serum concentrations of IR drugs have more frequent peaks and troughs, where peaks are associated with adverse events and troughs are associated with seizures, especially in patients having a lower seizure threshold [21]. In addition to the above-mentioned reports, previous studies showed that oxcarbazepine ER had a better tolerability and lamotrigine ER had a better efficacy than IR formulation [22,23]. Currently, there is no evidence that LEV ER is more effective in seizure control than IR. In one placebo-controlled trial using indirect comparisons and meta-analytic techniques, LEV ER had significantly lower rates of treatment-emergent adverse events in the nervous system, psychiatric disorders, and metabolic and nutritional disorders than IR [24]. Our study showed a lower average seizure frequency during the ER period than the IR period, but it was not statistically significant. As most of the included patients were taking LEV IR for a long time without complications, we cannot compare the probability of adverse events between IR and ER. However, LEV ER could be associated with following adverse events. Two patients had dyspepsia and dizziness, which were tolerable and became normal as medication compliance continued. Two other patients had serious psychiatric adverse events, which were suicidal ideations and visual hallucinations, respectively. Both patients had been taking LEV IR over 5 months, and we could not evaluate serum concentrations of LEV at the time of the adverse events. It is unclear if LEV ER was the causative agent; however, it cannot be overlooked, since LEV is commonly associated with behavioral adverse effects, such as depression, hostility, and anxiety [15]. Many studies showed that ER drug has better tolerability than IR drug [25]. However, they also showed that many patients reported adverse events in the ER period, although less frequently than IR period [26-28]. Short-term follow-up and careful monitoring may be needed during the transition time. Although pharmacokinetic profiles of LEV ER are similar to IR, IR medication still leads to more peaks and troughs of serum concentration than ER-like drugs [12]. It may be possible to enhance our understanding of the efficacy of switching patients from LEV IR to LEV ER by performing larger-scale long-term follow-up studies.

It has previously been believed that there is a higher probability of seizures when patients missed an ER drug that is 100% of the entire day’s dose, instead of 50% in a twice-daily dose with an IR drug [29]. However, in a pharmacokinetic simulation study of serum drug concentrations following dosing irregularities with topiramate IR and ER, serum trough levels were only slightly lower for the ER drug when comparing missed doses [30]. In our study, missing dose-related seizures were four of 39 seizures in the IR period and two of 29 in the ER period. Although these data were not statistically evaluated, more missing dose-related seizures occurred in the IR period.

To our knowledge, this is the first study directly comparing LEV IR and ER from the aspect of compliance. Furthermore, there was no significant difference in seizure frequency, which was independent of other clinical characteristics, especially seizure type. The results of our study suggested that LEV ER is a better choice than IR, especially in patients with poor compliance.

Our study had some limitations. First, since this is a retrospective study, it was possible to omit events if the patient did not report them at that time. In particular, some patients might want to hide incorrect behavior, such as skipping medicine. However, similar to other epilepsy clinics, our clinic routinely check compliance, seizure occurrence, and adverse events. Second, many enrolled subjects were relatively well-controlled for seizures. There might be a selection bias toward patients having a higher seizure threshold. These patients were less likely to be affected by the trough level of serum drug concentration. This could be one reason for the results of similar efficacy between IR and ER. Furthermore, improved compliance was not led to better efficacy. These patients may be less affected by improved compliance in 3 months which was relatively short time. Third, because this study is from a single center.
and it is a small-scale study, the statistical power is limited.

Our study shows that LEV ER has an efficacy similar to that of IR formulations. The reduced number of medication doses improved patient satisfaction and medication compliance. LEV ER may be the better option for adolescent epilepsy patients, especially those who have poor medication compliance.

Conflicts of interest

Joon Won Kang is an editorial board member of the journal, but he was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Conceptualization: JWK. Data curation: SYJ. Formal analysis: SYJ. Funding acquisition: JWK. Methodology: JWK. Project administration: JWK. Visualization: YYY. Writing-original draft: SYJ. Writing-review & editing: YYY and JWK.

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Clinical and Genetic Spectrum of STXBP1 Encephalopathy in the Korean Pediatric Population

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Purpose: Syntaxin-binding protein 1 (STXBP1) mutations are known to result in various phenotypes including Ohtahara syndrome, West syndrome, and autism, collectively referred as STXBP1 encephalopathy. This study aimed to expand our understanding of the genotype–phenotype spectrum of STXBP1 encephalopathy in the Korean pediatric population.

Methods: Ten patients with STXBP1 mutations were enrolled for a retrospective chart review. The patients were investigated for developmental delay of unknown cause and epileptic encephalopathy at a single center.

Results: Ten different STXBP1 mutations were identified. Three mutations had not previously been reported (c.1212A>C, c.1497C>G, c1030-2A>G). Eight patients showed early-onset epileptic encephalopathy as the main feature, while the main feature was developmental delay and non-epileptic movements in two patients. The most commonly seen electroencephalographic change was focal/multifocal epileptiform discharges, which were observed in nine patients (90%). The classical burst-suppression pattern was observed in four patients, two of which evolved to show hypsarrhythmia. All patients with seizures had drug-resistant epilepsy. The patients suffered from severe developmental delay regardless of seizure frequency. Six patients showed an associated movement disorder or behavioral disorder.

Conclusion: This study describes the STXBP1 encephalopathy patients in Korean pediatric population, further expanding knowledge of its phenotype spectrum.

Keywords: STXBP1 protein, human; Pediatrics; Epilepsy; Developmental disabilities

Introduction

Developmental and epileptic encephalopathy is a concept to acknowledge that many genetic disorders show developmental impairment as a direct consequence of the genetic mutation, in addition to the detrimental effect of the frequent epileptic activity on
brain development [1]. In the era of next generation sequencing, increasing monogenic causes for developmental and epileptic encephalopathy are being discovered, providing us with new insight to its underlying patho-genetic mechanisms.

Syntaxin-binding protein 1 (STXBP1) (also known as MUNC18-1) is a member of the membrane trafficking proteins predominantly expressed in the brain, which is important for docking and fusion of the synaptic vesicles [2]. There is approximately 200 cases reported in the literature until date, mostly being de novo heterozygous mutations [3]. STXBP1 mutation was first described in 2008, in patients with early infantile epileptic encephalopathy with suppression-burst (EIEE), or Ohtahara syndrome [4]. Subsequent studies showed that STXBP1 mutation is responsible for approximately 22% of cases with Ohtahara syndrome, 6% of non-syndromic early onset epileptic encephalopathy and 2% of West syndrome [4-7]. Due to the expanding phenotype STXBP1 encephalopathy was suggested to be more appropriate [8].

Recognition and classification of diverse phenotypes arising in STXBP1 mutation is crucial to guide future management options. Here, we report 10 patients with STXBP1 mutation from a single center and summarize the detailed clinical features, with the aim to expand its phenotypic spectrum in Korean pediatric population.

Materials and Methods

Patients with STXBP1 mutation were identified from a cohort of 198 pediatric patients with developmental and epileptic encephalopathy of unknown etiology. Patient cohort was selected from the Division of Pediatric Neurology of the Seoul National University Children's Hospital from September 2012 to May 2019. All the patients had no obvious etiology based on clinical features, neuroimaging and metabolic screening. The first-tier test included chromosomal microarray or targeted multi-gene panel. Whole exome sequencing was conducted as a first-tier test as well as a second-tier test, in selected cases based on the decision of the child neurology expert consortium. Variants were evaluated and classified according to the guideline proposed by American College of Medical Genetics (ACMG) [9]. ClinVar database was searched for past variant reports [10]. Population frequency of variants were determined using 1000genomes, ExAC, and gNomad database.

STXBP1 sequencing was performed on DNA from probands and family members using the Sanger method to identify parental origin whenever possible. Information obtained from medical records include age at seizure onset, symptoms at onset, duration from onset to diagnosis, previous diagnosis and treatment, associated psychiatric and behavior symptoms, response to antiepileptic drug (AED) treatment and developmental outcome. Each patient’s electroencephalography (EEG) was obtained and analyzed. Seizure types and epileptic syndromes were classified according to the 2017 International League Against Epilepsy guidelines [1].

The study protocol was approved by the Institutional Review Board of Seoul National University Hospital (IRB No.H-2001-134-1096), and the study was conducted in accordance with relevant guidelines and regulations. Written informed consent by the patients was waived due to the retrospective nature of our study.

Results

1. Genetic identification of STXBP1 mutations

Total of 10 different variants were identified in 10 patients (Table 1 and Fig. 1). Four variants were missense mutations, another four variants were nonsense mutations, and two variants were splicing mutations. Three novel variants (c.1212A>C, c.1497C>G, c.1030-2 A>G) and seven variants previously reported as pathogenic [3,8,11-15] were identified. All novel variants were not found in either 1000Genomes or ExAC control database. Total of eight patients’ parental DNA sample were available for segregation analysis. All eight patients tested harbored de novo mutation.

2. Clinical characteristic of STXBP1 mutations

The clinical characteristics of 10 patients with STXBP1 mutations are summarized in Table 1. Patient’s median age was 7 years old (range, 1 to 11). Head circumference was normal in all patients. Brain magnetic resonance imaging from all patients did not show remarkable abnormalities. Among 10 patients, nine patients had confirmed electroclinical seizures. The median age of seizure onset was 6 months old (range, 3 days to 7 years). Three patients showed neonatal onset seizures, presenting within 1 month of age and other five patients presented as early onset epilepsy, presenting with seizures before the age of 3 years. In remaining two patients seizure was not a main clinical feature; patient 6 was being followed up for global developmental delay and ataxia before his first seizure at age of 7 years. Patient 7 never had clinical seizures until the age of 8 years at last follow-up, but suffered from head dyskinesia, bruxism, and hand stereotypy.

All patients showed significant global developmental delay regardless of seizure frequency. All domains of development were delayed, but the language domain was always more severely affected compared to the motor domain. Only one patient was able to achieve any word output, whereas four patients achieved the milestone of walking. Three patients (patient 4, 6, 7) were already being followed for a global developmental disorder before seizure occurrence. Two patients (patient 6, 7), who's seizure was not a dominant feature, showed clear developmental regression during fol-
<table>
<thead>
<tr>
<th>Variable</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>P7</th>
<th>P8</th>
<th>P9</th>
<th>P10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex/age</td>
<td>F/7 years</td>
<td>F/5 years</td>
<td>F/3 years</td>
<td>M/7 years</td>
<td>F/1 year</td>
<td>M/11 years</td>
<td>F/8 years</td>
<td>F/2 years</td>
<td>F/3 years</td>
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<tr>
<td>Seizure onset</td>
<td>3 days</td>
<td>13 months</td>
<td>3 months</td>
<td>24 months</td>
<td>10 days</td>
<td>7 years</td>
<td>Never</td>
<td>10 days</td>
<td>2 months</td>
<td>2 months</td>
</tr>
<tr>
<td>Seizure type</td>
<td>T</td>
<td>Ba, T</td>
<td>T, Es, My, At</td>
<td>T, Ba</td>
<td>T, Es</td>
<td>My</td>
<td>NA</td>
<td>T, Es</td>
<td>GTC</td>
<td>Es</td>
</tr>
<tr>
<td>Seizure frequency</td>
<td>Daily&gt;weekly</td>
<td>Daily&gt;weekly</td>
<td>Weekly&gt;daily</td>
<td>Weekly&gt;daily</td>
<td>Daily&gt;weekly</td>
<td>Monthly</td>
<td>Never</td>
<td>Frequent daily&gt;weekly</td>
<td>Daily&gt;Monthly</td>
<td>Daily&gt;Seize-free</td>
</tr>
<tr>
<td>EEG</td>
<td>B-S with multifocal spikes+focal spikes</td>
<td>Focal and generalized spikes</td>
<td>B-S with multifocal spikes+focal spikes</td>
<td>Focal spikes</td>
<td>B-S with multifocal spikes+focal spikes</td>
<td>Focal spike</td>
<td>Diffuse background slowing</td>
<td>B-S with multifocal spikes</td>
<td>Focal spikes</td>
<td>Focal spikes</td>
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<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
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<td>Normal</td>
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</tr>
<tr>
<td>DD before seizure</td>
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<td>No</td>
<td>Yes</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Regression</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Neurologic state at the last follow-up</td>
<td>Severe GDD</td>
<td>Few words, climbed stairs</td>
<td>Pointed to objects, walked while holding</td>
<td>No word output, walked alone</td>
<td>Severe GDD</td>
<td>No word output but walked alone&gt;bedridden state</td>
<td>Rolled over, babbling&gt;bedridden state, no word output</td>
<td>Bedridden state, no eye contact</td>
<td>Severe GDD, standing up by self</td>
<td>Severe GDD, bedridden state</td>
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<tr>
<td>Other symptoms</td>
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<td>Hyperactivity, disruptive behavior</td>
<td>None</td>
<td>Hyperactivity, disruptive behavior</td>
<td>None</td>
<td>Hyperactivity, disruptive behavior</td>
<td>Head tremor, dyskinesia, bruxism, hand stereotypy</td>
<td>Truncal dystonia, hypotonia</td>
<td>Bruxism, hand stereotypy</td>
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<td>VPA, LEV, OXC, TPM</td>
<td>VPA, VGB, LEV</td>
<td>VPA, VGB, LEV</td>
<td>VPA, VGB, LEV</td>
<td>TPM, LEV, CLB, VPA</td>
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<td>LEV, VGB, CLB</td>
<td>VPA, CLB</td>
<td>None</td>
</tr>
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<td>EE, unspecified</td>
<td>EE &gt; WS</td>
<td>EE, unspecified</td>
<td>EE &gt; WS</td>
<td>EE, unspecified</td>
<td>EE, unspecified</td>
<td>EIEE</td>
<td>EE, unspecified</td>
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</tr>
<tr>
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<td>De novo</td>
<td>De novo</td>
<td>De novo</td>
<td>De novo</td>
<td>De novo</td>
<td>NA</td>
<td>NA</td>
<td>De novo</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>Pathogenicity (ACMG criteria)</td>
<td>Pathogenic</td>
<td>Likely pathogenic (PS2, PM2)</td>
<td>Pathogenic (PS2, PM1, PM2, PM3, PP3)</td>
<td>Pathogenic (PS2, PM1, PM2, PM3, PP3)</td>
<td>Pathogenic (PS2, PM1, PM2, PM3, PP3)</td>
<td>Pathogenic (PS2, PM1, PM2, PM3, PP3)</td>
<td>Pathogenic (PS2, PM1, PM2, PM3, PP3)</td>
<td>Pathogenic (PS2, PM1, PM2, PM3, PP3)</td>
<td>Pathogenic (PS2, PM1, PM2, PM3, PP3)</td>
<td>Pathogenic (PS2, PM1, PM2, PM3, PP3)</td>
</tr>
</tbody>
</table>

STXBP1, syntaxin-binding protein 1; T, tonic; Ba, behavior arrest; Es, epileptic spasm; My, myoclonic; At, atonic; NA, not available; GTC, generalized tonic-clonic; EEG, electroencephalography; B-S, burst-suppression; H, hypsarrhythmia; MRI, magnetic resonance imaging; DD, developmental delay; GDD, global developmental delay; AED, antiepileptic drug; LEV, levetiracetam; CNZ, clonazepam; VPA, valproic acid; OXC, oxcarbazepine; TPM, topiramate; VGB, vigabatrin; LCS, lacosamide; ClB, cllobazam; EIEE, early infantile epileptic encephalopathy; EE, epileptic encephalopathy; WS, West syndrome; ACMG, American College of Medical Genetics; PVs, pathogenic very strong; PS, pathogenic strong; PM, pathogenic moderate; PP, pathogenic supporting.
low-up. In patient 6, motor function deterioration was observed after seizure onset, which progressed to worsening ataxia and dysphagia. Patient 7 showed developmental regression being evident at the age of 2 years without any clinical seizures, which lead to initial misdiagnosis as Rett syndrome.

Six patients showed additional symptoms other than seizure. Behavior problem was seen in five patients showing autistic like features including hyperactivity/disruptive behavior (n = 3), bruxism (n = 2), hand stereotypy (n = 2). Neurologic symptoms were seen in three patients, including tremor (n = 2), ataxia (n = 1), dystonia (n = 1), hypotonia (n = 1), and dyskinesia (n = 1).

Four patients (patient 1, 3, 5, 8) were initially diagnosed as EIEE (or Ohtahara syndrome) with background burst-suppression pattern with multifocal spikes. Two patients (patient 3, 5) went on to develop hypsarrythmia with epileptic spasms and diagnosis was changed to West syndrome. A total of six patients (patient 1, 2, 4, 6, 9, 10) eventually evolved to unspecified epileptic encephalopathy with focal and multifocal spike discharges (Fig. 2). Patient 7 showed irregular high amplitude delta activities in the background activity. All patients except patient 7 (n = 9) showed either focal or multifocal epileptiform discharges in EEG.

Tonic seizure was the most common type seen (n = 8), but multiple types of seizure semiology are observed including epileptic spasms (n = 4), myoclonic (n = 2), and behavior arrest (n = 2) seizure. Six patients overall showed more than one type of seizure semiology.

Regarding treatments, all patients with epilepsy were refractory to antiepileptic medications, requiring more than two types of AEDs, and none achieved seizure freedom through medication. Of note, patient 10 showed significance response to ketogenic diet, leading to cessation of all AEDs.

**Discussion**

Here we identified 10 Korean patients with STXBP1 mutation, which included three novel mutations. We described detailed phenotypes and genotypes of the patients with STXBP1 encephalopathy. Due to its rarity, the clinical spectrum of STXBP1 encephalopathy is not yet well known, but recent large cohort study suggests that intellectual disability and epilepsy is the two main major components of STXBP1 encephalopathy.

Epilepsy was observed in 95% of STXBP1 encephalopathy patients. Among the patients with available information, about half was taking more than three AEDs and one-third was suffering from frequent seizure. On the other hand approximately one-third achieved seizure freedom [3]. Consistent with the above observation, nine patients (90%) in the current cohort showed epilepsy and all patients required more than two AEDs.

In the same study, moderate to severe intellectual disability was observed in 88.4% of patient. Notably, developmental delay was present before seizure onset in 64.3%, and 7% of patients were observed to have just developmental delay without any seizure. Other previous studies also observed that some degree of developmental delay was often observed prior to any seizure onset, thus it is considered an independent domain from epilepsy [3,12]. Indeed, in our cohort all patients showed profound developmental delay or severe intellectual disability and 30% of patients showed developmental delay before seizure onset.

However, it is often difficult to assess whether seizure activity or epileptiform discharges have any effect on developmental process or whether developmental delay is totally separate phenotype in STXBP1 encephalopathy. It was suggested that developmental regression is rarely seen and does not seem to be related to seizure activity [3]. In the current cohort, patient 6 and 7 showed clear regression during follow-up. Interestingly, both patients were different from typical patients presenting with early onset epileptic encephalopathy, as seizure was not their main feature and both showed predominantly non-epileptic movement symptoms. Patient 7 followed Rett syndrome like features with regression starting at age of 3 with autistic features, and patient 6 showed many resemblances to the phenotype previously described as ataxia-tremor-retardation syndrome [16]. Patient 6 showed clear motor func-

Fig. 1. Positions of syntaxin-binding protein 1 (STXBP1) variants.
tion regression after seizure onset with worsened ataxia and newly developed dysphagia. Above two patients support that seizure activity and intellectual development are indeed independent domains.

STXBP1 encephalopathy was known to be most commonly associated with burst suppression and hypsarrhythmia, as it was initially described mostly in Ohtahara syndrome [11,12,17]. However recent large cohort study suggest most commonly described changes are focal or multifocal activities, seen in 64% of the cases [3]. Our patients also showed that focal or multifocal epileptiform discharges were the most common finding (60%), and classical burst-suppression patterns were seen in only proportion of the patients (40%). However, patients who were followed up long term showed that patients with burst-suppression pattern often evolve to hypsarrhythmia or non-specific focal patterns. Thus it is possible some patients did have burst-suppression pattern at one point but was never recorded. The range of abnormal EEG findings was wide in the current cohort, and they showed little correlation with their developmental status or seizure activity, in accordance to previous reports [3,18].

The reason why the clinical presentation of patients with STXBP1 mutation varies so much is yet unknown. Previous evidence supported the hypothesis that haploinsufficiency is the main pathogenic mechanism underlying STXBP1 encephalopathy, which may explain for such phenotypic heterogeneity [19]. However, there is growing evidence that STXBP1 mutation have more than one mechanism of pathophysiology. Recently, a homozygous STXBP1 mutation was found to cause Lennox-Gastaut syndrome, showing that STXBP1 also have a dominant-negative effect [20]. Thus mechanism of STXBP1 encephalopathy still needs much further research in the future.

Another important aspect of STXBP1 mutation to consider is mosaicism. Both somatic and germline mosaicism of STXBP1 mutation has been reported. It is interesting that focal epileptiform discharges are commonly seen in STXBP1 encephalopathy, especially since there were two reports of significant improvement of seizure with epilepsy surgery [8,21]. According to these reports, presence of focal cortical dysplasia have been confirmed by tissue pathology in both cases. One patient was confirmed to also harbor somatic mosaicism for homozygosity of STXBP1 mutations in the dysplastic tissue.

Pathogenic role of somatic mosaicism on STXBP1 encephalopathy is still unknown, but this finding suggest they may play an important role in cortical development.

Fig. 2. Electroencephalograms of patients with syntaxin-binding protein 1 (STXBP1) mutations showing focal epileptiform discharges. (A) Patient 1, central area. (B) Patient 2, right fronto-temporal area. (C) Patient 3, right or left occipital area. (D) Patient 4, left temporo-occipital area. (E) Patient 6, right frontal area.
On the other hand, germline mosaicism can cause major problem in genetic diagnosis of STXBP1 mutation and in family genetic counseling. Germline mosaicism of STXBP1 has been reported previously from an unaffected parent of a patient [22,23]. Assumption of de novo mutation based on parental Sanger sequencing maybe incorrect, as it is unlikely to detect low-rate mosaicism [24]. Therefore it is possible that unrecognized parental STXBP1 mosaicisms are present among the current cohort as well. Recently, the high fold coverage of next-generation sequencing allow for the detection of even very low levels of mosaicism in the blood cells [25]. This has opened a new era of genetic testing, which will hopefully broaden our knowledge of mosaicism in STXBP1 encephalopathy.

Current study has several limiting factors. Selection bias is present due to primary identification of cases from pediatric clinics with significant epilepsy or developmental delay. Given the wide variety of phenotypes, it is plausible that there are cases of STXBP1 encephalopathy with milder symptoms or different phenotype were overlooked. There are also possibility that patients with focal cortical dysplasia or other cortical malformation harbor STXBP1 mutation and were never considered for genetic testing, in the absence of the classical features.

In conclusion, STXBP1 encephalopathy showed a wide clinical spectrum of phenotypes from severe epileptic encephalopathy such as Ohtahara syndrome or West syndrome to unknown neurodevelopmental retardation without epilepsy. Their seizure types and EEG findings are also diverse. Therefore it is important to consider STXBP1 mutation even in patients without seizure, or predominantly focal EEG changes without history of burst suppression. Further research is needed to discover the full range of phenotypes of STXBP1 encephalopathy in order to elucidate underlying disease mechanism.

Conflicts of interest

Jieun Choi is an associate editor, Ki Joong Kim and Jong-Hee Chae are the editorial board members of the journal, but they were not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Conceptualization: WJK and JHC. Data curation: WJK, YKS, YJK, and SYK. Formal analysis: WJK and YKS. Funding acquisition: KJK and JHC. Methodology: WJK and YKS. Project administration: HK, BCL HH, JC, and JHC. Visualization: WJK and YJK. Writing-original draft: WJK. Writing-review & editing: BCL, KJK, and JHC.

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References


Comparison of the Demographics and Ratio of Rotavirus-Associated Benign Convulsions with Mild Gastroenteritis to Rotavirus Gastroenteritis before and after Rotavirus Vaccination over a Period of 20 Years

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Purpose: Through a study of rotavirus gastroenteritis (RVGE) cases experienced over 20 years at our center, we aimed to investigate changes in the ratio of rotavirus-associated benign convulsions with mild gastroenteritis (RaCwG) to RVGE and in patients’ demographics after rotavirus vaccination.

Methods: We analyzed the data of patients aged ≤6 years who visited Inha University Hospital between January 1999 and December 2019 and were confirmed to have RVGE. Patients were divided according to whether they had convulsions with mild gastroenteritis, and their demographics were compared. The yearly and monthly ratios of RaCwG to RVGE were evaluated. To investigate the effects of rotavirus vaccination, data regarding demographics and prevalence were divided into periods I (pre-vaccination, 1999–2009) and II (post-vaccination, 2010–2019) and compared.

Results: Altogether, 2,100 children had RVGE, and 50 (2.4%) had RaCwG. RaCwG occurred frequently every 4 to 6 years. Although the total number of RVGE and RaCwG cases significantly decreased in period II versus period I, the ratio of RaCwG to RVGE did not differ between the two groups (P=0.921). The age distribution shifted upwards in period II versus period I (P=0.001), but the sex ratio and seasonal distribution showed no significant difference.

Conclusion: Considering that the ratio of RaCwG to RVGE is dynamic, an increase in the ratio of RaCwG may be possible in the future. Although there was no change in the ratio of RaCwG to RVGE, the number of RVGE and RaCwG patients decreased simultaneously, suggesting that rotavirus vaccination was effective in preventing RaCwG.

Keywords: Rotavirus vaccines; Rotavirus infections; Seizures

Introduction

Rotavirus gastroenteritis (RVGE) is a major form of acute gastroenteritis that is associated with a high hospitalization rate, resulting in 215,000 deaths in children under 5 years of age each year [1]. Children infected with rotavirus may have watery diarrhea, vomit-
ing, and fever but symptoms are less severe in infants because of the protective effect of the maternal antibodies that are transferred through the placenta or through breast milk [2]. Prevention is important, as rotavirus infection is associated not only with enteric symptoms, but also with neurologic disorders such as seizures, leukoencephalopathy, meningoencephalitis and cerebelitis [3,4].

The most common neurologic complication of rotavirus infection is benign convulsions with mild gastroenteritis (CwG). CwG was reported for the first time by Morooka [5] in 1982 as mild gastroenteritis that causes afebrile convulsions without severe dehydration, electrolyte imbalance, or hypoglycemia. Rotavirus-associated benign convulsions with mild gastroenteritis (RaCwG) is characterized by a short duration, and all episodes of clustered seizures usually stop within 24 hours of seizure onset [6-9]. The underlying pathological mechanisms remain unclear. Some researchers have attempted to detect neurologic disorders based on the presence of rotavirus RNA and antigens in the cerebrospinal fluid of infected patients [10-12]. Although CwG is frequently reported in East Asian countries, including Japan, South Korea, and Taiwan, it has also been recently reported in the United States and Europe [3,13-15]. Such convulsions are often associated with RVGE [5,16].

The prevalence of RVGE has decreased since the introduction of rotavirus vaccines [17,18]. In South Korea, RotaTeq (Merck & Co. Inc., West Point, PA, USA), a pentavalent vaccine, and Rotarix (GlaxoSmithKline Biologicals, Rixensart, Belgium), a monovalent vaccine, became available in June 2007 and March 2008, respectively. However, rotavirus vaccines have not yet been incorporated into a national immunization program. Since then, the rotavirus vaccination rate exceeded 50% in 2009 and reached 84.5% in 2016 [19,20].

Emerging data suggest that vaccines may not protect all population equally. Females, in particular, typically develop higher antibody responses after vaccination [21]. Moreover, after rotavirus vaccination, changes in the age and the seasonal distribution were founded and a decreased prevalence of RVGE was observed [22,23]. However, studies investigating the demographic changes and ratio of RaCwG to RVGE have been scantily conducted.

Thus, in this study, we focused on changes in the ratio of RaCwG to RVGE and the demographics after rotavirus vaccination through the examination of cases of RVGE that we have experienced over the past 20 years at a single center.

Materials and Methods

1. Participants and sample collection
We assessed the medical records of patients with RVGE aged ≤ 6 years who visited Inha University Hospital, which is a tertiary hospital in Incheon with a population of 2.9 million people, between January 1999 and December 2019. RVGE was confirmed using immunochromatography, enzyme immunoassay, or reverse transcription-polymerase chain reaction (RT-PCR). Patients infected with norovirus, enteric adenovirus or astrovirus were excluded.

With reference to the diagnostic criteria suggested by Komori et al. [16], CwG was defined as follows: (1) afebrile seizure occurring within 5 days of acute viral gastroenteritis in previously healthy infants and children; (2) absence of moderate or severe dehydration; (3) absence of abnormal findings in cerebrospinal fluid analyses, serum electrolytes, and blood glucose; and (4) cases with good prognosis. Being afebrile was defined as a body temperature below 38.0°C when measured on the tympanic membrane or axilla. Findings of seizure were confirmed through the statement of caregivers or others that observed the seizure, and neurologic imaging and electroencephalography results were confirmed through consultation with the Radiology and Pediatric Neurology Departments.

To compare the demographics and ratio of RaCwG to RVGE before and after rotavirus vaccination, data were divided into period I (January 1999 to December 2009) and period II (January 2010 to December 2019), because prescription of rotavirus vaccines was started in 2009 in our hospital and the rotavirus vaccination rate reached 50% in South Korea [19].

The rotavirus antigen detection test for stool samples was conducted using immunochromatography assay kits (SD BIOLINE Rotavirus, Standard Diagnostics Inc., Yongin, Korea) until 2010 and using enzyme immunoassay (RIDASCREEN Rotavirus, R-Biopharm Aktiengesellschaft, Darmstadt, Germany) between 2011 and 2019. Rotavirus RT-PCR has been conducted using Allplex GI-Virus Assay (Seegene, Seoul, Korea) since June 2014.

This study was approved by the Institutional Review Board of Inha University Hospital (IRB No. 2020-04-012). Written informed consent by the patients was waived due to a retrospective nature of our study.

2. Statistical analyses
Statistical analyses were performed using SPSS version 19.0 (IBM Corp., Armonk, NY, USA). Changes in the ratio of RaCwG to RVGE and the sex ratio were compared between periods I and II using chi-square tests, and the changes in season were compared using linear by linear association test. The age at onset was compared between the periods using the Mann-Whitney U tests. P values < 0.05 were considered statistically significant.
Results

1. Demographics of patients with RVGE and RaCwG

Overall, 2,100 children were confirmed to have RVGE between 1999 and 2019. Of these, 50 had RaCwG while 2,050 did not. The male-to-female ratio in children with CwG was 1:1.1 and of RVGE without CwG was 1:0.8. The median age was 23.0 and 10.0 months in children with CwG and RVGE without CwG, respectively. The age at onset was significantly lower in children without CwG than in those with CwG (P < 0.01). Seasonally, RaCwG was most common in winter (n = 28, 56.0%), followed by spring (n = 17, 34.0%) but RVGE without CwG was most common in spring (n = 772, 37.7%), followed by winter (n = 755, 36.8%) (Table 1).

2. Yearly and monthly prevalence of RaCwG

The ratio of RaCwG to RVGE was 2.43 per 100 RVGE cases. The ratio of RaCwG to RVGE increased most noticeably in 2011, reaching 6.19 per 100 RVGE patients and RaCwG occurred periodically, almost every 4 to 6 years (Fig. 1). Regarding the monthly number of patients, RaCwG was common between December and April and was most common in January (n = 11, 22%). In contrast, no cases were noted in June, August, and October. RVGE without CwG was most common in March (n = 343, 16.7%) and least common in September (n = 58, 2.8%) (Fig. 2).

3. Comparison of period I and period II

There were 31 cases of RaCwG in period I compared with 19 cases in period II. The male-to-female ratio was 1:1.4 in period I, with more female patients affected by RaCwG. Although there were more male patients in period II (male-to-female ratio, 1:0.7), the difference was not statistically significant (P = 0.273). The median age of the patient in period I and period II were 21 and 31 months, respectively. The age at onset was significantly higher in period II than in period I (P = 0.001). The ratio of RaCwG to RVGE was 2.4% in period I as well as in period II, without any significant difference (P = 0.921). Regarding seasonality, RaCwG was common in winter during period I (n = 20, 64.5%) and in the spring during period II (n = 10, 52.6%), but there was no statistical significance (P = 0.052) (Table 2). Regarding monthly prevalence, in period I, the numbers of patients with RVGE with and without CwG were highest in January. In period II, the numbers of patients with RVGE with and without CwG were highest in March and April, respectively (Fig. 3).

Table 1. Demographic characteristics of patients with RaCwG and RVGE without CwG

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RaCwG</th>
<th>RVGE without CwG</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>50</td>
<td>2,050</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>24 (48)</td>
<td>1,160 (56.6)</td>
</tr>
<tr>
<td>Girls</td>
<td>26 (52)</td>
<td>890 (43.4)</td>
</tr>
<tr>
<td>Age (mo)</td>
<td>23 (17–27.8)</td>
<td>10.0 (0–21)</td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td>1 (2)</td>
<td>137 (6.7)</td>
</tr>
<tr>
<td>2000</td>
<td>1 (2)</td>
<td>43 (2.1)</td>
</tr>
<tr>
<td>2001</td>
<td>4 (8)</td>
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<tr>
<td>2002</td>
<td>3 (6)</td>
<td>181 (8.8)</td>
</tr>
<tr>
<td>2003</td>
<td>0</td>
<td>131 (6.4)</td>
</tr>
<tr>
<td>2004</td>
<td>5 (10)</td>
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<td>2005</td>
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<tr>
<td>2006</td>
<td>4 (8)</td>
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</tr>
<tr>
<td>2007</td>
<td>3 (6)</td>
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</tr>
<tr>
<td>2008</td>
<td>3 (6)</td>
<td>94 (4.6)</td>
</tr>
<tr>
<td>2009</td>
<td>1 (2)</td>
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<td>2010</td>
<td>3 (6)</td>
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<td>2011</td>
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<td>2014</td>
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</tr>
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<td>2015</td>
<td>2 (4)</td>
<td>42 (2)</td>
</tr>
<tr>
<td>2016</td>
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</tr>
<tr>
<td>2017</td>
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</tr>
<tr>
<td>2019</td>
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<td>53 (2.6)</td>
</tr>
<tr>
<td>Season</td>
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<td></td>
</tr>
<tr>
<td>Spring</td>
<td>17 (34)</td>
<td>772 (37.7)</td>
</tr>
<tr>
<td>Summer</td>
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</tr>
<tr>
<td>Autumn</td>
<td>4 (8)</td>
<td>254 (12.4)</td>
</tr>
<tr>
<td>Winter</td>
<td>28 (56)</td>
<td>755 (36.8)</td>
</tr>
</tbody>
</table>

Values are presented as number (%) or median (interquartile range). RaCwG, rotavirus-associated benign convulsions with mild gastroenteritis; RVGE, rotavirus gastroenteritis; CwG, convulsions with mild gastroenteritis.

Discussion

This retrospective study included patients with RaCwG patients from a single center over an examined period of 20 years to determine changes in demographics and ratio of RaCwG to RVGE since the introduction of rotavirus vaccination. As a result, we found that RaCwG caused cyclic epidemics. After vaccination, the total number of patients with RaCwG decreased but there was no change in prevalence. In addition, change in demographics was found.

RaCwG commonly occurs in winter and early spring in temper-
Fig. 1. The ratio of rotavirus-associated benign convulsions with mild gastroenteritis (RaCwG) to rotavirus gastroenteritis (RVGE).

Fig. 2. Monthly distributions of rotavirus-associated benign convulsions with mild gastroenteritis (RaCwG) and rotavirus gastroenteritis (RVGE) without convulsions with mild gastroenteritis (CwG).
The ratio of RaCwG to RVGE differs by country: 2.6% to 2.9% in Japan, 3.7% in India, 2.1% to 5.0% in Taiwan, and 1.29% in Hong Kong [6,24,25]. In Korea, the reported incidence was 5.6% [25]. In this study, the ratio of RaCwG to RVGE was 2.4%, similar to those reported by previous studies.

RaCwG occurs between age 1 month and 6 years and peaks at age 1 to 2 years, [13,14,16,24,27] Similar to previous study, RaCwG was most common in 1-year-old patients, and the median age at onset was 23 months in this study. In addition, patients with RVGE without CwG were significantly younger than those with RaCwG. This result is different from the recent study using nationwide data in South Korea that reported the age of RaCwG was younger than RVGE. We suspected that this is due to this research being conducted at a single center and the total number of patients being small. Further studies on the age of RaCwG patients compared to RVGE patients will be needed [28].

In this study, the ratio of RaCwG occurred frequently every 4 to 6 years. Mycoplasma infection, which causes outbreaks every 3 to 5 years, is known to contribute by gene divergences within the P1 gene divergences.
adhesin. Thus, we suspected that this was because of the increased prevalence in the local community of a certain genotype such as CwG. Previously, Yang et al. [29] found no significant difference between RaCwG and genotype in an analysis of 13 patients with RaCwG. However, Choi et al. [30] compared the genotype of 82 rotavirus positive patients, of which 11 had neurologic complications, and G2P [4] was found to be significantly associated with neurologic complication. Considering the periodic outbreaks of RaCwG, an outbreak is possible in the future. Therefore, continuous monitoring of RaCwG and genotypes is required.

In this study, we observed cases for longer than 20 years and found that the ratio of RaCwG to RVGE, at 2.4%, was identical in periods I and II. However, the numbers of patients with RVGE and RaCwG decreased simultaneously. Moreover, patients with RaCwG were poorly observed after 2016. These results appear to be due to an increase in the vaccination rate in Korea. Therefore, we expect that the number of patients with RaCwG will decrease significantly as the use of rotavirus vaccines increases.

After vaccination, previous studies reported changes in the demographics of rotavirus. In Finland, rotavirus infection was most common in children aged < 5 years, but after the introduction of the vaccine, it became most common in children between the ages of 6 and 16 years and individuals over 70 years of age [31]. In addition, the start of the rotavirus season was delayed and the duration of the season was shortened after the rotavirus vaccination [32]. In this study, the age at onset of RaCwG was greater in period II than in period I. This may have been influenced by the higher prevalence in older children who have not received rotavirus vaccination in period II. In terms of seasons, RaCwG was more common in winter in period I and in spring in period II. This may be associated with the shift in peak in all RVGE cases from January in period I to March in period II.

In RaCwG, previous studies reported female-dominant prevalence, with male-to-female ratios of 1:1.5 to 1.8 [24,25]. However, data in these studies were mostly from pre-rotavirus vaccination days. Similar to previous studies, the male-to-female ratio was 1:1.4 in period I in this study, but RaCwG more commonly occurred in male patients in period II at a ratio of 1:0.7. Although this difference was not statistically significant, post-vaccination changes in the sex ratio should be monitored continuously.

This study had some limitations. Data were collected only from a single center. Although G1P is the most common genotype in South Korea [33,34], common genotypes of rotavirus change depending on season and geography [25,29]. Our study used data collected over 20 years, but an analysis of genotypes of RaCwG in various areas of South Korea is necessary. Furthermore, whether patients received vaccination could not be confirmed in their electronic chart data. If vaccination data were available, we would have been able to compare vaccinated and unvaccinated patients to investigate the effects of rotavirus vaccination, but we had to set different periods as the data were unavailable.

Considering the ratio of RaCwG to RVGE over the past 20 years and its fluctuation, it may be possible in the future to increase the ratio of RaCwG. Therefore, continuous monitoring of RaCwG and its genotyping will be required. Although there was no change in the prevalence of RaCwG, both RVGE, and RaCwG decreased simultaneously. Thus, rotavirus vaccination was effective in preventing RaCwG. As demographics such as patient sex, age, and season of RaCwG changed after rotavirus vaccination, further research is required on this aspect.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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Author contribution

Conceptualization: YSK. Methodology: YSL. Data curation: YSL and DJH. Formal analysis: DHK. Validation: DHK. Writing-original draft: YSL and DHK. Writing-review & editing: YSK.

Acknowledgements

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29. Yang HR, Lee YM, Ko JS, Seo JK. Detection and genotyping of viruses detected in children with benign afebrile seizures associ-
Characteristics of Meningitis in Febrile Infants Aged ≤90 Days

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Department of Pediatrics, Inje University Sanggye Paik Hospital, Seoul, Korea

Purpose: This study evaluated the clinical and laboratory characteristics of infants ≤90 days old with meningitis who presented to the hospital with a fever. We also investigated whether initial C-reactive protein levels and white blood cell counts were reliable predictors of bacterial meningitis.

Methods: The medical records of 1,151 infants aged ≤90 days who visited our hospital with a fever between October 2009 and October 2019 were retrospectively evaluated.

Results: Of the 1,151 patients, 274 (23.8%) had meningitis (bacterial, n=7; viral, n=206; pleocytosis in the cerebrospinal fluid, n=136). Thirty-seven viral meningitis patients (18.0%) had a positive polymerase chain reaction result without pleocytosis in the cerebrospinal fluid. The patients without pleocytosis were significantly younger. Among the patients with only pleocytosis, 46 had a urinary tract infection, 22 had other viral infections, and the etiology was unknown in 68. Among patients with urinary tract infections, infants without pleocytosis were younger than those with pleocytosis. Low white blood cell counts (<5,000/mm³) were more frequently found in bacterial meningitis patients (n=7) than in viral meningitis patients. Furthermore, there were normal C-reactive protein levels (42.9%) and no pleocytosis (20%) in some cases of bacterial meningitis.

Conclusion: Our findings show that meningitis is not uncommon among infants ≤90 days old who were brought to the hospital with complaints of fever. Furthermore, younger patients may not have cerebrospinal fluid pleocytosis, even if they have bacterial meningitis. Therefore, the patient’s condition should be monitored closely and, if necessary, a re-examination should be considered.

Keywords: Infant; Fever; Meningitis, bacterial; Meningitis, viral; Urinary tract infections

Introduction

Fever is the most common symptom indicative of serious infections among young infants, including neonates [1]. Although most febrile young infants have simple viral infections [2], bacterial infections, such as urinary tract infections (UTIs), meningitis, and bacteremia, are not uncommon.

Central nervous system infections, such as bacterial or viral meningitis, frequently occur in young infants due to immature humoral and cellular immunity. Depending on the age at diagnosis, type of identified organism(s), and delay in treatment, central nervous system infections can lead to several acute comorbidities, severe com-
plications, and long-term disabilities, ranging from hearing loss to a permanent motor or cognitive impairment [3]. An early diagnosis of meningitis in younger infants is essential for correct treatment so as to reduce mortality and complications.

Clinical signs and symptoms of central nervous system infections are often nonspecific in young infants. These symptoms include fever, hypothermia, food retention, skin lesions, irritability, or general malaise [4,5]. Therefore, blood and cerebrospinal fluid (CSF) analyses are performed to diagnose or rule out severe central nervous system infections. A lumbar puncture (LP) with an analysis of the CSF profile might help the clinician differentiate between the presence or absence of meningitis and between bacterial or viral meningitis. CSF white blood cell (WBC) counts > 1,000 cells/mm$^3$ suggest bacterial meningitis [6]. The presence of CSF pleocytosis is usually diagnosed with meningitis. However, the absence of CSF pleocytosis in younger infants with enteroviral meningitis has been previously reported [7-9].

In addition, serum C-reactive protein (CRP) levels, WBC count, and procalcitonin are commonly used inflammatory biomarkers in the blood. However, there is no consensus regarding their ability to distinguish between bacterial and viral meningitis [10].

In this study, we aimed to describe the biochemical characteristics of CSF, blood, and urine in febrile infants ≤ 90 days old with meningitis. Furthermore, we compared the characteristics of the laboratory results in patients with meningitis after dividing them into two groups based on the presence or absence of pleocytosis in the CSF.

Materials and Methods

1. Study population

The medical records of 1,172 patients of age less than 90 days who visited Inje University Sanggye Paik Hospital with a complaint of fever between October 2009 and October 2019 were retrospectively evaluated. The study protocol was approved by the Institutional Review Board of Inje University Sanggye Paik Hospital (2020-03-015). The requirement for informed consent was waived due to the retrospective nature of the study.

Patients < 35 weeks gestational age (n = 7) and those with underlying conditions (n = 14), including Cornelia de Lange syndrome (n = 1), cerebral infarction (n = 2), periventricular leukomalacia (n = 1), Prader-Willi syndrome (n = 1), congenital cytomegalovirus infection (n = 1), and congenital hypothyroidism (n = 1), were excluded. Data regarding age, gestational age, sex, clinical characteristics, diagnosis, and all laboratory profiles, including blood, CSF, urine, and sputum were collected from all the patients.

2. Laboratory tests and microbiological tests of CSF, blood, urine, and sputum

For all included patients, all blood laboratory tests including initial WBC and CRP level checks were performed immediately after visiting the hospital. The peak CRP was defined as the highest numerical value by comparing all tests performed during hospitalization, including the initial CRP.

For all the included patients (n = 1,151), LP is usually performed after the initial blood test following which the platelet level is confirmed. In our study, LP was performed on 867 patients (74.3%). Of those 867 patients, 281 did not undergo CSF analysis due to traumatic tap or insufficient sample quantity, and one patient had a positive result for virus polymerase chain reaction (PCR) of enterovirus. Patients with traumatic LP were excluded from the CSF analysis. The detailed results are described in Fig. 1.

All patients who received the LP were tested for bacterial culture and underwent real-time multiplex PCR (Seegene Inc., Seoul, Korea) for six types of viruses—cytomegalovirus, human herpesvirus 6, Epstein-Barr virus, herpes simplex viruses 1 and 2, and varicella-zoster virus—or reverse transcriptase PCR for enteroviruses (Seegene Inc.).

Bacterial meningitis was defined as bacterial growth in the CSF. Viral meningitis was defined as either a positive PCR result or patients with age-adjusted CSF pleocytosis with a negative CSF culture or PCR results for a virus without UTIs or bacteremia. Coinfections were defined as meningitis accompanied by bacteremia, UTI, or other viral infections.

CSF pleocytosis was defined as a CSF WBC count > 22 WBCs/mm$^3$ if the patient was < 4 weeks old; > 15 WBCs/mm$^3$ if the pa-

Fig. 1. Flow diagram depicting the selection of the study population.
tient was between 4 and 7 weeks of age; and > 5 WBCs/mm³ if the patient was ≥ 8 weeks old. UTI was diagnosed as a urine culture (collected by urethral catheterization) with more than 100,000 colony-forming units/mL [11]. The growth of bacteria that are not commonly considered as pathogens (Staphylococcus epidermidis or coagulase-negative Staphylococcus) was classified as a priori as contamination [12].

Patients with various types of meningitis were compared and analyzed based on the presence or absence of pleocytosis in the CSF.

3. Statistical analysis
Continuous variables, including age and CRP, are expressed as the mean ± standard deviation and were compared using t-tests. Categorical variables, including sex and age group, were compared using a chi-square test or Fisher’s exact test. The comparison between the viral meningitis group and bacterial meningitis was performed as a nonparametric test, Mann-Whitney U test. All statistical analyses were performed using IBM SPSS Statistics for Windows version 25.0 (IBM Corp., Armonk, NY, USA). Statistical significance was defined as a P < 0.05.

Results

1. Characteristics of all included patients
During the study period, 1,151 infants (630 [54.74%] males and 521 [45.26%] females) were included. The cause of infection was identified in 724 (432 [59.67%] males and 292 [40.33%] females). LP was performed on 867 patients: meningitis (n = 274, 23.8%); UTI (n = 180); bacteremia (n = 24); and other viral infections (n = 347). The remaining 503 patients with unexplained fever were suspected of having a viral infection and they recovered without complications (Fig. 2).

2. Characteristics of patients with all kinds of meningitis
Of the 274 meningitis patients, 158 (57.7%) were males and 116 (42.3%) were females. The mean age at symptom onset was 52.85 ± 23.42 days (range, 1 to 90). Fifty-nine (21.5%) infants were ≤ 30 days old, 94 (34.3%) were 31 to 60 days old, and 121 (44.2%) were 61 to 90 days old. Seven patients had bacterial meningitis (Group B Streptococcus [GBS], n = 5; Escherichia coli, n = 2), 260 had viral meningitis, and 136 had only pleocytosis in the CSF (Table 2). Of the 130 patients with viral meningitis who had a positive viral PCR test, 37 (17.9%) had a positive PCR result without pleocytosis in the CSF. Of the 136 patients with meningitis with only pleocytosis, 46 had a UTI, 22 were reactive in other viral infections, and 68 were of an unknown etiology. The detailed information is described in Fig. 2.

3. Bacterial infections
Among the patients with bacterial meningitis, except for two patients with traumatic tap who could not be included in the analysis, 80% (4/5) had pleocytosis and 20% (1/5) had non-pleocytosis CSF. One patient who had non-pleocytosis with the initial WBC count and initial CRP in a normal range was later diagnosed with GBS meningitis. In addition, another patient with traumatic tap had CSF non-pleocytosis (CSF red blood cell count, > 20,000/mm³; CSF WBC, 3/mm³). Therefore, 28.6% (2/7) of bacterial meningitis patients had non-pleocytosis.

A comparison of the bacteremia patients with and without pleocytosis in the CSF revealed that a significantly large proportion of patients without pleocytosis underwent LP < 24 hours after the onset of fever (10/11, 90.9%; chi-square test, P = 0.046).

4. Comparison of viral meningitis with and without CSF pleocytosis
Of the 191 children diagnosed with viral meningitis without traumatic tap, 81.7% (156/191) had CSF pleocytosis and 18.3% (35/191) had CSF non-pleocytosis (Table 2). The comparison of viral meningitis with and without pleocytosis showed no significant difference in CRP (Table 2). However, patients without pleocytosis were significantly younger (39.20 ± 22.64 days vs. 54.19 ± 22.16 days, t-test P < 0.05) and had lower WBC counts (12,757 ± 5,358.75/mm³ vs. 9,134 ± 3,507.66/mm³, t-test P < 0.05). A greater proportion of patients without pleocytosis underwent LP < 24 hours after the onset of fever (10/11, 90.9%; chi-square test, P = 0.006) than patients with pleocytosis (Table 2). The multivariate logistic regression analysis showed that younger age, shorter interval from fever onset to LP, lower WBC in the peripheral blood, lower CSF protein, and higher CSF glucose were independent predictors of CSF non-pleocytosis in viral meningitis in infants ≤ 90 days old (Table 2).

There were no patients with bad prognosis from viral meningitis. However, one of the patients with bacterial meningitis died and one suffered from subdural empyema, but is developing normally without any sequelae.

5. Characteristics of patients with UTIs
Among the patients with UTIs, 7.8% had coinfection with PCR-positive viral meningitis, 25.6% had reactive pleocytosis in the CSF, and one patient (0.5%) had a co-infection with bacterial meningitis. A comparison of the patients with UTI with and without pleocytosis in the CSF revealed that younger patients had a lower rate of pleocytosis occurrence (56.23 ± 20.41 days vs. 67.61 ± 13.59 days, t-test P < 0.05). A greater proportion of patients without pleocytosis underwent LP < 24 hours after the onset of fever (chi-square test, P = 0.006).
Fig. 2. Flow diagram illustrating the strategy to identify the cause of fever.
Table 1. Clinical characteristics of febrile infants with meningitis (n=274)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
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</tr>
<tr>
<td>Age (day)</td>
<td>52.85±23.42 (1–90)</td>
</tr>
<tr>
<td>Age group (day)</td>
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<tr>
<td>0–30</td>
<td>59 (21.5)</td>
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<td>31–60</td>
<td>94 (34.3)</td>
</tr>
<tr>
<td>61–90</td>
<td>121 (44.2)</td>
</tr>
<tr>
<td>Sex, male:female</td>
<td>158 (57.7):116 (42.3)</td>
</tr>
<tr>
<td>Bacterial meningitisa</td>
<td>7</td>
</tr>
<tr>
<td>Group B Streptococcus (Streptococcus agalactiae)</td>
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<tr>
<td>Escherichia coli</td>
<td>2</td>
</tr>
<tr>
<td>Viral meningitis with virus PCR positive in CSFb</td>
<td>130</td>
</tr>
<tr>
<td>Type of virus in cases with positive PCR in CSF</td>
<td></td>
</tr>
<tr>
<td>Human herpesvirus-6 meningitis</td>
<td>1</td>
</tr>
<tr>
<td>Enteroviral meningitis</td>
<td>129</td>
</tr>
<tr>
<td>Type of viral meningitis excluding patients without CSF analysis</td>
<td>113</td>
</tr>
<tr>
<td>Viral PCR (+) in CSF with pleocytosis</td>
<td>76</td>
</tr>
<tr>
<td>Viral PCR (+) in CSF without pleocytosis</td>
<td>37</td>
</tr>
<tr>
<td>Traumatic tap</td>
<td>16</td>
</tr>
<tr>
<td>Viral PCR (+) without CSF analysis</td>
<td>1</td>
</tr>
<tr>
<td>Only pleocytosis in CSF</td>
<td>136</td>
</tr>
<tr>
<td>Unknown pleocytosis</td>
<td>68</td>
</tr>
<tr>
<td>Reactive in urinary tract infection</td>
<td>46</td>
</tr>
<tr>
<td>Reactive in other viral infection</td>
<td>22</td>
</tr>
<tr>
<td>Reactive in bacteremia</td>
<td>1</td>
</tr>
<tr>
<td>Urinary tract infection with viral meningitis (virus PCR positive)</td>
<td>14</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation (range) or number (%). PCR, polymerase chain reaction; CSF, cerebrospinal fluid.

aThis test includes Streptococcus pneumoniae, Haemophilus influenzae type b, Neisseria meningitides, group B Streptococcus, and Listeria monocytogenes;
bThis test includes cytomegalovirus (CMV), human herpes virus 6 (HHV6), Epstein–Barr virus (EBV), herpes simplex virus 2 (HSV2), varicella-zoster virus (VZV), herpes simplex virus 1 (HSV1), and enterovirus.

test, P=0.001) compared to patients with pleocytosis. This further indicates that younger age and early examination correlate with the absence of pleocytosis.

6. Comparison between bacterial and viral meningitis

When comparing bacterial meningitis with viral meningitis, there were more cases of WBC count < 5,000/mm³ in patients with bacterial meningitis (chi-square test, P < 0.05) (Table 3). Moreover, the initial CRP levels (0.50 mg/dL [0.3–11.3] vs. 1.00 mg/dL [0.3–13.9], respectively; Mann-Whitney U test, P = 0.024) (Table 3) and peak CRP levels (0.60 mg/dL [0.3–14.7] vs. 12.90 mg/dL [6.8–26.7], respectively; Mann-Whitney U test, P = 0.000) (Table 3) were significantly different. Initial WBC counts < 5,000/mm³ were more frequent in bacterial meningitis cases but CRP levels were significantly different between viral and bacterial meningitis. However, among bacterial meningitis (n = 7), three patients (42.9%) had normal CRP level initially.

Discussion

It is challenging to differentiate between severe infection and benign viral infections among infants aged ≤ 90 days reporting with a fever. Of the severe infections in young infants, central nervous system infections are the most serious as they may lead to long-term morbidities. However, the most common infection of the central nervous system is aseptic meningitis. In a study of young infants with acute meningitis, almost 80% of infants were found to have aseptic meningitis [3]. In our study, 274 patients (31.6%) were diagnosed with meningitis. Of these, seven had bacterial meningitis, 206 had viral meningitis, and 68 had pleocytosis in the CSF with an unknown cause (Table 1 and Fig. 1).

Of the 191 children diagnosed with viral meningitis without traumatic tap, 18.3% had CSF non-pleocytosis. In particular, infants with CSF non-pleocytosis were younger than those with CSF pleocytosis, suggesting a negative correlation with age (Table 2). Univariate analysis showed that a shorter interval from symptom onset to LP, lower CSF glucose, and lower WBC count in the peripheral blood were significantly associated with viral meningitis with CSF non-pleocytosis (Table 3). In our study, 28.6% of bacterial meningitis patients did not have pleocytosis; however, the number of patients was too small to compare pleocytosis and no pleocytosis. Thus, it might be challenging to differentiate between bacterial and viral meningitis based on initial CSF analysis, as there might be overlaps in the CSF white cell count.

Previous studies also showed some cases of enteroviral meningitis without pleocytosis [7]. The proportion of patients with CSF non-pleocytosis in the case of enteroviral meningitis was approximately 30% of infants < 2 months of age in New Zealand [13] and in Missouri (USA) [14], and 31% in Pennsylvania (USA) [15]. In Korea, approximately 28% of infants < 3 months of age [16], 63.3% of neonates, and 38.3% of young infants aged 29 to 56 days [8] had CSF non-pleocytosis in enteroviral meningitis. Mulford et al. [17] reported that 30% of infants < 2 months of age with enteroviral meningitis did not have pleocytosis. Although studies published so far have focused upon enteroviral meningitis and CSF pleocytosis, this study shows that various types of meningitis and not only meningitis caused by an enterovirus, may be related to meningitis without CSF pleocytosis in younger patients who were ≤ 90 days old.
Table 2. Comparison of viral meningitis with and without pleocytosis in cerebrospinal fluid

<table>
<thead>
<tr>
<th>Variable</th>
<th>With pleocytosis (n = 156)</th>
<th>Without pleocytosis (n = 35)</th>
<th>P value</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>84</td>
<td>12</td>
<td>0.04*</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Female</td>
<td>72</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (day)</td>
<td>54.18 ± 22.23</td>
<td>39.20 ± 22.64</td>
<td>0.00*</td>
<td></td>
</tr>
<tr>
<td>Age group (day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60</td>
<td>86</td>
<td>29</td>
<td>0.00*</td>
<td></td>
</tr>
<tr>
<td>&gt; 60</td>
<td>70</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time interval from onset to LP (day)</td>
<td>0.84 ± 1.09</td>
<td>0.43 ± 0.82</td>
<td>0.04*</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>&lt; 24 hours</td>
<td>67 (42.95)</td>
<td>24 (68.57)</td>
<td>0.01*</td>
<td></td>
</tr>
<tr>
<td>CSF WBC (l/mm³)</td>
<td>182.94 ± 277.05</td>
<td>3.94 ± 4.26</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>CSF protein (mg/dL)</td>
<td>78.01 ± 34.15</td>
<td>70.27 ± 27.05</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>CSF glucose (mg/dL)</td>
<td>49.39 ± 12.56</td>
<td>55.11 ± 11.02</td>
<td>0.01*</td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (l/mm³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5,000</td>
<td>6</td>
<td>3</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>&gt; 15,000</td>
<td>49</td>
<td>3</td>
<td>0.01*</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>12,792.56 ± 5,357.56</td>
<td>9,134.57 ± 3,507.66</td>
<td>0.00*</td>
<td></td>
</tr>
<tr>
<td>Initial CRP (mg/dL)</td>
<td>1.10 ± 1.64</td>
<td>1.06 ± 1.02</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Peak CRP (mg/dL)</td>
<td>1.40 ± 2.61</td>
<td>1.45 ± 1.46</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation or number (%).

Table 3. Comparison between viral meningitis and bacterial meningitis patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Viral meningitis (n = 206)</th>
<th>Bacterial meningitis (n = 7)</th>
<th>Comparison P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (day)</td>
<td>53.00 (1–90)</td>
<td>27.00 (2–74)</td>
<td>0.03*</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Male</td>
<td>103</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>103</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Time interval from onset to LP (day)</td>
<td>1.00 (0–7)</td>
<td>0.00 (0–1)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>&lt; 24 hours</td>
<td>100 (48.54)</td>
<td>6 (85.71)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>CSF WBC (l/mm³)</td>
<td>40.00 (0–1,360)</td>
<td>230.00 (2–1,970)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>CSF protein (mg/dL)</td>
<td>74.65 (19–501.2)</td>
<td>111.40 (60.3–566.6)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>CSF glucose (mg/dL)</td>
<td>49.00 (29–127)</td>
<td>52.30 (7.6–85.7)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Initial white blood cell count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5,000/mm³</td>
<td>10 (4.83)</td>
<td>2 (28.57)</td>
<td>0.01*</td>
</tr>
<tr>
<td>&gt; 15,000/mm³</td>
<td>53 (25.60)</td>
<td>2 (28.57)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Median</td>
<td>11,125 (1,540–27,120)</td>
<td>10,210 (1,790–31,810)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Initial C-reactive protein</td>
<td>0.5 (0.3–11.3)</td>
<td>1.0 (0.3–13.9)</td>
<td>0.02*</td>
</tr>
<tr>
<td>Peak C-reactive protein</td>
<td>0.6 (0.3–14.7)</td>
<td>12.9 (6.8–26.7)</td>
<td>0.00*</td>
</tr>
</tbody>
</table>

Values are presented as median (range) or number (%).

References:
https://doi.org/10.26815/acn.2020.00318

Cho KU et al. • Meningitis and Febrile Infants

ANNALS OF CHILD NEUROLOGY
The mechanism of meningitis with non-pleocytosis in infants is still unclear. Seiden et al. [9] reported that CSF non-pleocytosis is associated with lower peripheral WBC counts and suggested this may result from a lack of sufficient cellular response to infections. In our study, the mean time interval from onset to LP was significantly shorter in viral meningitis without pleocytosis. These results suggest that sufficient time may be needed for CSF pleocytosis to develop. In other words, in the early stages of central nervous system infections, results may indicate non-pleocytosis if sufficient time has not passed to allow pleocytosis to develop [8,18,19].

Our study showed that 7.8% of UTI patients had coinfection with PCR-positive viral meningitis, 25.6% had reactive pleocytosis in CSF, and one patient (0.5%) had bacterial meningitis (E. coli). Previous studies have shown that sterile CSF pleocytosis is not rare in infants with a UTI undergoing LP (18% to 29% of patients); however, UTIs are rarely associated with bacterial meningitis [20,21]. It is thought that pleocytosis is a type of inflammatory response to UTI and is the result of undetected viral infections [22,23]. In this study, among the patients with UTIs, infants without pleocytosis were younger than those with pleocytosis. It is unknown whether sterile CSF pleocytosis in UTIs is significant and further research on this topic is warranted.

Meningitis may not always be accompanied by pleocytosis. Pleocytosis can be related to age, the interval from symptom onset to LP, and peripheral WBC counts. A comparison between viral meningitis and bacterial meningitis revealed that there were more cases of WBC count < 5,000/mm³ in patients with bacterial meningitis (chi-square test, P < 0.05) (Table 3). This suggests that bacterial meningitis may present with relatively low WBC counts. Furthermore, CSF non-pleocytosis may also be present. Although the initial CRP levels were significantly different between viral and bacterial meningitis (0.50 mg/dL [0.3–11.3] vs. 1.00 mg/dL [0.3–13.9], respectively; Mann-Whitney U test, P = 0.024) (Table 3), initial CRP levels were normal in three cases of seven bacterial meningitis (3/7, 42.9%). Together, these results suggest that the distinction between viral and bacterial meningitis may be potentially difficult in the early stages of disease onset, leading to delayed diagnosis. Therefore, the patient’s condition should be monitored closely and, if necessary, re-examinations should be considered.

Excluding herpes simplex virus infections, most viral meningitis is known to be non-fatal. However, some cases of enterovirus, human herpesvirus 6 infections, and parechovirus [6,24,25] may be accompanied by CSF non-pleocytosis and may cause severe encephalitis, which can be fatal or lead to further complications. Therefore, PCR tests to identify the specific virus that caused meningitis are needed; these tests have the added benefits of reducing the need for further investigations, the number of medications prescribed, and the length of hospital stay.

This study has some limitations. As our work is retrospective in nature, a generalization of the results may not be appropriate. We did not obtain data of CSF examinations in all the included infants. Therefore, further prospective studies are necessary.

In conclusion, we showed that meningitis is not uncommon among infants < 90 days old who have come to the hospital with a fever. Furthermore, younger age may be negatively correlated with CSF pleocytosis in meningitis. Our results indicate initial CRP levels may not be reliable predictors of bacterial meningitis and that initial leukopenia may be more dependable. Analysis of CSF or inflammatory markers in the peripheral blood is insufficient to diagnose meningitis and to also differentiate between viral and bacterial source, and therefore, PCR tests for various viruses and bacteria may be necessary. Meningitis patients < 90 days old have non-specific symptoms and signs and often do not have CSF pleocytosis. It is crucial to closely monitor the patient’s condition by analyzing whether the laboratory findings and clinical symptoms are consistent.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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References


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Filamin A (FLNA) is involved in several fundamental processes surrounding cellular protrusion and motility [1]. Recent studies have shown that FLNA mutations can result in periventricular nodular heterotopia (PNH) [1], which is characterized by the failure of neurons to migrate appropriately during corticogenesis. This failure in migration causes nodular tissue formation in the ventricular lining of the brain [1]. It is known that FLNA mutation (Mendelian Inheritance in Man #3000049)-related PNH is usually inherited in an X-linked manner [2], such that hemizygous males run a high risk of intrauterine and perinatal mortality, whereas heterozygous variant-affected females present variously from asymptomatic to neurological symptoms (e.g., seizure and weakness) that preserve psychomotor function [3]. However, the mechanism of FLNA interaction with migrating neurons remain unknown [1].

Here, we report a case of an 8-month-old girl with FLNA-related PNH who was admitted for ocular deviation and unilateral motor weakness. She was previously healthy without a history of seizures and intellectual disabilities and had appropriately reached developmental milestones. She had received an influenza vaccination 1 week prior to admission and experienced a fever for 3 days after vaccination. A neurological examination showed reduced motor tone (grade 4) in the left arm and leg. Cerebrospinal fluid analysis and laboratory results were normal; however, magnetic resonance imaging (MRI) of the brain revealed PNH in the bilateral ventricles as well as acute disseminated encephalomyelitis (ADEM) (Fig. 1). She presented no abnormal range of motion and definite skeletal abnormalities such as shortened digits or hyperflexible joints. Her echocardiogram showed mild aortic valve regurgitation (trivial–grade 1). Ophthalmologic evaluation was within the normal range without symptoms such as strabismus. She was administered high-dose intravenous glucocorticoids therapy, and since it had little effect, was added intravenous immunoglobulin. In a follow-up brain MRI scan taken eight days later, corresponding improvements were observed with respect to acute neurological symptoms and ADEM lesions; however, the bilateral PNH remained. To evaluate the genetic cause of the cerebral lesions, we performed whole exome sequencing, but no significant single nucleotide variants were detected. So, copy number variation analysis were performed using the whole exome sequencing data. Heterozygous FLNA duplication (hg19 chrX: g.153,599,242–153,609,557) was identified and confirmed by quantitative real-time polymerase chain reaction validation. Unfortunately, this report has limitations in that parental genetic testing has not been performed.
Few cases in male and female PNH with FLNA deletion have recently been found. However, although there has been one reported case of PNH in a patient with a large duplication copy number variant that included the FLNA gene [3], this is the first reported case of a female patient with PNH who had duplications in exons 1 and 2 of the FLNA gene. Thus, this study expands the genotype-phenotype spectrum for FLNA variants associated with PNH. It also demonstrates the importance of using diagnostic tools for detecting copy number gain as well as deletion and sequencing variants in the FLNA gene.

Informed consent was waived by the board and this study was approved by the Institutional Review Board of Yonsei University Health System (IRB, 3-2020-0373).

Conflicts of interest

Young-Mock Lee is an editorial board member of the journal, but he was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

Author contribution

Conceptualization: HL and YML. Data curation: NSK, HL, and YML. Visualization: HL. Writing-original draft: NSK and HL. Writing-review & editing: HL and YML.

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Fig. 1. Brain magnetic resonance imaging scan in a female patient with filamin A (FLNA) gene duplication who had acute disseminated encephalomyelitis (ADEM) and periventricular nodular heterotopia. (A, C) T2-weighted images showing periventricular and subcortical white matter lesions in both the basal ganglia and thalami. (B, D) After 1 year, bilateral periventricular nodular heterotopia remained. the ADEM resolved.
The misuse and abuse of fentanyl patches for chronic pain have been frequently reported. Such reports show side effects ranging from asymptomatic to death [1]. However, there are no reports on brain and neurological damages resulting from oral ingestion of such patches. Thus, we would like to report on this subject.

A 10-month-old normally developed boy was admitted to emergency room of another hospital. He took a nap at 3:00 PM; however, did not wake up 3 hours. His presenting complaint upon arrival was as follows: severe drowsiness with inappropriate response to pain equivalent to a score of 6 on the Glasgow Coma Scale (GCS). His pupils were fixed, and both lower extremities showed spasticity on neurologic examination. The initial laboratory results showed lactic acidosis. Following oxygen supply, hydration, and sodium bicarbonate infusion, he showed irritability and a weak response to pain. Nonetheless, there was no spontaneous eye opening. He underwent emergent brain magnetic resonance imaging (MRI) that showed diffuse brain damage at the both frontal lobe, para-sagittal area, basal ganglia, occipital, and cerebellum (Fig. 1A and B). He was referred to Gangnam Severance Hospital for further treatment.

The initial GCS score at Gangnam Severance Hospital was 8 (eye opening response, 2; best verbal response, 2; best motor response, 4). We found a 12 µg fentanyl patch in his mouth during physical examination. After removing this patch, we administered naloxone for detoxification. An electroencephalogram (EEG) performed on the day 2 showed a diffusely slow and disorganized background, without epileptiform discharges. The GCS score increased to 10 (eye opening response, 4; best verbal response, 2; best motor response, 4). He had a seizure on the day 3. His arms alternatively became rigid, which later advanced to generalized tonic-clonic movement. He had ophthalmic examination due to lack of eye contact, which showed intact optic nerve. The brain MRI did not show any interval change on the day 11 (Fig. 1C and D). He could not control his neck or crawl. Despite residual neurologic and visual impairment, his general condition improved, and he was discharged from the hospital.

He received follow-up brain MRI and EEG after 2 months. The EEG showed no significant change. However, there were symmetrical cystic changes in the entire brain areas, mimicking the improved MRI results in hypoxic insults (Fig. 1E and F). His visual impairments improved enough for his eyes to follow objects, and his neurological deficit improved as well allowing him to control his neck or crawl.

After 8 months, the brain MRI showed no interval changes compared to the earlier brain
Furthermore, frequent sharp waves were seen in his EEG but there were no clinical seizures. Nevertheless, he could toddle and utter about three words. His sight improved enough to recognize and hold objects. The brain damage persisted while his neurological and visual deficiency was recovering.

In our case, the patient reported a neurologic change after orally ingesting a fentanyl patch. When a fentanyl patch is absorbed orally, the absorption of fentanyl is three times more than that absorbed through the stomach [2], which may be fatal to young children. A few numbers of accidental misuse of opiate pills revealed cerebellar damages and edema that can be attributed to opioid mu receptor toxicity [3,4]. However, unlike previous cases, our case illustrates overall brain damage from toxic leukoencephalopathy resulting in neurological and visual deficits. Such injury was caused by the toxic leukoencephalopathy from fentanyl intoxication.

This case of a 10-month-old illustrates that fentanyl intoxication may not only result in cerebellar damages and edema but also cause overall brain damage and neurological deficit in pediatric patients. We suggest that oral fentanyl intoxication should be considered as a differential diagnosis for children presenting with acute neurologic deficits showing diffuse brain damage due to toxic leukoencephalopathy on the brain MRI.

This study was approved by the Institutional Review Board of Yonsei University Health System (IRB, 3-2020-0358). Informed consent was waived by the board.

Conflicts of interest

Young-Mock Lee is an editorial board member of the journal, but he was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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References

Unusual Clinical Presentations in a Patient with Novel ADCK3 Variants

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The AarF domain containing kinase 3 (ADCK3) is a mitochondrial protein required for coenzyme Q (CoQ) biosynthesis. Loss of function mutations in the ADCK3 gene cause coenzyme Q10 (CoQ10) deficiency (#MIM 612016), resulting in mitochondrial dysfunction. Cerebella ataxia is its most common phenotype [1]. However, acute epileptic encephalopathy with stroke-like episodes has been reported in few cases [2]. Here, we report the case of a patient with novel ADCK3 mutations who presented with a homonymous hemianopsia, stroke like brain lesions in brain magnetic resonance imaging (MRI).

A 3-year-old girl presented with exercise intolerance, gait disturbance and occasional dysarthria following normal birth and development. Serum creatinine kinase and lactate dehydrogenase were elevated at 964 U/L (21 to 215) and 3,427 IU/L (255 to 455). And serum lactate was high at 8.3 mmol/L (0.5 to 1.6). We observed ragged red fibers on muscle biopsy and identified mitochondrial respiratory chain complex 1 deficiency from biochemical tests. She was diagnosed with a mitochondrial disorder. The first generalized tonic seizure occurred at the age of 6 years and was administered anti-epileptic drugs. At 14 years of age, she presented with stroke-like episodes with severe headache, nausea, dysarthria, and left-sided hemianopsia. However, her brain MRI findings were normal. Electroencephalogram (EEG) showed frequent epileptic discharges in the right occipital area. Those acute symptoms improved after 7 days of conservative management to reduce cerebral edema with hypertonic agent (mannitol) and high-dose corticosteroids. She reported another stroke-like episode with similar symptoms at the age of 18 years. In addition, she experienced sudden left-sided homonymous hemianopsia and dysarthria concomitant with ataxic gait and exercise intolerance. We detected generalized tonic seizure during her hospitalization. Repeated brain MRI showed focal areas of high-signal intensity in the right occipital lobe and dentate nucleus. Nonetheless, brain magnetic resonance angiography showed no abnormal vascular lesion. While there were frequent sharp wave discharges from both occipital areas in EEG, a visual field examination showed ipsilateral left hemianopsia.

Whole-exome sequencing was performed for accurate diagnosis because no significant variant was detected in the previous whole mitochondrial genome test. In this study, the compound heterozygous mutations were identified, namely c.655+1G > A and c.1015G > A (p.Ala339Thr) in the ADCK3 gene (NM_020247.4 and NP_064632.2). According to the American College of Medical Genetics and Genomics guidelines, c.655+1G > A and c.1015G > A (p.Ala339Thr) in the ADCK3 gene (NM_020247.4 and NP_064632.2). According to the American College of Medical Genetics and Genomics guidelines, c.655+1G > A and c.1015G > A is a novel pathogenic variant and c.1015G > A is a likely pathogenic variant which has been
reported in a patient with a homozygote mutation [3]. The variants were confirmed by Sanger sequencing in the proband and both parents and considering the autosomal recessive inheritance of the ADCK3 gene, it is likely that the asymptomatic parents were carriers. After genetic diagnosis, she has been taking CoQ10 supplements with 400 mg daily.

Hemianopia and stroke-like lesions are common symptoms of mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). In a patient suspected of mitochondrial cytopathy with stroke-like lesions on brain MRI without pathogenic variants related to MELAS, it is recommended to consider testing for mitochondrial disorders caused by nuclear DNA mutations, including the ADCK3 gene.

ADCK3-related CoQ10 deficiency is a rare disease. Furthermore, the phenotype is neither classified systematically nor well known. Patients with ADCK3 mutations usually present with progressive cerebellar ataxia, exercise intolerance, and high-signal intensity of the dentate nucleus. However, they rarely report cardiomyopathy and nephrotic syndrome. In addition, her abdominal radiography revealed ovarian calcification and a mature ovarian teratoma was confirmed by ultrasound findings incidentally (Fig. 1). There are no reports of a definite association with the ADCK3 gene, which causes the deficiency of CoQ10, which plays an important role in antioxidant.

To our knowledge, this is the first report of a patient with novel compound heterozygous ADCK3 variants in Korea who showed signs of ataxia, exercise intolerance, homonymous hemianopsia, dysarthria, and teratoma. This report aims to expand the understanding of the genotype-phenotype spectrum associated with ADCK3-related CoQ10 deficiency.

This study was approved by the Institutional Review Board of Gangnam Severance Hospital (approval number: 3-2020-0393). Informed consent was waived by the board.

Fig. 1. A patient with coenzyme Q10 deficiency diagnosed with compound heterozygous variants in the AarF domain containing kinase 3 (ADCK3) gene: (A) a focal abnormal lesion with restricted diffusion at right occipital lobe; (B) increased signal intensity at right occipital lobe on T2 weighted image; (C) increased signal intensity at dentate nucleus on T2 weighted image; (D) homonymous hemianopia; and (E) ovarian teratoma.
Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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References

Chorea is uncommon clinical presentation in moyamoya disease (MMD); it can be present in 3% to 6% of patients [1]. Although the mechanism of development of chorea is unclear, ischemia of the basal ganglia, which could lead to post stroke abnormal movements is considered a dominant mechanism. A majority of chorea symptoms improve within 1 year of surgical revascularization of the ischemic region. However, it is very rare that chorea develops after surgical revascularization. We report two adolescent cases of new-onset hemichorea after diagnosis of MMD and bilateral bypass surgery.

An 11-year-old boy (Patient A) had undergone bilateral bypass surgery for MMD. Four years after the bypass surgery, the patient was referred to our department for new-onset hemichorea. Intermittent tremors began in the right arm and gradually worsened to become involuntary movement of right arm. We performed bilateral internal carotid artery angiography and perfusion magnetic resonance imaging (MRI) of the brain with gadolinium. At the first diagnosis, MRI of both patients revealed prominent network of collateral vessels in the basal ganglia as well as stenosis of the internal carotid artery. However, it was confirmed that there were no new lesions in the blood vessels and there was no reduction in brain diffusion and blood flow reserve compared with the previous findings (Figs. 1 and 2). We approached the cause of chorea not from the perspective of new brain vasculopathy but from that of the neurotransmission system. We considered the age variation of neuronal structure in the basal ganglia was the cause of chorea, which was not present at the time of diagnosis in children with MMD, occurs newly in adolescence.

Neurons in the striatum in the basal nucleus exclusively regulate the activation and suppression of spontaneous behavior through direct and indirect pathways, respectively [2]. These pathways are regulated by the neurotransmitter dopamine. The possible pathophysiological mecha-
nism of chorea appears to be inactivity of the indirect pathways in the basal ganglia-thalamocortical motor circuits [3]. In the presence of striatal lesions, the inhibition to the lateral globus pallidus is weakened and the inhibition to the subthalamic nucleus is activated. Suppressed subthalamic nucleus results in less inhibition of the thalamus by the globus pallidus pars interna, thus, resulting in unwanted movements. This is the currently accepted hypothesis for basal ganglia dysfunction that causes chorea based on the excitation of the thalamus against the cerebral cortex.

In our patients, there was no chorea at the time of diagnosis of MMD; however, after bilateral bypass surgeries, there was new onset of chorea in adolescence. We believe that the cause was related to the changes in the pathways of the basal ganglia as the patients grew older. The nigrostriatal dopamine neurons and the components of the basal ganglia demonstrate marked age-related variations in their functions and morphologies [4]. Striatal indirect pathways are functionally immature in the childhood and attain adult levels approximately around the middle of the second decade. Therefore, even if there was a lesion in this area, if the structure of the lesion site was immature, the related symptoms would not appear initially. However, it is assumed that the associated symptoms appear when the lesion site matures with age. This is also consistent with the fact that the onset time of hemichorea in our patients was after the appearance of secondary sexual characteristics.

Cases of chorea are still difficult to manage because optimal therapy has not been established. Prior studies have reported that anticonvulsants including clonazepam, valproic acid, and carbamazepine can successfully control myoclonic hyperkinesia in huntington's disease and paroxysmal kinesigenic dyskinesia [5]. The mechanism of action of these agents in chorea is not well understood. Inducing neuronal excitability appears to be involved with an imbalance of both dopaminergic and GABAergic neurotransmissions. Clonazepam has antidopaminergic effects that are medi-
ated predominantly by the central-type benzodiazepine receptors in the central nervous system via GABAergic mechanisms. The mechanism of action of valproic acid and carbamazepine is believed to be via increasing brain GABA levels either with sodium channel blockage or with GABA agonistic effects. So we decided to use anticonvulsants with antidopaminergic effect.

Patient A was started on clonazepam 0.5 mg and valproic acid 600 mg per day. A week after starting the medications, the patient demonstrated significant improvements in hemichorea; the frequency had decreased from approximately 20 to 10 episodes per day and the intensity was much weaker. Seven months later, his symptoms completely disappeared and the drug was stopped.

Patient B was started on clonazepam 0.5 mg and carbamazepine 400 mg per day. On the 4th day of starting the treatment, the patient demonstrated significant improvements; the right-hand tremor did not occur even once during the day or occurred weakly once or twice when there were many. After 4 months, the symptoms completely disappeared and the clonazepam was stopped. Since then, she is currently under outpatient follow-up.

In conclusion, our case report suggests that anticonvulsants with antidopaminergic effects can be considered as a treatment for new-onset chorea in patients who have undergone bilateral surgical revascularization in MMD. This study was approved by the Research Ethics Committee of Inha University Hospital (approval number: 2020-04-006). Written informed consent by the patients was waived due to a retrospective nature of our study.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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References

A *MAST1* Mutation Underlying Mega–Corpus Callosum Syndrome with Extended Phenotypes: The First Case in Korea

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Mega-corpus callosum is a rare radiological finding. It is a characteristic finding of diseases such as Cohen syndrome, neurofibromatosis (NF), megalencephaly-polymicrogyria-mega-corpus callosum (MEG-PMG-MegaCC, MIM#603387) syndrome or mega-corpus callosum syndrome with cortical malformations (MCC-CM, MIM #618273) [1]. Such diseases are characterized by specific phenotypes such as neurofibromas in NF; non-progressive mental retardation and microcephaly in Cohen syndrome; megalocephaly and polymicrogyria in MEG-PMG-MegaCC; and normocephaly and cortical malformation, with or without cerebellar hypoplasia in MCC-CM. The knowledge of these specific phenotypes could facilitate the differentiation of these conditions from each other. However, MCC-CM has been reported to be a phenotype of MEG-PMG-MegaCC [2]. MCC-CM, which is caused by autosomal dominant heterozygous mutations in the *MAST1* gene, has been reported in less than 10 patients to date. Herein, we describe the first case of a male patient with MCC-CM with cerebellar hypoplasia in Korea. The diagnosis was based on the detection of *MAST1* mutation via whole exome sequencing (WES). The patient’s other manifestations such as microcephaly and lack of secondary sexual characteristics have never been reported thus far. This study was approved by the Institutional Review Board of Inje University Busan Paik Hospital (18-0041), and the data were presented without divulging the patient’s identity. Written informed consent was obtained from a patient.

A 19-year-old man was the first of three children of healthy non-consanguineous parents. The patient was born via vaginal birth at 40 weeks’ gestational age, with a birth weight of 2.8 kg. Although his fetal movements were reduced, perinatal problems were not detected. He showed poor feeding and hypotonia with upward gaze during the neonatal period. He started to control his head at 1
year of age and sit without support when he was 3 years old. He experienced his first generalized tonic clonic seizure at the age of 8 years, which recurred after 1 year. His mother recollected that magnetic resonance imaging (MRI) performed at that time revealed ventriculomegaly, and electroencephalography (EEG) showed slow background rhythms. He was administered topiramate, an antiseizure drug, for 2 years but had been weaned off the medication. At the age of 17 years, he was referred to our hospital for the evaluation of the recurrence of two febrile generalized tonic clonic seizures lasting for 2 minutes. His development progressed slowly, and he could stand up with support. However, he exhibited language and intellectual impairments and his speech consisted only of babbling. He had spastic diplegia with muscular atrophy in the lower extremities. He also exhibited microcephaly (52 cm, < the third percentile), low weight (32 kg, < the third percentile), and short stature (152 cm, < the 10th percentile). He also had facial dysmorphism (Fig. 1A), dolichocephaly, scoliosis, oculomotor apraxia, strabismus, and small testes without secondary sexual characteristics. MRI revealed a mega-corpus callosum, focal cortical dysplasia, and cerebellar hypoplasia (Fig. 1B-D). Slow and disorganized background rhythms with parietal vertex sharp waves were observed on EEG. Laboratory investigations revealed vitamin D deficiency (25-hydroxy vitamin D3, 6.44 ng/mL; reference range, 30 to 150 ng/mL), abnormal gonadotropin-releasing hormone stimulation (peak simulated luteinizing hormone, 16.5 mIU/mL; reference range, 3.3 to 5 mIU/mL) and testosterone levels (0.039 ng/mL; reference range, 2.49 to 8.36 ng/mL), normal insulin-like growth factor (IGF)-1, and low IGF-binding protein-3 (2,860 ng/mL; range, 3,200 to 8,700 ng/mL). His karyotype was 46,XY, but microarray testing was not performed due to parental refusal. WES showed de novo heterozygous variants in MAST1 (NM_014975.2:c.1549G > A). Sanger sequencing of these variants obtained from the peripheral blood of patient and his parents revealed a de novo mutation in the patient, while both parents had the wild-type gene (Fig. 1E). He received valproic acid (750 mg daily) for recurring seizures and was seizure-free for 18 months. He also received vitamin D owing to deficiency detected on laboratory testing and testosterone hormonal therapy for delayed puberty.

MCC-CM due to MAST1 mutation is characterized by delayed development, intellectual disability, impaired language abilities, and specific brain abnormalities (Table 1) [2-4]. Patients with MAST1 mutations can present with varying phenotypes, including MCC-CM, microcephaly and cerebellar hypoplasia, autism spectrum disorder, global developmental delay, and cerebral palsy [2]. Our patient exhibited MCC-CM and microcephaly, which were diagnosed by the detection of heterozygous variants in MAST1 (c.1549G > A). MAST1 is expressed in multiple organs, including the spleen, kidney, testis, and skeletal muscle [2]. Our patient exhibited small testes and lack of secondary sexual characteristics, which could be due to MAST1 mutations in the testes.

MAST1, located on chromosome 19p13.13, encodes a microtubule-associated serine/threonine kinase protein expressed in the
postmitotic neurons in the developing brain. This protein consists of an N-terminal domain of the unknown function (DUF1908), a serine/threonine kinase domain and a post-synaptic density protein-95/discs large/zona occludens-1 (PDZ) domain. It supports a PDZ-dependent interaction with other proteins and also interacts with tumor suppressor phosphatase and tension homolog (PTEN) via the PDZ domain to facilitate the functioning of the phosphorylation of the interacting proteins [2,5]. We suggest that the reduction in PTEN function, which encodes a negative regulator of the mechanistic target of rapamycin signaling pathway, may influence cortical malformation and social-behavioral problems because a significant reduction was observed in the levels of the MAST family of proteins in MAST1 mutant rodents compared to their wild-type counterparts [2]. Further research is needed to determine the genetic function and mechanisms affecting the disease phenotypes.

In conclusion, we report the first case of a Korean patient with MAST1 gene mutation leading to MCC-CM without secondary sexual characteristics.

**Conflicts of interest**

No potential conflict of interest relevant to this article was reported.

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**References**

Clinical Manifestations of Hydranencephaly: A Case in Monochorionic-Diamniotic Twin

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Hydranencephaly is a rare and fatal central nervous system (CNS) disorder, occurring in less than 1 per 10,000 births [1]. Cerebral hemispheres are almost absent and replaced with cerebrospinal fluid, but there is typically intact falx cerebri, thalamus, and cerebellum. Facial features and midline structures generally appear normal, distinguishing it from other CNS anomalies [1]. Most patients are stillborn or die within a few hours to months after birth, though long-term survival has been reported in rare cases [2]. Etiologies may be related to infections, toxins, genetics, or occlusive vascular lesion, but there are still many doubts about exact etiopathogenic mechanisms [1]. A previous case report briefly described the survival of a patient with hydranencephaly born as one of monochorionic-diamniotic (MCDA) twins [3]. However, there have been few reports on the neurological manifestations after survival especially as an extremely low birth weight (ELBW) infant of MCDA twins. Here, we report the clinical manifestations in a survived ELBW case of hydranencephaly, in MCDA twins.

The male patient was born at 33 weeks and 2 days of gestation. The birth weight was 840 g, height 33 cm, and head circumference (HC) 20.8 cm. His identical twin was born without any congenital anomalies and his birth weight was 1.7 kg (20th percentile). Prenatal ultrasound revealed the presence of fetal hydrops and brain ischemic insult. There was no maternal history of infections or exposure to toxins during pregnancy. Termination of pregnancy was avoided due to the possibility of intrauterine fetal death of the co-twin. The patient’s Apgar score was 7 at 1 minute and 9 at 5 minutes. Physical examination at birth showed microcephaly, corneal opacities, anonychia of feet, but normal facial appearance (Fig. 1). He had stable spontaneous breathing and actively responded to external stimuli. Diagnostic tests were delayed due to his parents’ initial refusal. As he was still alive with minimal care, further tests were performed. Brain ultrasound performed at 18 days of age showed that supratentorial brain structures were almost absent, with the presence of only a small remnant of frontal lobe-like areas. At 28 days of age, echocardiography was normal. Glaucoma on both eyes was detected by eye consultation at 38 days of age showed that supratentorial brain structures were almost absent, with the presence of only a small remnant of frontal lobe-like areas. At 28 days of age, 37\(\frac{1}{2}\) weeks of postmenstrual age (PMA), he started showing some sucking movements, and echocardiography was normal. Glaucoma on both eyes was detected by eye consultation at 38 days of age. At 41\(\frac{1}{2}\) weeks of PMA, a full dose of bottle feeding was possible, and automated auditory brainstem response test was normal. At 46\(\frac{3}{4}\) weeks of PMA, brain magnetic resonance imaging revealed the presence of the bilateral frontal lobes, small in size and displaced. A large cystic...
area was observed in the brain, extending to the 4th ventricle with an asymmetric enlargement, with spared falx cerebri. The cerebellum and brainstem were within normal limits, and the lens in the left eyeball was not visualized (Fig. 2). Karyotype was a normal 46XY. On neurological examination, motor power showed grade 5 in all extremities, the muscle tone and deep tendon reflexes were normal. No pathologic reflexes were present. At 47\textsuperscript{+2} weeks of PMA, he was discharged with a weight of 3.8 kg ( < 3rd percentile), height 47.8 cm ( < 3rd percentile), and HC of 31.5 cm ( < 3rd percentile). At 6 months of age, on the last outpatient visit, he showed a weight of 6.7 kg (10th percentile), height 59.7 cm ( < 3rd percentile), and HC of 37.5 cm ( < 3rd percentile). He was still actively responded to external stimuli but showed fontanelle bulging and mild bilateral proptosis. He couldn’t control his head and showed truncal hypotonia, but no spasticity of extremities.

In this case, we describe the clinical course of ELBW infant with hydranencephaly, in MCDA twins. Previous studies suggested that early severe bilateral internal carotid artery occlusion may induce hydranencephaly [1,2]. However, MCDA twins have unique, different mechanisms, such as selective fetal growth restriction and twin-to-twin transfusion syndrome [4]. In an adverse position of the MCDA twin, exposure to hypoxia at some point in the fetal development process may lead to massive tissue necrosis with large cavitation. Rarely, patients with prolonged survival have been reported. Hence, documentation of neurologic conditions of survival in infants with hydranencephaly is essential for appropriate parental counseling on the possibility of prolonged survival and about the decision making of medical treatment.

Ventriculoperitoneal shunt or choroid plexus cauterization in infants can reduce brainstem compression and improve survival [2]. A retrospective study on 50 cases of hydranencephaly showed that the average life expectancy had increased due to appropriate medical and surgical treatments to 7.5 years in 58% of the subjects [1]. Bae et al. [5] reported the case of a patient with hydranencephaly who survived for 22 years and 6 months. They suggest that such patients can live beyond adolescence to adulthood. However, even
if surgery may prolong their lives, patients and their families face serious medical conditions due to recurrent pneumonia, seizures, intellectual disabilities, and spastic paralysis throughout the rest of their lives [1]. Counseling parents about poor prognosis and possible management options helps them to prepare for potential outcomes.

This study was approved by the Institutional Review Board of Kandong Sacred Heart Hospital (2020-12-004). Written informed consent by the patients was waived due to the retrospective nature of our study.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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References

Instructions to authors

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   2) Log in (or click the 'registration' option, if you are a first-time user of http://submit.annchildneurol.org).
   3) Click on 'new submissions'.
   4) Check and confirm 'author’s manuscript check list'.
   5) Proceed with the following 8-step process.
      - Step 1. Fill in the manuscript type, title, running title, abstract, keywords and corresponding author.
      - Step 2. Fill in the author names and affiliation.
      - Step 3. Writer down the additional notes to Editor-in-Chief in cover letter field and respond to the additional information below.
      - Step 4. Suggest reviewers. Suggesting 2 reviewer(s) is required for submission.
      - Step 5. Upload manuscript file and copyright transfer form.
      - Step 6. When the conversion is completed, please click the "Make PDF" button.
      - Step 7. Confirm preview contents. If you agree to submit the manuscript, please click "submit" button.
      - Step 8. Your submission is completed. You will receive your registration number or return notice via email.
4. If you have any questions about the online submission process, contact the Editorial Office by e-mail at editor@annchildneurol.org or by telephone at +82-2-2228-2050.

Editorial and peer review process

All manuscripts are initially reviewed by a Annals of Child Neurology editor. Submissions that are clearly outside the scope of Annals of Child Neurology will be declined without further review. Manuscripts that are so poorly written or incomplete that it hampers the review process will also be declined but with the option of resubmission if the concerns have been addressed. All submitted manuscripts are analyzed with plagiarism detection software prior to undergoing editorial review. Manuscripts are sent to the two most relevant investigators available for review of the contents. The editor selects peer referees by recommendation of Annals of Child Neurology's editorial board members or from the Board’s specialist database.

The journal uses a single-blind peer review process: peer reviewer identities are kept confidential (unless reviewers choose to reveal their names in their formal reviews); author identities are made known to reviewers. The existence of a manuscript under review is not revealed to anyone other than peer reviewers and editorial staff. Peer reviewers are required to maintain confidentiality about the manuscripts they review and must not divulge any information about a specific manuscript or its content to any third party without prior permission from the journal editors. Information from submitted manuscripts may be systematically collected and analyzed as part of research to improve the quality of the editorial or peer review process. Identifying information remains confidential. Final decisions regarding manuscript publication are made by an editor who does not have any relevant conflicts of interest. All correspondence, including the editor’s decision and requests for revisions, will be conducted by e-mail.

Accepted: The manuscript will be forwarded to the publisher without further corrections.

Minor revisions: The author should address the comments from the reviewers, which will be confirmed by the reviewers before being sent to the publisher.

Major revisions: The author should address the comments from the reviewers and make the appropriate corrections for review by the three reviewers.

Rejection: When one out of the two reviewers rejects the manuscript, the final decision is made by the editorial committee.

The time to first decision without review will normally be made within 5 days (median). Within 14 days after the agreement of review by the reviewers, the reviewers’ comments will then be
sent to the corresponding authors. Revised manuscripts must be submitted online by the corresponding author. Failure to resubmit the revised manuscript within 4 weeks of the editorial decision is regarded as a withdrawal. The editorial office should be notified if additional time is needed or if an author chooses not to submit a revision.

All authors are required to confirm the following conditions of publication prior to their manuscript being considered:

a. If the manuscript does not have a new result or conclusion, then it should not have the same title as a previously published review article.

b. Once a case has been published in an original paper, it may not be reproduced as a case report. However, only in circumstances in which a novel diagnostic method, a novel therapeutic trial, or a previously unknown accompanying condition is found will the editorial board determine the possibility of acceptance.

c. Clinical trials on drugs with commercial implications will be reviewed by the proper subcommittee or subspecialty before being reviewed for publication.

d. Clinical letters of previously published cases will not be accepted. The editorial board will make an exception only if the case is very rare. The Annals of Child Neurology index should be reviewed before the submission of clinical letters.

e. Rejected manuscripts may not be resubmitted.

f. The manuscript will be rejected if the author does not address the comments made by the reviewer or the manuscript does not follow the required guidelines.

Manuscript preparation

1. General principles

1) Annals of Child Neurology publishes original articles, reviews, letters to the editor, and editorials.

2) The manuscript should not have been published previously, and not have been submitted for publication elsewhere. Any conflicts of interest of all listed authors should be stated.

3) The manuscript should be written according to the prescribed format. If not, the editorial board may return it before reviewing. The editorial board decides on publication and may modify a portion of the text with little effect on the original.

4) The manuscript must be written in English. Authors (particularly non-native English speakers) who submit the original article or letters to editor should check their manuscript by using professional editing service and submit the manuscript with a certificate of English review, including the name, institution, position, statement of approval, and signature with unstructured format.

5) The text of the manuscript, including tables and their footnotes and figure legends, must be double-spaced and in standard 12-point font on A4 paper size with left and right margin spaces of 2 cm and top and bottom margins of 3 cm.

6) Except for units of measurement, abbreviations are strongly discouraged. Do not use abbreviations in the title or abstract and limit their use in the text. Expand all abbreviations at first mention in the text.

7) Measurements of length, height, weight, and volume should be reported in metric units (meter, kilogram, or liter) and laboratory values should be displayed in International System of Units (SI).

8) The number of pages of manuscripts of reviews and original articles has no limitation but no more than 10 printed pages are recommended. Letters to editor should be written in a maximum of 2 printed pages.

2. Cover letter

The cover letter accompanying the manuscript must specify the type of manuscript and include statements on ethical issues and conflicts of interest, and complete contact information for the corresponding author.

The cover letter should include the following statement: “All authors have read and approved the submitted manuscript, the manuscript has not been submitted elsewhere nor published elsewhere in whole or in part, except as an abstract (if relevant).” The cover letter may include the names of up to 3 potential reviewers whom the authors would like to suggest, especially members of the editorial board. The authors may also include the names of up to 3 reviewers whom they would like not to evaluate their submission. The editor ultimately decides who will review the manuscript.

3. Original articles

Original articles are papers reporting the results of basic or clinical investigations, which are sufficiently well documented to be acceptable to critical readers. The manuscript should be prepared according to Recommendations from ICMJE. The manuscript should have the following sequence: Title page, Abstract and Keywords, Introduction, Materials and Methods, Results, Discussion, Acknowledgment, References, Tables, and Figure Legends. All pages should be numbered consecutively in the middle of the bottom margin, starting with the title page.

Title page

The title page should contain the following information: (1) title; (2) author list (full names of authors); (3) name of the institutions
Abstract and keywords
The abstract should be a single paragraph of less than 250 words, and describe concisely, the purpose, methods, results, and conclusion of the study, in a structured format. Abbreviations, if needed, should be kept to an absolute minimum, and their first use should be preceded by the full term in words. The abstract should not include footnotes, references, or tables. The abstract can be modified by an English language reviewer who is appointed by the editorial board. A maximum of 5 keywords should be listed at the end of the abstract to be used as index terms. For the selection of keywords, refer to Medical Subject Headings (MeSH; https://meshb.nlm.nih.gov/search).

Introduction
The introduction should provide the background of the study and state the specific purpose of research or hypothesis tested by the study. It may mention previous publications most closely related to the article.

Materials and Methods
The materials and study design should be presented in detail. In experimental research, methods should be described in such a manner that the experiments can be reproduced by the readers. The sources of special chemicals or preparations should be given (name of company, city and state, and country). Clinical studies or experiments using laboratory animals or pathogens should include approval of the studies by relevant committees. A statement concerning IRB approval and consent procedures must be presented.

Clearly describe the selection of observational or experimental participants (healthy individuals or patients, including controls), including eligibility and exclusion criteria and a description of the source population. Because the relevance of such variables as age, sex, or ethnicity is not always known at the time of study design, researchers should aim for inclusion of representative populations into all study types and at a minimum provide descriptive data for these and other relevant demographic variables.

Ensure correct use of the terms sex (when reporting biological factors) and gender (identity, psychosocial or cultural factors), and, unless inappropriate, report the sex and/or gender of study participants, the sex of animals or cells, and describe the methods used to determine sex and gender. If the study was done involving an exclusive population, for example in only one sex, authors should justify why, except in obvious cases (e.g., prostate cancer). Authors should define how they determined race or ethnicity and justify their relevance.

Results
This section should include a concise textual description of the data presented in the tables and figures. Excessive repetition of table or figure contents should be avoided.

Discussion
Observations pertaining to the results of research and other related materials should be interpreted for your readers. Emphasize new and important observations; do not merely repeat the contents of the results. Explain the meaning of the observed opinion along with its limits, and within the limits of the research results connect the conclusion to the purpose of the research. In a concluding paragraph, summarize the result and its meaning.

Acknowledgment
The acknowledgments section should contain brief statements of assistance and financial support. Any other matters associated with research funds, facilities and drugs that were used in the study should also be given.

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Open researcher and contributor IDs (ORCID) are recommended for authors. To receive ORCID, authors should register on the ORCID website available from: https://orcid.org.

References
Reference citations in the text should be made with consecutive numbers in parenthesis (Vancouver style). References should be listed in the order of citation in the text, with the corresponding number. The reference style for journal articles is as follows: names of authors, full title of article, journal name abbreviated in accordance with MEDLINE, year, volume, and page numbers. List all authors when they are six or less; when they are seven or more, list the first six and add ‘et al.’ The names of all authors must be listed by the last name and the initials of the first and middle names. Papers in press may be listed with the journal name and tentative year of publication. The style for a chapter of a book is as follows: author and title of the chapter, editor of the book, title of the book, edition, volume, place, publisher, year, and page numbers. Cite unpublished data or personal communications in the text only and not in the reference list. Internet URLs should be as follows: authors’ names, website title, URL and the time of.
the latest update. All other references should be listed as shown in the Recommendations from ICMJE. Authors are responsible for the accuracy and completeness of their references. The maximum number of cited references should be 40 for original articles and 10 for letters to editor.

Examples of reference styles

1) **Journal article**

2) **Book**
   - Book
   - Book chapter
   - Abstract book or conference proceedings
   - Thesis

3) **Website**

**Tables**

1) Each table should be inserted on a separate page, with the table number, table title and legend.
2) The numbers of tables should be in Arabic numerals in their order of citation.
3) Titles of tables should be concise using a phrase or a clause. The first character should be capitalized.
4) Tables should be concise and not duplicate information found in figures.
5) The significance of results should be indicated by appropriate statistical analysis.
6) Unnecessary longitudinal lines should not be drawn. Horizontal lines should be used as sparingly as possible.
7) All symbols and abbreviations should be described below the table.
8) Use superscript letters (a, b, c) to mark each footnote and be sure each footnote in the table has a corresponding note. List abbreviations in the footnote section and explain any empty cells.
9) All units of measurements and concentrations should be designated.

**Figures and figure legends**

1) Figures should be submitted separately from the text the manuscript. All pictures and photographs should be of excellent quality and supplied as JPEG or TIFF files with resolution of more than 300 dpi. The preferred size of figure is 7.4×10.0 cm (3×4 inches). Except for particularly complicated drawings that show large amounts of data, all figures are published at one page or one column width. All kinds of figures may be reduced, enlarged, or trimmed for publication by the editor.
2) Color figures and pictures will be published if the editor decides it is absolutely necessary.
3) Figure numbers, in Arabic numerals, should appear in the figure legends. Arabic numerals should be used in the order in which the figures are referred to in the main text. In cases where more than two photographs are used with the same number, alphabet characters should be used next to the Arabic numeral (e.g.: Fig. 1A, Fig. 1B).
4) All pictures and photographs should be described in the legend with complete sentences rather than incomplete phrases or a clause.
5) All symbols and abbreviations should be described below the figure.
4. Other types of manuscripts

All other types of manuscripts should meet the above mentioned requirements.

1) Reviews articles

Reviews may be written by invitation by the editorial board and provide concise reviews of important subjects to medical researchers. They are organized as follows: title page, abstract and keywords, introduction, main text, conclusion, acknowledgments, references, tables, figure legends, and figures. An abstract is required but it need not be structured.

Reviews should not exceed 7,000 words, include no more than 6 figures or tables and 150 references.

2) Letters to the editor

Letters to the editor is a type of brief communication on any topics that attract attention of journal readers. It should be brief, clear and conclusive. And it should be accompanied by a statement that written consent to publish was obtained from the patient(s). No abstraction is required. Body of the letter has no structure and the word count is limited to 1,500 words. It should be written in a maximum of 3 printed pages, less than 10 references, less than 2 table or figures, and less than 5 authors.

3) Editorial

Editorials are invited by the editors and should be commentaries on articles published recently in the journal. Editorial topics could include active areas of research, fresh insights, and debates in all fields of child neurology. Editorials should not exceed 1,000 words, excluding references, tables, and figures, and no more than 2 figures or tables and 10 references.

Manuscripts accepted for publication

1. Final version

After the paper has been accepted for publication, the author(s) should submit the final version of the manuscript. The names and affiliations of the authors should be double-checked and if the originally submitted image files were of poor resolution, higher resolution image files should be submitted at this time. The EPS, JPG, PPT, or TIF formats are the preferred digital files for photographic images. Symbols (e.g., circles, triangles, squares), letters (e.g., words, abbreviations), and numbers should be large enough to be legible even after reduction to the journal’s column widths. All symbols must be defined in the figure captions. If references, tables, or figures are moved, added, or deleted during the revision process, renumber them to reflect the changes so that all tables, references, and figures are cited in numeric order.

2. Manuscript corrections

Before publication, the manuscript editor will correct the manuscript such that it meets the standard publication format. The author(s) must respond within 7 days when the manuscript editor contacts the author for revisions. If the response is delayed, the manuscript’s publication may be postponed to the next issue. The author should double-check for corrections in the content, title, affiliation, capitalization, locations of figures, and references. Corresponding authors are responsible for further corrections made after printing.

3. Gallery proof

The author(s) will receive the final version of the manuscript as a PDF file. Upon receipt, within 2 days, the editorial office (or printing office) must be notified of any errors found in the file. Any errors found after this time are the responsibility of the author(s) and will have to be corrected as an erratum.

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If the authors or readers find any errors, or contents that should be revised, it can be requested from the Editorial Board. The Editorial Board may consider erratum, corrigendum or a retraction. If there are any revisions to the article, there will be a CrossMark description to announce the final draft. If there is a reader’s opinion on the published article with the form of Letters to the editor, it will be forwarded to the authors. The authors can reply to the reader’s letter. Letters to the editor and the author’s reply may be also published.

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The policy of Annals of Child Neurology is primarily aimed at protecting the authors, reviewers, editors, and the publisher of the journal. If not described below, the process of handling complaints and appeals follows the guidelines of the Committee of Publication Ethics available from: https://publicationethics.org/appeals.

Who complains or makes an appeal?

Submitters, authors, reviewers, and readers may register complaints and appeals in a variety of cases as follows: falsification, fabrication, plagiarism, duplicate publication, authorship dispute, conflict of interest, ethical treatment of animals, informed consent, bias or unfair/inappropriate competitive acts, copyright, stolen data, defamation, and legal problem. If any individuals or institutions want to inform the cases, they can send a letter via the contact page on our website: http://annchlidneurol.org. For the complaints or
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Who is responsible to resolve and handle complaints and appeals? The Editor, Editorial Board, or Editorial Office is responsible for them. A legal consultant or ethics editor may be able to help with the decision making.

What may be the consequence of remedy? It depends on the type or degree of misconduct. The consequence of resolution will follow the guidelines of the Committee of Publication Ethics (COPE).

6. Page charge
There are no page charges to authors. All color figures and tables will be reproduced in full color in the online edition of *Annals of Child Neurology* at no cost to authors, but the complete cost in the printed version of the journal will be charged to the authors. Please contact the Editorial Office if you have any questions about potential fees.

7. Confirmation of acceptance
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8. E-publication ahead of print
All accepted manuscripts are subject to copyediting. Before publication, page proofs are sent to the corresponding author, who is responsible for verifying the final manuscript contents, including all copyediting changes. Once a manuscript has been typeset, copyedited, and approved by the editor and the authors, it will soon appear online in our "Ahead-of-Print" section.

Further information
Any correspondence, queries or additional requests for information on the manuscript submission process should be sent to *Annals of Child Neurology* editorial office as follows:
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E-mail: editor@annchilneurol.org
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1. General provisions
☐ The authors should ensure that the contents of the present manuscript have not been published nor intended to be published in other journals.
☐ The manuscript should be formatted as follows: A4 paper, 12 point font, left-aligned, double-spaced.
☐ An original article should be presented in the following order: cover page, abstract, keywords, introduction, methods, results, discussion, references, and captions and legends for tables and figures.

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☐ This section should indicate the contact information of the corresponding author: postal code, address, phone number, fax number, and email address.
☐ A running title should be given in 10 words or less.

3. Abstract and Keywords
☐ The abstract should be divided into Background and Purpose, Methods, Results, and Conclusions; it should be written in one paragraph that is within 250 words.
☐ Three to six keywords should be included (preferably those recommended in MeSH of Index Medicus; the first letter of each key word should be capitalized).

4. Main text
☐ The title should not include abbreviations; all the words must be spelled out.
☐ Information regarding approval of an institutional review board and obtaining informed consent should be mentioned in the Method section.
☐ References should be numbered in Arabic numbers in the order they are cited.
☐ Superscript numbers should come after commas and periods according to submission rules.
☐ When using abbreviations, their full forms should be used at first mention; abbreviations/acronyms should then be used consistently in further occurrences.
☐ Units of measure should be written in accordance with submission rules (except for % and °C, a space should come between the number and the unit of measure).
☐ For numbers, a comma should be inserted after every third digit.
☐ All statistical methods used should be described accurately in detail.

5. References
☐ In-text citations should be numbered and should correspond to the numbers in the references.
☐ Up to six authors should be mentioned. In case there are seven or more authors, "et al." must come after the primary author.
☐ Official abbreviations of quoted journals must be used.
☐ Year, volume, and start page–end page of the quoted literature should be accurately mentioned.
☐ The first letter of the title of the quoted article should be capitalized.
☐ Compliance with quotation styles should be observed.
☐ The manuscript should comply with quotation rules in case a book has separate authors by chapter.

6. Table
☐ Each table must have its own title and be given a separate page.
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7. Figures
☐ Each figure should be produced in a separate file and should not be included in the main text.
☐ The file name of each figure should be the figure number.
☐ Figures can be black and white or in color; they will be published as submitted.
☐ The titles and legends of the figures should be concisely drafted on a separate page in English.
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☐ All abbreviations should be written out.
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Translation: Safety Information

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REFERENCES 1) Kwon and Son, J Inno Ther 21(3), 181-189(2013), in vivo : rat, dog
2) DA9701_FU_II_CSR_Final (Ver 2.0), Phased III Clinical Trial Comparing the Efficacy and Safety of DA-9701 and itopride in Patients with Functional Dyspepsia.
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서방정 제형으로 하루 한 번 간편하게 복용하세요!

✓ 하루 한 번 복용의 편의성
✓ 다양한 seizure type의 치료
✓ 24시간 안정적인 혈중농도 유지
✓ 우수한 내약성

함께하는 value

Various

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TEL : (02)405-3000, FAX : (02)404-2518

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서울특별시 강남구 영동대로 421 삼탄빌딩 3F 한국애보트(유)
TEL : (02)3429-3500, FAX : (02)565-3197

Reference
1. Depakote ER FDA label NDA 20-782
2. 대한뇌전증학회, 뇌전증의 약물치료지침 2015
3. Depakote ER 한국어 사용자 설명서

[전문의약품]

Selected Prescribing Information

[전문의약품]
Earlier Line, Better Aiming

Fycompa is now available on monotherapy
**THE RELIABLE PARTNER YOU CAN TRUST**

- Ultra-broad spectrum of activity
- High stability to ESBLs
- Well tolerated, Low incidence of nausea and vomiting
- The carbapenem indicated in meningitis

ESBL: Extended-spectrum β-lactamase
*Meropenem has a very broad spectrum of in vitro activity against Gram-positive and Gram-negative pathogens, including extended-spectrum β-lactamases (ESBLs) and AmpC-producing Enterobacteriaceae.

References:
미토콘드리아 내 지방대사와 당대사 활성을 깨우세요!¹

REFERENCES

¹ 시험 환자 5명 중 비시모바르디리더성질환성당 93mg (비시모바르디리더성질환성당 150mg)

제품명: 베시브행
제품성분: 베시모바르디리더성질환성당 330mg (비시모바르디리더성질환성당성당)
Fast & Safe Care for Seizure

CEREBYX® inj
(Fosphenytoin Sod.)

▶ 국내 최초로 도입되는 Phenytoin Prodrug입니다.
▶ 약알카리성으로 용해성이 개선되었습니다.
▶ 수액 혼합 후 안정성이 개선되어 적응이 편리합니다.
▶ IV 주입속도가 페니토인에 비해 3배 정도 빨라졌습니다.
▶ 주사부위의 국소피사 부작용이 개선되어 안전합니다.
▶ IV 및 IM 모두 적용 가능합니다.
▶ 간질증협증 치료 및 발작의 예방에 효과적입니다.

세레 vidé스주 2mL/10mL

표준코드 2mL: 645304030
          10mL: 645304040

#제품에 대한 모든 자세한 정보는 원하시는 소비자 상담장 02-3449-6114로 문의해 주시기 바랍니다.
위염예방을 위한 레바미피드의 새로운 패러다임
하루 두번으로 복용 편의성을 높였습니다

1일 2회 복용으로 24시간

뮤코트라 레바미피트 150mg

서방정
하루, 한번

큐티스테서캡슐은 Topiramate 서방형 제제입니다.¹

국내 최초의 Topiramate 서방형 제제입니다.¹

복약편의성을 개선한 Topiramate 서방형 제제입니다.²

FDA 허가를 받은 Topiramate 서방형 제제입니다.³