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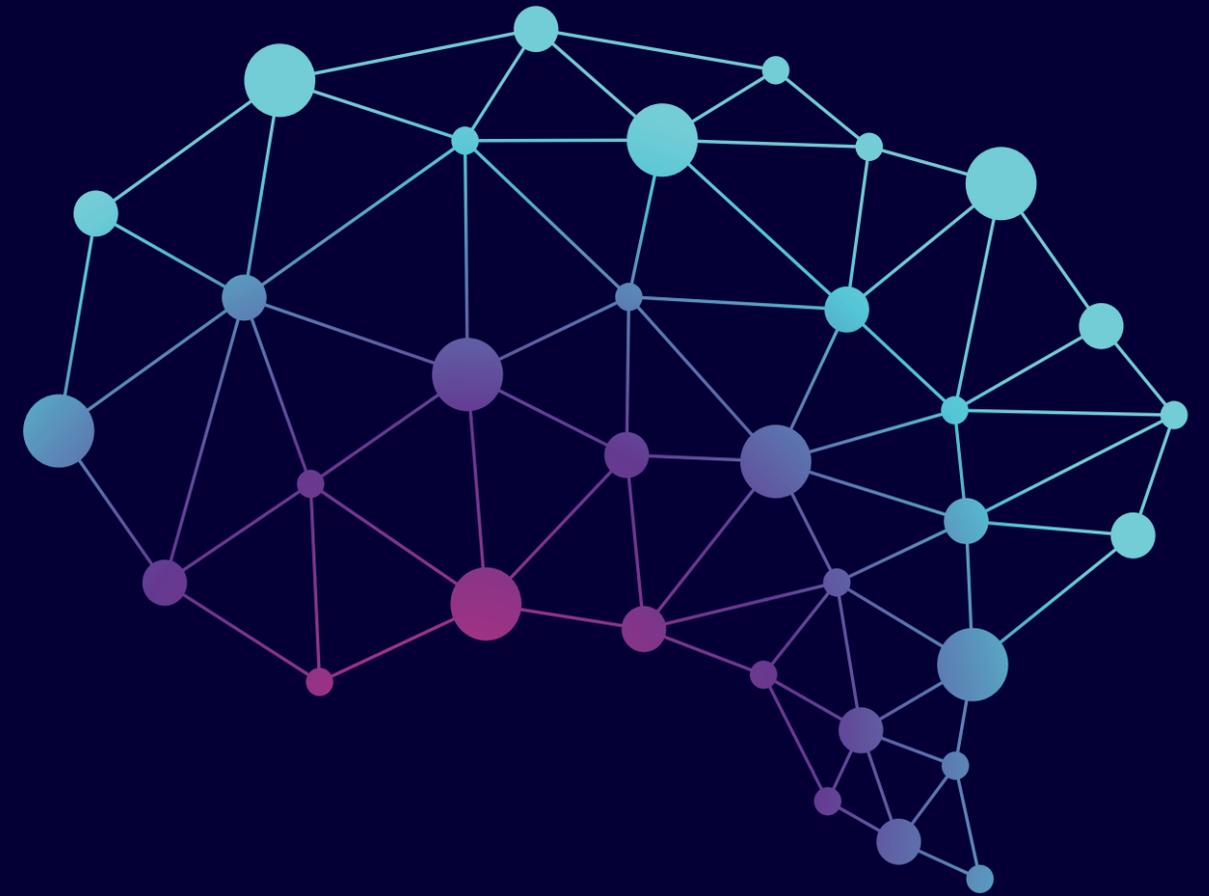
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Aims and scope

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, neuroimaging, 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, brief communications, reviews, letters to the editor, and editorials. 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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Screening Children with Epilepsy for Cognitive Deficits Using the Modified Mini-Mental Scale Examination and the Digit Letter Substitution Test

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Purpose: Children with epilepsy commonly have cognitive deficits; however, full-length neuropsychological testing is time- and resource-intensive. Therefore, we evaluated the feasibility of using the modified Mini-Mental Scale Examination (MMSE) and the Digit Letter Substitution Test (DLST) to screen children with epilepsy for cognitive deficits.

Methods: This was a prospective case-control study comparing scores on the MMSE and the DLST in children with epilepsy with normal age-matched controls between 8 and 12 years of age.

Results: In 35 cases and 36 controls, the cases had significantly lower ($P<0.05$) mean scores than the controls. The correlation coefficient between the MMSE and DLST scores was 0.902 ($P<0.001$). Children with developmental or speech delays and an epilepsy duration ≥ 5 years had lower scores than those without the corresponding risk factors.

Conclusion: This study demonstrated significantly lower scores on the MMSE and DLST in children with epilepsy than in controls, as well as significantly lower scores in patients with developmental or speech delays and an epilepsy duration ≥ 5 years.

Keywords: Epilepsy; Cognition; Child; Neuropsychological tests

Introduction

Cognitive deficits are seen in approximately half of patients with epilepsy, even at diagnosis [1]. A community-based cohort study of children with epilepsy demonstrated that 26.4% had subnormal global cognitive function [2]. Over time, while the majority of these children maintain their cognitive levels, children with uncontrolled seizures, especially younger children, have shown more than a 10-point drop in the full-scale intelligence quotient [3]. Analyses of the adult outcomes of childhood epilepsy have shown that those with good cognitive skills fare significantly better than their counterparts with poor cognitive skills in several domains, in-

cluding education, employment, marriage and parenthood [4].

The etiology of epilepsy determines cognitive functioning to a large extent. However, seizures also interfere with learning and behaviour, especially in the developing brain, most likely by interfering with signalling pathways and neuronal networks [5]. The degree and progression of cognitive deficits in children with epilepsy are important to identify and monitor. Hence, the International League Against Epilepsy (ILAE) Neuropsychology Task Force recommends that all children with newly diagnosed epilepsy should receive routine screening for cognitive difficulties [6]. Detailed neuropsychological function testing, if done thoroughly, requires 6 to 8 hours of contact between the patient and examiner.

Even a highly abbreviated assessment takes at least 2 hours [7]. While these comprehensive test batteries are the gold standard for evaluation, they are unavailable at most centres, especially in developing countries. To date, few studies have used screening tools to detect cognitive dysfunction in children with epilepsy. Even in the United States, a survey including 25 unique epilepsy programs showed that 50% of them used non-standardised informal questioning to screen for cognitive deficits [8]. There is an urgent need for a simple, quick, and repeatable screening tool that clinicians may use. Abnormal results on a screening tool could prompt a recommendation for detailed neuropsychological testing.

The first test we chose was the modified Mini-Mental Scale Examination (MMSE). The MMSE evaluates orientation, attention and concentration, registration, recall, and language and has been widely used as a short screening tool for cognitive impairment, especially in those above 4 years of age [9]. An evaluation of 181 Spanish children found that it correlated well with both the chronological age and Kaufman Brief Intelligence test [10]. A modification of the MMSE called School Years Screening Test for Evaluation of Mental Status (SYSTEMS) was tested in Australian school children and showed adequate sensitivity and specificity in detecting cognitive dysfunction [11]. We used the MMSE, which was standardised for Indian children and further modified for Brazilian children [12,13].

The broad subgroups of cognitive function that are commonly tested include intelligence, language, memory, executive function, and psychomotor speed. Of these subgroups, psychomotor processing speed has been shown to be the most affected in children with both localised and generalised epilepsy [14]. The Digit Letter Substitution Test (DLST) requires participants to quickly match various symbols such as letters with other symbols such as numbers in a limited time. It is one of the commonest tests in neuropsychology, and it is considered a good test for monitoring information processing speed in addition to a range of other cognitive functions including motor speed, attention, visuo-perceptual functions, visual scanning, and manual dexterity. The DLST has been shown to yield clinically meaningful results when serially monitoring patients [15]. Normative data for the DLST in Indian children are available; hence, this was the second test we chose to evaluate [16].

The present study aimed to evaluate the feasibility of using these two simple screening cognitive tests (the MMSE and DLST) in children with epilepsy. Each of these tests requires about 10 to 15 minutes to administer and score.

Materials and Methods

1. Patient selection

This was a prospective case-control study. The inclusion criteria for cases were (1) children with epilepsy; (2) age between 8 and 12 years; and (3) the ability to read and write letters and numbers. Cases were excluded if they refused consent or were unable to use paper and pencil due to motor deficits. Cases were selected among children with epilepsy attending the Pediatric Neurology Clinic of Choithram Hospital and Research Centre between July 2019 and June 2021. Controls included children between 8 and 12 years of age without neurological illnesses who attended the general pediatric outpatient department or were admitted to the pediatric ward for non-neurological illnesses.

2. Assessment of cases and controls

The following data were collected from cases: age, sex, significant perinatal events, age at onset of epilepsy, duration of epilepsy, etiology of epilepsy, type of seizures (focal or generalized), seizure burden, age of acquisition of developmental milestones, date of last seizure, electroencephalogram (EEG) reports, magnetic resonance imaging (MRI) brain reports, and the number of anti-epileptic drugs (AEDs). Data collected from controls included age, sex, age of acquisition of developmental milestones, and significant perinatal events.

3. Evaluation

All children were evaluated using the MMSE and the DLST. Testing was done in a quiet room after a careful explanation of the test to the child. The MMSE for children was calculated based on a set of questions and tasks given to the child, and their answers were scored based on a test previously standardised in Indian children [12]. It included five subtests to assess orientation, attention/concentration, registration, recall, and language. The cut-off scores for abnormal results were 28, 30, and 35 in the age groups of 6–8, 9–11, and 12–14 years, respectively [12].

The DLST is a paper-and-pencil cognitive test in which the patient is asked to match a set of digits with letters according to a key shown at the top of the page. The children were asked to match as many target digits as possible in the specified time of 90 seconds. The net score was obtained by deducting wrong substitutions from the total substitutions attempted. Scores below 2 standard deviations for age and sex were considered abnormal [16,17].

4. Ethics

The study was approved by the local institutional ethics committee

(IER/2021/41). Informed consent was obtained from the parents or guardians accompanying the children.

5. Statistical analysis

The data were analysed using GraphPad and Epi Info as online-available software. Comparisons of means between the two groups were conducted using the unpaired t-test. The Glass delta value was used to calculate the effect size when the standard deviation of the study and control groups differed, and the Hedge g value was used if the sample sizes were different [18]. Correlations between pairs of parametric variables were calculated using the Pearson coefficient of correlation test. A *P* value of < 0.05 was interpreted as statistically significant. Scores on the MMSE and DLST were compared in cases with and without various risk factors that have been described in literature as being associated with cognitive deficits, such as the age of onset of seizures below 2 years, a high seizure frequency, long duration of epilepsy, MRI brain abnormalities, and a relatively high number of AEDs.

Results

1. Baseline data

There were 35 cases (62.9% male) and 36 controls (66.7% male) (Table 1). The mean age of cases was 10.53 ± 1.47 years, and that of controls was 10.39 ± 1.48 years. Global developmental delay was seen in 16 (45.7%) cases and one (2.8%) control ($P < 0.001$). Speech delay was seen in 22 (34.4%) cases and in none of the controls ($P < 0.001$).

2. Details of cases

Generalised-onset epilepsy was seen in 23 (65.7%) cases, while 12 (34.3%) had focal onset epilepsy (Table 2). Significant perinatal events, including birth asphyxia and neonatal hypoglycemia, were noted in 16 patients. The etiology of epilepsy was idiopathic in 22 cases (62.9%), perinatal hypoxia in 10 (28.6%) and structural in three (8.6%). MRI brain abnormalities were seen in 20 patients

(57.1%) and EEG abnormalities in 15 (42.9%). The age at onset of epilepsy was below 2 years in 15 (42.9%), between 2 and 5 years in 11 (31.4%), and above 5 years in nine (25.7%). The seizure burden was 0–5 per month in 20 patients (57.1%), 6–20 per month in 10 (28.6%), and more than 20 per month in 5 (14.3%). Ten children (28.6%) were taking one AED, 16 (45.7%) were taking two AEDs, six (17.1%) were taking three AEDs, and three (8.6%) were taking no AEDs. Fifteen (42.8%) children had experienced no seizures for the past 1 year or more and were classified as having controlled epilepsy.

3. Scores on cognitive screening tests in cases and controls

The mean scores of children with epilepsy were significantly lower ($P < 0.05$) than those of controls on the MMSE (28 vs. 35) and DLST (24 vs. 41) (Table 3). Subnormal cognitive scores were seen

Table 2. Details of children with epilepsy

Variable	No. (%)
Generalised epilepsy	23 (65.0)
Significant perinatal events	16 (45.7)
Aetiology of epilepsy	
Perinatal asphyxia	10 (28.6)
Structural	3 (8.6)
Unknown	22 (62.9)
MRI abnormalities	20 (57.1)
Abnormal EEG	15 (42.9)
No seizures in the last 1 year	15 (42.9)
Age at onset of seizures (yr)	
≤ 2	15 (42.9)
2–5	11 (31.4)
> 5	9 (25.7)
Seizure burden (/mo)	
0–5	20 (57.1)
6–20	10 (28.6)
> 20	5 (14.3)
No. of anti-seizure medications being used when tested	
0	3 (8.6)
1	10 (28.6)
2	16 (45.7)
3	6 (17.1)

MRI, magnetic resonance imaging; EEG, electroencephalography.

Table 1. Baseline data of children with epilepsy (cases) and children without epilepsy (controls)

Variable	Cases	Controls	<i>P</i> value
Number	35	36	NS
Male sex	22 (62.9)	24 (66.7)	NS
Mean age (yr)	10.53 ± 1.47	10.39 ± 1.48	NS
Global developmental delay	16 (45.7)	1 (2.8)	< 0.001
Speech delay	22 (34.4)	0	< 0.001

Values are presented as number (%) or mean \pm standard deviation. NS, not significant.

Table 3. Mean MMSE and DLST scores in children with epilepsy (cases) versus children without epilepsy (controls)

Group	No. of children	MMSE scores	DLST scores
Cases	35	27.7 ± 7.04	24.23 ± 10.62
Controls	36	35.11 ± 1.26	41.28 ± 5.67
<i>P</i> value		0.001 ^a	0.001 ^a

Values are presented as mean \pm standard deviation.

MMSE, Modified Mini-Mental Scale Examination; DLST, Digit Letter Substitution Test.

^aStatistically significant results.

in 21 (60%) and 13 (37%) of the case group using the MMSE, and DLST, respectively, while none of the children in the control group had subnormal scores on any of the tests.

4. Comparison of scores on the MMSE versus the DLST

The correlation coefficient between MMSE and DLST scores was 0.902 ($P < 0.001$), demonstrating a very high correlation between the two tests.

5. Comparison of MMSE and DLST scores in children with epilepsy with and without various risk factors for poor cognition

The MMSE and DLST scores in children with epilepsy with various risk factors for poor cognition were compared with those of children with epilepsy who did not have those risk factors. The risk factors included developmental delay, speech delay, duration of epilepsy ≥ 5 years, uncontrolled epilepsy (defined as the presence of seizures in the past 1 year), age of onset of epilepsy ≤ 2 years, focal-onset epilepsy, seizure burden ≥ 1 /month, and abnormal brain

MRI. Children with developmental delay, speech delay, and duration of epilepsy ≥ 5 years all showed significantly lower scores on both the MMSE and DLST than their counterparts without these risk factors (Table 4). Children with uncontrolled seizures and abnormal MRI findings showed significantly lower scores on the DLST, but not on the MMSE, as compared to children without these risk factors. However, children with an age of onset of epilepsy ≤ 2 years, focal-onset epilepsy and those with a high seizure burden did not show significantly lower scores than controls. All scores fell uniformly as the number of AEDs rose from 1 to 3. The mean scores of epileptic children on no drugs were lower than those on one or two drugs but higher than those on three drugs (probably because two patients were non-compliant and one had not begun anti-seizure medication) (Table 5).

Discussion

In this study, we found that it was feasible to use two previously validated quick tests for cognitive deficits (namely, the MMSE and

Table 4. Mean MMSE and DLST scores in children with epilepsy with various risk factors

Risk factor	No. of patients	MMSE scores	DLST scores
Developmental delay present	16	22.88 \pm 7.05	17.19 \pm 9.81
No developmental delay	19	31.79 \pm 3.74	30.16 \pm 7.18
<i>P</i> value		0.001 ^a	0.001 ^a
Speech delay present	21	24 \pm 7.9	18.43 \pm 11.53
No speech delay	14	30.19 \pm 5.25	28.09 \pm 8.13
<i>P</i> value		0.009 ^a	0.006 ^a
Duration of epilepsy ≥ 5 years	17	31.12 \pm 5.37	29.47 \pm 8.01
Duration of epilepsy < 5 years	18	24.50 \pm 7.04	19.28 \pm 10.57
<i>P</i> value		0.004 ^a	0.003 ^a
Epilepsy not controlled	20	25.90 \pm 6.92	20.65 \pm 11.05
Epilepsy controlled	15	30.13 \pm 6.67	29.00 \pm 8.09
<i>P</i> value		0.078, NS	0.019 ^a
Epilepsy onset ≤ 2 years	15	25.40 \pm 6.93	21.40 \pm 9.96
Epilepsy onset > 2 years	20	29.45 \pm 6.79	26.35 \pm 10.84
<i>P</i> value		0.09, NS	0.2, NS
Focal-onset epilepsy	12	27.42 \pm 7.54	24.08 \pm 8.66
Generalised onset	23	27.87 \pm 6.94	24.30 \pm 11.69
<i>P</i> value		0.8, NS	0.9, NS
Seizure burden ≥ 1 /month	12	26.74 \pm 7.74	22.87 \pm 11.92
Seizure burden < 1 /month	23	29.58 \pm 5.26	26.83 \pm 7.28
<i>P</i> value		0.3, NS	0.3, NS
Abnormal brain MRI	20	27.45 \pm 6.67	24.20 \pm 9.40
Normal brain MRI	10	31.80 \pm 5.55	31.30 \pm 6.46
<i>P</i> value (MRI was not done in 5)		0.087, NS	0.041 ^a

Values are presented as mean \pm standard deviation.

MMSE, Modified Mini-Mental Scale Examination; DLST, Digit Letter Substitution Test; NS, not significant; MRI, magnetic resonance imaging.

^aStatistically significant values.

Table 5. Comparison of MMSE and DLST scores in relation to the number of anti-seizure medications in cases versus controls

No. of drugs	No.	MMSE	DLST
Control group	36	35.11 ± 1.26	41.28 ± 5.67
Case group			
No drugs ^a	3	26.00 ± 10.15	25.00 ± 11.14
1 drug ^a	10	32.30 ± 3.80	31.80 ± 7.77
2 drugs ^a	16	28.25 ± 3.62	22.88 ± 8.35
3 drugs ^a	6	19.50 ± 10.25	14.83 ± 13.14
Comparisons			
Control vs. no drugs		<i>P</i> < 0.001 ^a	<i>P</i> < 0.001 ^a
Control vs. 1 drug		<i>P</i> < 0.001 ^a	<i>P</i> < 0.001 ^a
Control vs. 2 drugs		<i>P</i> < 0.001 ^a	<i>P</i> < 0.001 ^a
Control vs. 3 drugs		<i>P</i> < 0.001 ^a	<i>P</i> < 0.001 ^a

Values are presented as mean ± standard deviation.

MMSE, Modified Mini-Mental Scale Examination; DLST, Digit Letter Substitution Test.

^aStatistically significant results.

the DLST) in a clinical setting for children with epilepsy. Children with epilepsy had significantly lower mean scores than normal age-matched controls on both the MMSE and DLST. The scores on both tests correlated well with each other. Children with several known risk factors for low cognition scored lower than those without the corresponding risk factors.

While the ILAE Neuropsychology Task Force recommends routine screening for cognitive deficits in children with epilepsy, they do not mention any particular screening test. Nonetheless, they suggest that testing should cover most core cognitive domains such as general intellect, attention, speed of processing, memory, language, spatial function, executive function, and sensory and motor function [6]. The MMSE has the advantage of screening most cognitive domains. However, it is more complicated to use than the DLST, which has the advantage of simplicity but evaluates a narrower window of cognitive functions, focusing primarily on processing speed. There is always a trade-off between what is practical versus what is ideal; however, the high concurrent validity between the tests suggests that the DLST may be useful in a busy clinical setting. The DLST has been used to serially monitor cognitive decline in children with multiple sclerosis. An increase in DLST scores by 4 points or 10% is now being used to identify and define clinical responders in these patients [19].

There are few reports of the use of cognitive screening in children with epilepsy. Triplett and Asato [20] studied 39 children with medication-naïve epilepsy between 8 and 17 years of age using the computerised Central Nervous System (CNS) Vital Signs battery, which is a 30-minute assessment over multiple cognitive domains. Their pilot study found that this battery was time-effi-

cient, potentially usable in the clinical setting, and well tolerated by children. However, this computerised battery needs to be purchased and may not be accessible to clinicians working in developing countries. Both tests we used are brief and take 10 to 15 minutes to administer and score. Furthermore, they are freely available and need no additional infrastructure other than paper and pen. They have been validated in children of various age groups. While computerised testing has advantages in terms of the standardisation and accuracy of timing, it has inherent problems. Some limitations include its availability in low-resource countries and variation in stimuli due to differences in screen resolution. Moreover, much clinical information is garnered by observing the patient's interaction styles and the way a patient responds to difficulties, but those nuances are lost in computerised testing. The six-item PedsQL (<https://www.pedsq.org/>) cognitive functioning scale has recently been used as a brief generic outcome measure in children with epilepsy, but this is a patient-/parent-reported self-assessment scale [21].

Although the main goal of this study was to assess the feasibility of the use of two simple screening tests for cognitive deficits in children, we also explored risk factors for low scores to support construct validity. In the literature, risk factors for cognitive deficits include epilepsy onset in early childhood, uncontrolled epilepsy, symptomatic epilepsy, a higher seizure burden, poor seizure control, and relatively high AED exposure [3,22-25]. In our study, children with global developmental delay, delayed speech, and epilepsy duration ≥ 5 years all had significantly lower scores on the screening tests than the normal controls. However, in children with uncontrolled epilepsy and those with abnormal brain MRI, the scores of cases were lower than those of controls only in the DLST, while the MMSE failed to show a statistically significant difference. In contrast, seizure onset at ≤ 2 years of age, generalised epilepsy, and a seizure burden ≥ 1/month were not associated with significantly lower scores than controls on both tests. There is a clear need for studies including larger groups of children.

The lack of simultaneous testing of all children with a gold-standard test for cognition such as the Wechsler Full-Scale Intelligence Quotient and the relatively small sample size are the major limitations of the study. The study was carried out among children with epilepsy referred to a tertiary care centre, who therefore constituted a group with significant cognitive issues. It would be relevant to subsequently evaluate these tests in a larger group of children with epilepsy who have no apparent developmental or cognitive delays to evaluate its efficacy in identifying and monitoring children with subtle deficiencies who would benefit from a detailed evaluation and intervention.

In summary, this pilot study evaluated the feasibility of using the

MMSE and the DLST to screen children for cognitive deficits. We found the two tests easy to use, and their results were closely correlated with each other. These tests appear potentially useful to screen and monitor for cognitive deficits in children with epilepsy. Further comparisons with gold-standard full-length neuropsychological tests on larger groups of children with epilepsy will clarify their value and help us identify children at risk and intervene in time.

Conflicts of interest

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

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Conceptualization: GRP. Data curation: MB. Formal analysis: GRP. Methodology: MB and GRP. Project administration: GRP. Visualization: GRP. Writing-original draft: GRP. Writing-review & editing: GRP.

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Changes in Sleep Patterns in Korean Early Adolescents during Sexual Maturation

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Purpose: Teenagers' sleep patterns show physiological delays influenced by sexual maturation and other external time-related factors. However, Korean adolescents show differences in the onset of pubertal development and have shorter sleep durations than other adolescents worldwide. Therefore, we assessed sleep patterns and sexual maturation in Korean early adolescents to evaluate changes in sleep patterns in relation to sexual maturation in early adolescents with sleep deprivation.

Methods: From March to August 2017, we surveyed children aged 10 to 12 years in Seongnam (Seongnam Atopy Project). We evaluated items related to sleep and sexual maturation, assessed sleep duration and sleepiness scale scores, and analyzed the relationships of sleep parameters with sex, height, weight, and sexual maturation rating (SMR).

Results: In total, 620 children were included. Sleep duration was 8.63 ± 0.81 hours in boys and 8.40 ± 0.98 hours in girls. Sleep started from PM 11:00 \pm AM 0:47 in boys and PM 11:13 \pm AM 1:06 in girls, and ended at AM 7:38 \pm AM 0:27 in boys and AM 7:34 \pm AM 0:27 in girls. After adjusting for sex and standardized body mass index, bedtime was delayed as the SMR increased (mean delay for each rating increase, 0.251 hours; $P=0.001$; 95% confidence interval [CI], 0.105 to 0.397). SMR did not influence the wake-up time, although sleep duration decreased as the SMR increased (mean decrease for each rating increase, 0.258 hours; $P=0.001$; 95% CI, -0.403 to -0.114). The sleepiness scale scores showed no relationship with SMR.

Conclusion: Sleep patterns, especially sleep duration and bedtimes, show changes with sexual maturation in adolescents, who are vulnerable to sleep deprivation.

Keywords: Adolescent; Sleep; Sleepiness; Sexual maturation

Introduction

Sleep patterns in adolescents are physiologically delayed [1], and the mechanism underlying these delays is currently unclear. This

physiological delay is not caused by environmental factors, such as evening light exposure [2]. Moreover, internal sleep phases in teenagers are delayed compared to those in children, and these delays present as delayed dim light melatonin onset (DLMO) [3]. The

interval between DLMO and sleep onset is also prolonged in adolescents [4]. Individuals in the mature Tanner stage show increased sleep latency, delayed bedtime [1,5], and increased sensitivity of the circadian system to light in early puberty [6]. Similar to humans, numerous mammals also show physiological sleep delays in adolescence [7]. In combination with earlier school start times, this physiological delay in sleep can induce sleep deprivation in older adolescents.

Sleep patterns can also be affected by numerous external factors, especially in teenagers. For example, teenagers in East Asia experience more extreme sleep deprivation than those in other parts of the world. Numerous studies have shown that teenagers in Asia have a sleep duration of 6 to 7 hours on weekdays [8], and sleep deprivation becomes more severe as they get older. This pattern of severe sleep deprivation has been attributed to social and academic needs, which are potent regulators of sleep patterns in school children.

The age of onset of sexual development in adolescents in Korea has been reported to be different. Moreover, compared to a study published in 2006 [9], a more recent study from 2020 reported earlier pubertal development onset in Korean adolescents [10]. However, studies on the relationship between the Tanner stage and sleep have not been conducted in Korean adolescents. Therefore, considering the differences in life patterns of East Asian adolescents from those of adolescents in other countries, we believe that the findings of this study will provide useful insights into the relationship between sexual development represented by Tanner stage and sleep patterns in early adolescents, who often experience sleep deprivation.

Materials and Methods

1. Participants

This study surveyed children aged between 10 and 12 years who attended 11 elementary schools in Seongnam from March to August 2017 as part of the Seongnam Atopy Project (SAP). In the SAP, we surveyed the students' characteristics, body weight and height, date of birth, questionnaires about allergic disease and gastrointestinal disease, routine diet, environmental factors, feeding history, sleep and sexual maturation with figures (Fig. 1). We selected questionnaire items to survey the participants' sleep patterns, sleepiness, anthropometric data, and baseline characteristics (Appendix 1). We asked the children's parents to complete the questionnaires prior to physical examinations. The parents of 621 children agreed to participate in this study, and 620 children returned completed demographic questionnaires, including information regarding school year and sex. The physical examination,

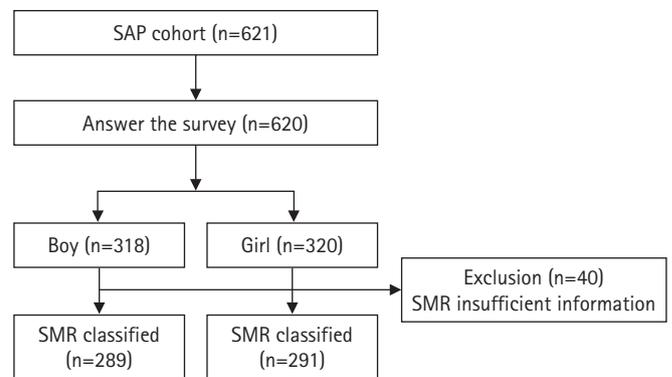


Fig. 1. Flow diagram. Among 621 children in the Seongnam Atopy Project (SAP) cohort, 620 children answered questions about age and gender. We analyzed 289 boys and 291 girls who responded to the sexual maturation rating scale. SMR, sexual maturation rating.

which included measurement of weight and height, was performed by a pediatrician and a well-trained technician. Body mass index (BMI) was calculated from the measured height and weight and standardized with reference to the age and sex (BMI-z) [11,12]. Sex and BMI are known to affect sleep based on previous studies, so they were used as correction variables.

2. Sleep duration and daytime sleepiness

Participants' usual sleep onset and wake time for the last 7 days were determined using direct questions. Sleep duration was computed from the sleep onset and wake times. Responses describing unusual times of sleep onset and waking, such as sleep onset in the afternoon, getting up in the evening, or sleep duration over 15 hours, were excluded. The degree of sleepiness was estimated using the Korean version of the Pediatric Daytime Sleepiness Scale (PDSS) [13]. The PDSS has eight questions, each of which is scored from 0 to 4 (total range, 0 to 32), and it assesses subjective sleepiness during classes, homework, daytime, and morning. A higher score indicates greater daytime sleepiness [13], and a score of 15 or more in the age group of 14 to 19 years old indicates excessive sleepiness [14].

3. Sexual maturity

The Tanner stage is a common parameter for evaluating children's pubertal development, which is classified using the sexual maturation rating (SMR) scale [15]. Parents answered questionnaires about their children's sexual maturity, which included pictures with serial stages of sexual maturity. The series of pictures outlined the degrees of development of pubic hair and breasts in girls and pubic hair, penis, and testes in boys. On the basis of the responses, for boys, an SMR of 2 implied visible signs of testicular enlargement or

development of pubic hair; an SMR of 3 indicated penile growth or further development of pubic hair; and an SMR of 4 indicated increased testes volume or a distributed pattern of pubic hair. For girls, an SMR of 2 indicated the presence of breast buds or development of pubic hair, and SMRs of 3 to 5 indicated patterns of pubic hair and breast development. The selected pattern of SMR was different; the average grade was used, whereas in cases with only one response for the queries regarding sexual development, the SMR was classified on the basis of this response.

4. Ethics statement

The survey was approved by the Institutional Review Board of CHA Bundang Medical Center (IRB No. 2017-04-049). Written informed consent was obtained from the parents or caregivers of all participants.

5. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA) and MS Excel version 2203 (Microsoft, Redmond, WA, USA). Frequencies and continuous variables were compared using the chi-square test and analysis of variance, respectively. Multiple linear regression models were used to estimate adjusted differences and 95% confidence intervals (CIs), with adjustments for sex and BMI-z. Sex and BMI have previously been reported as factors that influence sleep patterns and obstructive sleep apnea syndrome [15,16]. Each sleep-related variable was analyzed in a model with SMR, sex, and BMI-z. Statistical significance was defined as a $P < 0.05$.

Results

1. Sample characteristics

We evaluated the findings for 620 children (318 boys and 302 girls) who provided basic data such as sex and grade/school year. The mean ages were 11.51 ± 0.61 years for boys and 11.48 ± 0.90 years for girls. The 318 boys included four (1.3%) fourth-grade boys, 154 (48.6%) fifth-graders, and 159 (50.2%) sixth-graders, while the 302 girls included two (0.7%) fourth-graders, 152 (50.3%) fifth-graders, and 148 (49.0%) sixth-graders.

The mean heights were 149.15 ± 8.05 cm for boys and 149.43 ± 7.29 cm for girls, and the mean weights were 42.79 ± 9.61 kg for boys and 42.15 ± 8.57 kg for girls. The mean BMI was 19.1 ± 3.27 kg/m² for boys and 18.77 ± 3.01 kg/m² for girls, and the mean BMI-z was -0.08 ± 1.01 for boys and 0.03 ± 1.04 for girls.

2. Sleep patterns and sleepiness

We investigated sleep patterns and sleepiness using the Korean ver-

sion of the PDSS. The mean sleep duration was 8.63 ± 0.81 hours for boys and 8.4 ± 0.98 hours for girls, as calculated based on the bedtime (PM 11:00 \pm AM 0:47 for boys and PM 11:13 \pm AM 1:06 for girls) and wake time (AM 7:38 \pm AM 0:27 for boys and AM 7:34 \pm AM 0:27 for girls). The average PDSS score was 11.4 ± 5.02 for boys and 11.65 ± 5.24 for girls.

3. Sexual development

Among the 318 boys, 289 provided responses for pubic hair and 282 provided responses for genitalia, and the SMR was evaluated in 289 boys. Among the 302 girls, 287 provided responses for pubic hair and 291 provided responses for breasts, and the SMR was evaluated in 291 girls. Among the boys, 237 (82%), 42 (14.5%), and 10 (3.5%) were classified as SMR1, SMR2, and SMR3, respectively. None of the boys were categorized as SMR4. Among the girls, 102 (35.1%), 152 (52.2%), 31 (10.7%), and six (2.1%)

Table 1. Demographic characteristics according to sex

Characteristic	Boys	Girls
Age (yr)	11.51 ± 0.61	11.48 ± 0.9
School year (grade)		
4	4 (1.3)	2 (0.7)
5	154 (48.6)	152 (50.3)
6	159 (50.2)	148 (49)
Height (cm)	149.15 ± 8.05	149.43 ± 7.29
Weight (kg)	42.79 ± 9.61	42.15 ± 8.57
BMI (kg/m ²)	19.1 ± 3.27	18.77 ± 3.01
BMI-z	-0.08 ± 1.01	0.03 ± 1.04
Sleep duration (hr)	8.63 ± 0.81	8.4 ± 0.98
Bedtime	PM 11:00 \pm AM 0:47	PM 11:13 \pm AM 1:06
Wake time	AM 7:38 \pm AM 0:27	AM 7:34 \pm AM 0:27
PDSS	11.4 ± 5.02	11.65 ± 5.24
Pubic hair		
1	247 (85.5)	171 (59.6)
2	35 (12.1)	81 (28.2)
3	7 (2.4)	28 (9.8)
4	0	7 (2.4)
Genitalia		
1	204 (72.3)	30 (10.3)
2	43 (15.2)	82 (28.2)
3	28 (9.9)	164 (56.4)
4	7 (2.5)	12 (4.1)
5		3 (1)
SMR		
1	237 (82)	102 (35.1)
2	42 (14.5)	152 (52.2)
3	10 (3.5)	31 (10.7)
4	0	6 (2.1)

Values are presented as mean \pm standard deviation or number (%). BMI, body mass index; BMI-z, standardized body mass index; PDSS, Pediatric Daytime Sleepiness Scale; SMR, sexual maturation rating.

were classified as SMR1, SMR2, SMR3, and SMR4, respectively (Table 1).

4. Sleep duration and sleepiness in relation to SMR

Table 2 presents the sleep duration data for the 381 children who answered the survey for sleep patterns. Sleep duration in adolescents was inversely proportional to the SMR: 8.65 ± 0.81 hour/night in SMR1, 8.51 ± 0.80 hour/night in SMR2, 7.89 ± 1.02 hour/night in SMR3, and 7.67 ± 1.26 hour/night in SMR4 (Table 2). Similarly, the bedtime was PM 10:58 \pm AM 0:49 in SMR1, PM 11:07 \pm AM 0:48 in SMR2, PM 11:42 \pm AM 1:02 in SMR3, and PM 11:55 \pm AM 0:52 in SMR4. The wake times were similar and did not differ significantly among participants with different SMRs ($P=0.616$). The scores on the Korean version of the PDSS tended to increase with the SMR, but the differences were not significant: 11.44 ± 5.04 in SMR1, 11.34 ± 5.10 in SMR2, 12.05 ± 5.50 in SMR3, and 14.50 ± 3.99 in SMR4 ($P=0.423$). Even when the differences were analyzed based on values of 15 (the cutoff value of 14 to 19 years) [14] and 11 (the average value of the PDSS), no statistically significant results were obtained.

As shown in Table 3, after adjusting for sex and BMI-z, each rating increase in the SMR resulted in a delay of 0.251 hours in bedtime (95% CI, 0.105 to 0.397 hours; $P=0.001$), no significant changes in the wake time ($P=0.817$), a reduction of 0.258 hours in sleep duration (95% CI, -0.403 to -0.114 hours; $P=0.001$), and no significant change in the PDSS score ($P=0.310$).

Discussion

This study evaluated sleep patterns according to sexual maturation during early puberty in adolescents, who often experience sleep deprivation compared to the recommended sleep duration [16]. The sex- and BMI-adjusted findings suggested that sleep onset was delayed and sleep duration decreased with development of sexual maturity. However, daytime sleepiness showed no significant changes related to SMR. This study is the first to investigate the effect of sexual maturation in Asian adolescents, who often experience sleep deprivation.

Several previous studies have reported changes in sleep duration in adolescence. Rutter et al. [17] reported that sleep duration decreased according to the Tanner stage, after adjusting the findings for other confounding factors. Our findings showed that the total sleep duration per day decreased by approximately 0.258 hours for each stage increase in the SMR. Although this study had fewer adolescents with a mature SMR, it showed a similar pattern of reduction in sleep duration as other studies (0.27 to 0.33 hours per Tanner stage) [17,18]. However, a few studies did not observe significant differences in sleep duration in relation to the Tanner stage [19]. The reasons underlying these discrepancies across different studies are unclear.

Our findings also showed delayed sleep onset as the SMR increased. Delayed sleep onset manifests as a phase delay in the circadian timing system. Although such phase delays are known to be associated with pubertal and adolescent development, the under-

Table 2. Age, sleep pattern, and sleepiness according to sexual maturation

Variable	No.	SMR				P value
		1 (n=339)	2 (n=194)	3 (n=41)	4 (n=6)	
Sleep duration (hr)	358	8.65 ± 0.81	8.51 ± 0.8	7.89 ± 1.02	7.67 ± 1.26	<0.001
Bedtime	358	PM 10:58 \pm AM 0:49	PM 11:07 \pm AM 0:48	PM 11:42 \pm AM 1:02	PM 11:55 \pm AM 0:52	<0.001
Wake time	580	AM 7:36 \pm AM 0:27	AM 7:34 \pm AM 0:27	AM 7:34 \pm AM 0:26	AM 7:25 \pm AM 0:20	0.616
PDSS	580	11.44 ± 5.04	11.34 ± 5.10	12.05 ± 5.50	14.5 ± 3.99	0.423

Values are presented as mean \pm standard deviation.

SMR, sexual maturation rating; PDSS, Pediatric Daytime Sleepiness Scale.

Table 3. Sex and standardized BMI adjusted value according to the SMR

Variable	Coefficient (95% CI)	SEC	P value	Sex		BMI-z		Fit of model			
				Coefficient	P value	Coefficient	P value	SEE	R ²	MSE	MAE
Bedtime	0.251 (0.105 to 0.397)	0.074	0.001	0.024	0.804	0.068	0.139	0.833	0.05	0.689	0.635
Wake time	0.007 (-0.055 to 0.070)	0.032	0.817	-0.077	0.067	-0.051	0.007	0.454	0.02	0.198	0.331
Sleep duration	-0.258 (-0.403 to -0.114)	0.074	0.001	-0.048	0.620	-0.072	0.116	0.827	0.057	28.133	3.64
PDSS	0.361 (-0.336 to 1.057)	0.355	0.310	-0.046	0.921	-0.234	0.266	5.093	0.004	25.819	4.174

BMI, body mass index; SMR, sexual maturation rating; CI, confidence interval; SEC, standard error of coefficient; BMI-z, standardized body mass index; SEE, standard error of estimate; MSE, mean squared error; MAE, mean absolute error; PDSS, pediatric daytime sleepiness scale.

lying mechanism is unknown, and several attempts have been made to identify this mechanism. The intrinsic period of the circadian timing system may slow through puberty in adolescence, thereby delaying the circadian phase and sleep onset. This may be due to the increased sensitivity of light during DLMO [6]. Another study reported that human adolescents show a phase delay in DLMO according to age [4] and sexual maturation [5]. Delayed sleep onset in adolescence may also be caused by increased sleep latency [1,6]. Changes in phase-dependent sensitivity to light may cause phase delays, with strengthening of the delayed response to evening light and weakening of the advance response to morning light. Regarding DLMO, since homeostatic sleep pressure accumulates faster in younger adolescents than in older adolescents, sleep onset after DLMO is faster [4]. Therefore, the older one gets, the slower sleep onset becomes. Our study also showed that sleep onset was delayed by approximately 0.25 hours for each rating increase in SMR, supporting the findings of previous studies.

We did not observe significant differences in sleepiness according to the SMR. Delayed sleep onset causes a reduction in total sleep duration due to a fixed awake time, and various studies have shown sleepiness in individuals with a higher SMR [20]. Carskadon et al. [19] reported that teenagers in the higher SMR group were sleepier and presented short sleep latency. Another study also showed that the prevalence of excessive daytime sleepiness increased with advanced pubertal maturation [21]; however, a longitudinal cohort study reported that subjective sleepiness was more closely related to age than to pubertal development [22]. Our study included early adolescents with the same school hours, which are known to be one of the most influential factors for sleep [23]. Therefore, we suggested that our findings regarding the effect of sexual maturation on sleepiness in early adolescents are more relevant than those of previous studies.

Sleep deprivation may blunt the effects of sexual maturation on teenagers' sleepiness. Multiple previous studies have reported that Korean and East Asian teenagers have severely limited sleep due to academic reasons [23]. These patterns have also been observed in elementary school students [8,13]. Age has been reported to cause sleepiness in various studies [13,18,20]. Moreover, age or school year can influence sleep duration due to the school start time. Under conditions of sleep deprivation, age and school start time could have a greater influence on sleepiness than sexual maturation.

This study had several limitations. Although the inclusion of children with SMR1, SMR2, and SMR3 allowed us to evaluate the effects of sexual maturation in the early stages of puberty, it may have resulted in an underestimation of the effect of later SMR stages. In addition, we did not analyze adolescents' chronotype (i.e., eveningness or morningness), which is also a factor influencing

sleepiness in adolescents [24]. Moreover, we were not able to observe differences in the wake time according to the SMR, and this survey was conducted in a single season with participants who had a similar school start time.

In conclusion, sleep onset is delayed and sleep duration decreases with sexual maturation. However, this study found that puberty-related changes in sleepiness did not appear in early puberty or in adolescents, who often experience sleep deprivation. Understanding the physiological delays in sleep related to puberty will be helpful for improving teenagers' quality of life.

Conflicts of interest

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

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Conceptualization: TH and SR. Data curation: EKH, MYH, and HMJ. Formal analysis: TH and SR. Funding acquisition: EKH, MYH, and HMJ. Methodology: TH and SR. Project administration: MYH, HMJ, and SR. Visualization: TH and SR. Writing-original draft: TH. Writing-review & editing: TH, KYC, EGY, MKJ, and SR.

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Epilepsy with *SLC35A2* Brain Somatic Mutations in Mild Malformation of Cortical Development with Oligodendroglial Hyperplasia in Epilepsy (MOGHE)

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Purpose: This study presents the characteristics of patients with mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE) with *SLC35A2* somatic variants in the brain who underwent epilepsy surgery and showed clinical improvement in seizures.

Methods: We collected 10 patients with *SLC35A2* somatic mutations in the brain who underwent surgery to treat drug-resistant epilepsy at Severance Children's Hospital from 2014 to 2019 and retrospectively reviewed their genetic profiles, neuropathologic results, clinical features, pre-operative evaluations, and post-operative outcomes.

Results: Six of the 10 patients with *SCL35A2* somatic mutations in the brain had Lennox Gastaut syndrome (LGS) evolving from infantile spasms (IS), three had LGS, and one had IS. The median value of variant allele frequencies (VAFs) was 5.7% (1.7% to 5.8%; range, 1.4% to 22.9%). Non-sense mutations were the most common (50%), followed by missense mutations (40%) and a splicing site mutation (10%). Eight patients (80%) had good post-operative outcomes, with freedom from disabling seizures in five (Engel class I) and rare disabling seizures in three (Engel class II). Four of the eight patients who could be assessed for social quotient (SQ) after surgery showed SQ improvements by 12.2±6.4. Although all patients were finally diagnosed with MOGHE, seven (70%) were initially diagnosed with gliosis, two with mild malformation of cortical development, and one with no abnormality.

Conclusion: All patients with *SCL35A2* brain somatic mutations, even with low VAFs, had refractory epilepsy such as LGS or IS, and were finally diagnosed with MOGHE. This report is the first in Korea to our knowledge.

Keywords: Malformations of cortical development; Drug resistant epilepsy; Lennox Gastaut syndrome

Introduction

Malformations of cortical development (MCDs) refer to a wide range of cortical lesions from disruption of neurogenesis, proliferation, apoptosis, or migration [1,2]. The concept of MCDs was introduced in pediatric patients with developmental delay and epilepsy. A classification of those malformations was proposed in 1996 [3]. This classification was revised and updated in 2012 including mild MCD (mMCD) or focal cortical dysplasia (FCD), which features a small proportion of abnormal brain cells and disorganized cortical lamination [4]. Furthermore, mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE), which is characterized by increased proliferation of oligodendroglia in the white matter and deep gray matter, was proposed as a new histopathologic entity in 2017 [5].

Ample evidence has established that FCD is associated with intractable epilepsy requiring surgical resection of the lesion [1,6]. Remarkable advances in genetic technologies and molecular biology have revealed that somatic mutations in brain cells are associated with FCD [7,8]. Moreover, a low-level brain somatic mutation burden—even with a variant allele frequency (VAF) less than 1%—is enough to cause intractable epilepsy [9,10]. Therefore, deep sequencing with a high read depth (more than 1,000×) is necessary to identify the causative gene variant [10,11]. Genes related to epilepsy have been identified, including *SLC35A2* and genes encoding the mechanistic target of rapamycin (mTOR) pathway proteins (*AKT3*, *DEPDC5*, *MTOR*, *PIK3CA*, *TSC1*, and *TSC2*) [8,10,11].

SLC35A2, which is located at Xp11.23, encodes a uridine diphosphate (UDP)-galactose transporter, a member of the nucleotide-sugar transporter family that transports galactose from the cytosol or nucleus into Golgi vesicles [9,12]. Germline loss of function variants in *SLC35A2* resulting in producing abnormal truncated glycans that lack galactose have been identified in multiple patients with epileptic encephalopathy [13,14]. In addition, brain mosaic mutations in *SLC35A2* are now considered one of the major genetic causes of intractable epilepsy, and recent studies have reported the histopathologic features of *SLC35A2* somatic variants [12].

In this study, we present the clinical characteristics of patients with *SLC35A2* somatic mutations in the brain who were finally diagnosed with MOGHE.

Materials and Methods

1. Selection of subjects

We retrospectively collected a series of patients with intractable pe-

diatric epilepsy who underwent epilepsy resection surgery and were confirmed to have *SLC35A2* brain somatic mutations from January 2013 to December 2019 at the Epilepsy Research Institute of Severance Children's Hospital. Ten cases were enrolled, including six patients (patient numbers 1, 2, 3, 4, 5, and 7) who were previously reported in 2018, three patients (patient numbers 8, 9, and 10) reported in 2019, and one patient (patient number 6) reported in 2021 [10,12,15]. We reviewed all data available for these 10 patients, which comprised their demographic characteristics, clinical features, pre-operative investigations, genetic profiles, operation details (including the pathologic analysis), and post-operative outcomes with medical records.

All patients were informed about the study and agreed to the collection of human tissues, and the protocols were approved by Severance Hospital and the KAIST Institutional Review Board and Committee on Human Research (IRB No. 4-2017-0119).

2. Pre-operative evaluations

The pre-operative investigations included neurologic examinations, development tests, and evaluations for determining the surgical area. The epileptogenic area was determined through the interpretation of long-term video-electroencephalogram (EEG)-monitoring data and confirmed by imaging studies including high-resolution magnetic resonance imaging (MRI), fluoro-deoxy-glucose (FDG) positron emission tomography (PET), and subtraction ictal single-photon emission computed tomography (SPECT) co-registered to MRI (SISCOM).

The development level was measured considering both cognitive level and social function, and it was classified as follows: normal (intelligence quotient/developmental quotient > 70), mild delay (50 to 70), moderate delay (35 to 49), severe delay (20 to 34), and profound delay (< 20). The cognitive level was assessed using the Korean Bayley Scales of Infant Development-II, the Korean Wechsler Preschool & Primary Scale of Intelligence-IV or the Korean Wechsler Intelligence Scale for Children-IV according to the patient's age. The social quotient (SQ), as an indicator of general adaptive function, was scored in all patients using the Korean version of the Social Maturity Scale (SMS) based on the Vineland Social Maturity Scale, fifth version.

3. Genetic profiles and pathologic analysis through brain samples

Surgery proceeded in two stages: inserting intracranial EEG and determining the surgical margin according to the protocol of the Epilepsy Research Institute, Severance Children's Hospital [10,16]. All brain samples were freshly frozen (FFZ) or formalin-fixed paraffin-embedded (FFPE).

For DNA extraction from brain tissue, we used QIAamp DNA Mini kits (Qiagen, Germantown, MD, USA) from FFZ brain samples and QIAamp DNA FFPE kits (Qiagen, Hilden, Germany) from FFPE, as described in the previous report [10]. Site-specific amplicon sequencing for genetic analysis of *SLC35A2* with read depth $> 100,000\times$ and region-specific primers with the Illumina Nextera single index (Illumina, San Diego, CA, USA) for validation sequencing for somatic mutations was performed, as previously described [10,15].

Histopathologic analyses were conducted twice; the first one was done at the time of surgery by the Department of Pathology at Severance Children's Hospital with the International League against Epilepsy (ILAE) classification [17]. Because MOGHE was first introduced in 2017 [5] and has not yet been included in the ILAE classification, a reanalysis was performed with hematoxylin and eosin staining, as well as anti-NeuN to look for heterotopic neurons (Clone A60, Millipore, Temecula, CA, USA) and anti-olig2 to identify oligodendroglial cells (clone JP18953, IBL International, Hamburg, Germany) immunostaining. The reanalysis was conducted by an expert neuropathologist without knowing the genetic information and previous pathologic results at Schoen Klinik, Vogtareuth, Germany in 2020 [12].

Results

We enrolled a total of 10 patients with intractable pediatric epilepsy confirmed to have *SLC35A2* brain somatic variants.

1. Demographics and clinical features

As shown in Table 1, six of the 10 patients were male (60%). All patients suffered from daily seizures and had refractory epilepsy with focal epileptic findings on EEG, making them candidates for epilepsy surgery. The mean age of seizure onset was 12.2 ± 12.1

months old (range from 3 months to 3 years 2 months of age). Seventy percent of patients were diagnosed with infantile spasms presenting with spasms, and six of those patients progressed to Lennox Gastaut syndrome (LGS) with various types of seizures, including focal impaired-awareness seizures, focal motor seizures, and myoclonic seizures. Thirty percent of cases had only LGS, of whom two (2/3) experienced generalized seizures with head drop and one (1/3) had focal impaired-awareness seizures and spasms.

The degree of developmental delay was determined through an analysis of the cognitive level and social function; 10% of patients had low average development, 50% showed mild delay, 10% had moderate delay, 10% presented severe delay, and 20% had profound delay. The average SQ was 45.7 ± 22.2 , corresponding to a moderate delay.

2. Genetic profiles

SLC35A2 brain somatic mutations were confirmed by site-specific amplicon sequencing (read depth $> 100,000\times$). Nonsense mutations were found in 50% of patients, missense mutations in 40%, and a splicing site mutation of 10%. The *SLC35A2* VAFs ranged from 1.4% to 22.9% (median VAF, 5.7% [range, 1.7% to 5.8%]), and patients were assigned numbers based on the VAFs in descending order. Patients 1, 2, and 3, who had high VAFs (more than 15%), all had nonsense mutations, while patients 8, 9, and 10, who had low VAFs (less than 5%), were all identified as having missense mutations (Table 1).

3. Pre-operative evaluations, operation details, and post-operative outcomes

As presented in Table 2, four patients (40%) underwent epilepsy surgery twice, three of whom (3/4) had resection surgery after localization of the lesion following corpus callosotomy. All these patients initially had negative findings on MRI, but localization by

Table 1. Demographics, clinical features, and genetic profiles

No.	Sex	Age of seizure onset	Diagnosis of epilepsy	Type of seizures	Development level	Genomic variant	Protein change	Variant type	Brain VAF (%)
1	M	3 mo	LGS from IS	FIAS, spasm	Mild delay	c.589C>T	p.Gln197Ter	Nonsense	22.9
2	M	3 yr 2 mo	LGS	FIAS, spasm	Low average	c.502C>T	p.Gln168Ter	Nonsense	18.0
3	M	3 mo	LGS from IS	FIAS, focal motor, spasm	Profound delay	c.760G>T	p.Glu254Ter	Nonsense	15.8
4	F	13 mo	LGS from IS	FIAS, spasm	Severe delay	c.703A>C	p.Asn235His	Missense	10.0
5	F	5 mo	LGS from IS	Myoclonic, spasm	Mild delay	c.553C>T	p.Gln185Ter	Nonsense	6.0
6	F	7 mo	LGS from IS	FIAS, spasm	Moderate delay	c.359_360del	p.Leu120HisfsTer7	Nonsense	5.5
7	M	6 mo	LGS from IS	Spasm	Mild delay	c.275-1G>T	-	Splicing site	5.0
8	M	2 yr 6 mo	LGS	GT, head drop	Mild delay	c.842G>A	p.Gly281Asp	Missense	3.7
9	F	11 mo	LGS	GT, head drop	Profound delay	c.671T>C	p.Leu224Pro	Missense	3.7
10	M	6 mo	IS	Spasm	Mild delay	c.359T>C	p.Leu120Pro	Missense	1.4

VAF, variant allelic frequency; LGS, Lennox Gastaut syndrome; IS, infantile spasms; FIAS, focal impaired-awareness seizure; GT, generalized tonic.

Table 2. Pre-operative evaluations, operation details, and post-operative outcome

No.	Age at last surgery	Surgery count	Scalp EEG	MRI findings	PET findings (decreased FDG uptake area)	Resection topology	Initial histology	Histology reanalysis	Follow-up period	Engel class	No. of last AED	Pre-operative SQ (age of test)	Post-operative SQ (age of test)
1	3 yr 5 mo	1	Rt. posterior quadrant	Normal	Rt. inferior frontal and temporal	Rt. frontal and Rt. posterior quadrant	Gliosis	MOGHE	4 yr 1 mo	1C	3	55.26 (3 yr 4 mo)	48.28 (7 yr 0 mo)
2	6 yr 3 mo	2 (after resection surgery)	Lt. temporal	Normal	Lt. parietal and superior temporal	Lt. temporo-occipital	Gliosis	MOGHE	2 yr 8 mo	1A	1	88.55 (3 yr 8 mo)	88.4 (7 yr 6 mo)
3	5 yr 2 mo	2 (after CC)	Lt. posterior quadrant, Lt. frontal	Normal	Lt. hemisphere	Lt. fronto-temporal, Lt. amygdalo-hippocampus and Lt. posterior quadrant	Gliosis	MOGHE	6 yr 3 mo	2B	4	11.8 (5 yr 0 mo)	11.85 (7 yr 2 mo)
4	5 yr 1 mo	2 (after CC)	Lt. parietal	Normal	Lt. fronto-parietal	Lt. temporal	mMCD	MOGHE	5 yr 9 mo	1A	0	36.8 (5 yr 0 mo)	34.28 (8 yr 1 mo)
5	4 yr 0 mo	1	Rt. frontal	FCD on Rt. frontal lobe	Rt. frontal, Rt. parietal and Lt. inferior frontal	Rt. fronto-temporal and Rt. amygdalo-hippocampus	Gliosis	MOGHE	2 yr 8 mo	1A	2	54.56 (3 yr 10 mo)	66.04 (4 yr 10 mo)
6	9 yr 0 mo	1	Rt. posterior quadrant	Normal	Rt. anterior temporal	Rt. temporal	mMCD	MOGHE	11 mo	2B	1	46.43 (8 yr 10 mo)	-
7	2 yr 8 mo	1	Rt. posterior quadrant	Slightly increased white matter T2 signal around Rt. temporo-parietal lobes	no official reading	Rt. posterior quadrant	Normal	MOGHE	2 yr 2 mo	1A	0	50.4 (2 yr 6 mo)	71.28 (3 yr 10 mo)
8	6 yr 1 mo	1	Lt. frontal	FCD on Lt. frontal lobe	Lt. medio-anterior frontal and inferior parietal	Lt. fronto-temporal	Gliosis	MOGHE	2 yr 9 mo	2A	2	50.36 (5 yr 10 mo)	55.79 (7 yr 2 mo)
9	10 yr 2 mo	2 (after CC)	Lt. temporal (main), Lt. frontal and Lt. occipital	Mild cerebellum volume decrease	Lt. superior frontal and medial parietal	Lt. frontal, Lt. posterior quadrant, amygdalo-hippocampus, splenium, corpus callosum, disconnected occipital and temporal	Gliosis	MOGHE	2 yr 6 mo	3A	3	12 (10 yr 1 mo)	-
10	2 yr 1 mo	1	Rt. fronto-temporal	Normal	Lt. superior frontal, lateral inferior parietal, and lateral temporal	Rt. frontal and Rt. temporal	Gliosis	MOGHE	4 yr 0 mo	3A	2	50.48 (2 yr 1 mo)	61.54 (3 yr 2 mo)

EEG, electroencephalography; MRI, magnetic resonance imaging; PET, positron emission tomography; FDG, fluoro-deoxy-glucose; AED, anti-epileptic drug; SQ, social quotient; Rt, right; MOGHE, mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy; Lt, left; CC, corpus callosotomy; mMCD, mild malformation of cortical development; FCD, focal cortical dysplasia.

EEG was possible after the first operation. The mean age at the final operation was 5.4 years (range, 2 years and 1 month–10 years and 2 months of age).

Long-term video-EEG monitoring findings by both interictal and ictal localization demonstrated multiple ictal onset zones in three patients (30%). Three cases (30%) had cortical abnormality findings from pre-surgical MRI. All of them had previous MRI findings characteristic of MOGHE [18]; patient 5 had FCD on the right frontal lobe, patient 7 had a slightly increased white matter T2 signal around the right temporo-parietal lobe, and patient 8 had FCD on the left frontal lobe. FDG-PET was performed in all patients, and the hypometabolic lesions were concordant with the EEG results in seven patients (70%), showing more extensive lesions. SPECT was performed in only five patients because the other patients had an insufficient seizure duration to carry out the tests. SISCOM was obtained in only two of these patients, and all results were consistent with EEG localization.

The initial histopathologic diagnosis at the time of surgery was gliosis in 70%, mMCD in 20%, and normal findings in 10%. However, after a pathologic reanalysis in Germany in 2020, all cases were confirmed as MOGHE, featuring increased olig-2 immunostained cells not only in the gray-white matter junction, but also in the deep white matter, and heterotopic neurons in the white matter as previously described [5,12].

The mean follow-up period was 3.4 years (range, 11 months to 6 years and 3 months), and patients took an average of 1.8 anti-seizure medications (range, 0 to 4 at the time of the last follow-up). Eighty percent of cases reached Engel class I–II with complete freedom from seizures or rare disabling seizures, while the 20% of cases with the lowest VAFs (patients 9 and 10) showed worthwhile seizure reduction of Engel class III according to the Engel Epilepsy Surgery Outcome Scale [19]. Post-operative developmental tests were performed for eight patients, but as their age changed after surgery, it was difficult to make simple comparisons using a different tool from the test performed in a pre-operative cognitive evaluation. Nonetheless, the SMS was administered to all patients before and after the operation, and four patients showed improvement by an average of 12.2 ± 6.4 points in the SQ level.

Discussion

In this study, we presented the clinical features of patients with *SLC35A2* brain somatic variants showing the pathologic characteristics of MOGHE. *SCL35A2* somatic variants have been reported in correlation with neuropathologic phenotypes with MOGHE [12]. The definition, classification, clinical phenotypes, MRI findings, pathology, and correlated genetic variants of MCD remain a

challenging topic, and updates in this field continue [20]. As the concept of MOGHE has been introduced relatively recently, its clinical importance is underestimated in practice [5].

In our study, most of the mutations in *SLC35A2* were nonsense mutations (50%), including one frameshift deletion, and this category included the three patients with the highest VAFs. Brain tissues with the p.Gln197Ter variant from patient 1 and the p.Glu254Ter variant from patient 3 showed aberrant patterns in terms of pathogenicity, and patient 2, with the p.Gln168Ter variant, showed changes in UDP-galactose transport as previously reported [13,15]. *SLC35A2* is involved in transporting UDP-galactose from the cytosol to the Golgi apparatus and completing glycosylation by attaching galactose to N-acetylglucosamine. Loss of function of *SLC35A2* can cause problems with N-glycosylation in this process. Abnormal N-glycosylation of brain development affects neural transmission, myelination, and neuronal migration, leading to clinical neurologic symptoms such as congenital disorders of glycosylation (CDG) [21], and the phenotype of MOGHE involves increased heterotopic neurons in the white matter and oligo-2 positive cell clusters in the white matter and gray-white matter junction. Ultimately, these findings appear as a blurred gray-white matter junction and hypomyelination on brain MRI. Most patients in our study presented normal MRI findings (70%), while three patients had increased T2 signal intensity on the lesion. The MRI findings of MOGHE are known to involve an increased laminar signal at the corticomedullary junction on T2 and fluid attenuated inversion recovery in subtype I in younger children or an increased signal of the adjacent white matter in subtype II in older children [18]. Therefore, somatic brain variants of *SLC35A2*, which likely occur in a neuroglial progenitor cell during neurogenesis, are thought to have potential as a genetic marker for MOGHE [12].

In this study, the three patients with the low VAFs (less than 5%) were all missense mutations, which means that a low mutation burden is sufficient to cause intractable epilepsy. Of particular note, patient 10 with a VAF of 1.4% and patient 9 with a VAF of 3.7% belonged to Engel class III, with only partially controlled seizures after epilepsy surgery. The variant burden in the brain may also govern aspects of the clinical presentation, but this mechanism is still unclear [9]. Further research is needed to determine whether the VAF in a fraction of cells in the blood is proportional to the VAF in brain somatic mutations and could be used as a factor to determine clinical severity.

Considering the reports that the symptoms of *SLC35A2*-CDG patients improved after taking galactose supplementation, which may partially increase cytosolic UDP-galactose and thereby facilitate galactosylation through alternative UDP-galactose transport into the Golgi [12,22], precision treatment can be considered to

control the symptoms of patients with *SLC35A2* somatic brain mutations who cannot undergo epilepsy surgery or continue to have seizures after surgery.

Conflicts of interest

Hoon-Chul Kang is an associate editor, Ara Ko, Se Hee Kim, Joon Soo Lee and Heung Dong Kim are the editorial board members of the journal, but They 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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Expanding the Clinical and Genetic Spectrum of Caveolinopathy in Korea

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Purpose: Caveolinopathy is a disease caused by caveolin-3 (CAV3) mutations that shows a wide clinical spectrum, including isolated hyperCKemia and limb-girdle muscular dystrophy. While recent advances in next-generation sequencing (NGS) have enabled earlier diagnosis of this disease, it remains difficult to predict the clinical course of each patient.

Methods: This study summarizes the clinical presentations of 13 genetically confirmed caveolinopathy patients in four Korean families. Genetic diagnosis was performed using NGS technologies for probands and Sanger sequencing for the other family members.

Results: Four coding mutations were found (p.Val103_Val104del, p.Asp28Glu, p.Pro105Leu, and p.Arg27Gln), and each family showed autosomal dominant inheritance. While all 13 cases had hyperCKemia, only five of them showed some myopathic features including ankle contracture, calf hypertrophy, exercise intolerance, and muscle cramping. This high proportion of asymptomatic cases suggests both that these mutations may be associated with a mild phenotype and that caveolinopathy may be an underdiagnosed disease.

Conclusion: This study extends our understanding of caveolinopathy; in particular, the findings suggest the need to consider caveolinopathy in patients with incidental findings of creatine kinase elevation. NGS may be a useful method in the differential diagnosis of such cases.

Keywords: Muscular dystrophy, limb-girdle, type 1C; Caveolin 3; High-throughput nucleotide sequencing; Muscular dystrophies, limb-girdle; Creatine kinase

Introduction

Caveolinopathy is a disease caused by alterations in caveolin-3 (CAV3), a muscle-specific protein encoded by CAV3 [1]. It has been designated as LGMD1C, a subtype of limb-girdle muscular dystrophy (LGMD), but its clinical presentations are highly heterogeneous, including rippling muscle disease (RMD), LGMD,

long QT syndrome, distal myopathy, and isolated hyperCKemia [2]. Although the prevalence of caveolinopathy is unclear, it seems evident that it is a fairly rare disease.

LGMD1C accounts for fewer than 5% of cases of LGMD, whose prevalence ranges from 1 in 14,500 to 1 in 123,000 according to ethnicity [3]. One study on an LGMD cohort in the USA reported only one CAV3-affected case out of 4,656 patients [4],

and there are currently around 50 mutations of *CAV3* listed in the Human Gene Mutation Database (HGMD) [5].

The progression of muscle weakness may be slow or rapid. The age of onset varies greatly from early childhood to late adulthood, and initial symptoms also differ among individuals [6-8]. In addition, the same mutations of *CAV3* may result in quite different clinical features, showing evidence of intrafamilial phenotypic variability [2,9]. These diverse clinical presentations of caveolinopathy have posed a diagnostic challenge. However, in recent decades, advances in next-generation sequencing (NGS) technology have increased the diagnostic rate of caveolinopathy and further broadened its clinical spectrum. In addition to NGS, conventional diagnostic methods, such as muscle pathology and immunohistochemical staining, can still be helpful in confirming the pathogenicity of variants [10].

To our knowledge, few studies have investigated caveolinopathy in East Asian populations [11,12]. Although a couple of Korean patients have been reported previously [10,13], no caveolinopathy case series has been published to date. Considering its rarity and heterogeneity, a broad range of studies is warranted among different ethnicities to elucidate the clinical and genetic spectrum of caveolinopathy. In this study, we summarized the clinical manifestations of 13 caveolinopathy cases in four Korean families, which

were diagnosed using NGS technology and reviewed through muscle pathology. We investigated whether there were any differences in clinical presentations according to the different types of mutations.

Materials and Methods

1. Subjects

The study was approved by the Institutional Review Board (IRB) of Seoul National University Hospital (IRB no. 2008-135-115), and written informed consent was obtained from all patients and/or their parents. In total, 13 patients with proven mutations were enrolled in this study. They belonged to four families, and the largest family included eight affected patients (Fig. 1). The probands visited the Neuromuscular Clinic of Seoul National University Children's Hospital (SNUCH) with symptoms or signs suspicious of myopathy such as hyperCKemia and a tiptoeing gait, and they were all genetically diagnosed with caveolinopathy with *CAV3* mutations. We found nine cases with *CAV3* mutations in relatives through mutation screening in their family members. We retrospectively reviewed their medical records, including laboratory, radiographic, and pathologic findings during follow-up, and compared them within and be-

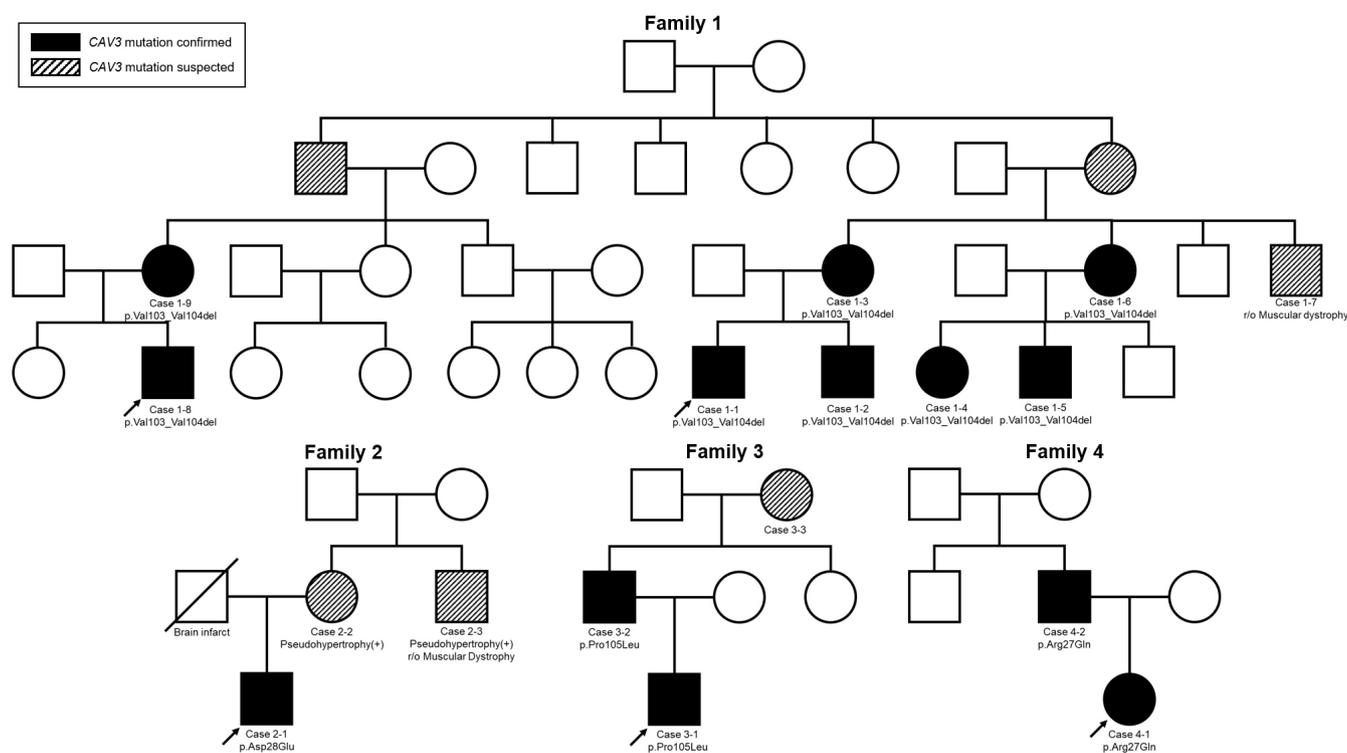


Fig. 1. Pedigrees of the families affected by caveolinopathy. Four independent families carried different caveolin-3 (*CAV3*) mutations, all inherited in an autosomal dominant manner.

tween families according to their mutations.

2. Mutation discovery

With the suspicion of isolated hyperCKemia or muscular dystrophy, we tried to find causal variants for each patient (Fig. 2). Single-gene analysis of the dystrophin (*DMD*) gene could not identify pathogenic variants, and the probands of each family were sequenced using different types of NGS technologies. Cases 1-1, 1-8, and 4-1 were sequenced through the LGMD panel of SNUCH. This panel is designed to include the exonic regions of 43 LGMD-related genes, using the Agilent SureSelectXT Custom kit (Agilent Technologies, Santa Clara, CA, USA). Case 2-1 was sequenced through whole-exome sequencing using the SureSelectXT2 Human All Exon v4+UTRs kit (Agilent Technologies) and HiSeq 2500 (Illumina, San Diego, CA, USA). The mutation in case 3-1 was found using a commercial NGS platform (Diagnostic Exome Sequencing, Green Cross Genome, Yongin, Korea).

The discovered variants were predicted to be “pathogenic” or “likely pathogenic” according to the guideline suggested by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology in 2015 [14]. HGMD was used to check whether the mutations had been previously reported.

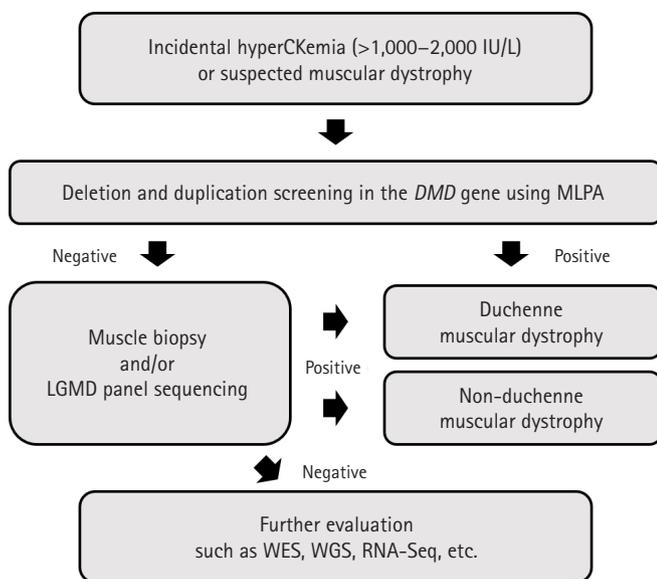


Fig. 2. Diagnostic approach to patients with incidental hyperCKemia or suspected muscular dystrophy. In general, we first apply multiplex ligation dependent probe amplification (MLPA) of dystrophin (*DMD*) to the patients. If negative, we conduct muscle biopsy or limb-girdle muscular dystrophy (LGMD) gene panel sequencing, sometimes both at the same time. Further studies such as whole-exome sequencing (WES), whole-genome sequencing (WGS), and RNA sequencing (RNA-Seq) are considered if the above studies fail to find causal mutations.

Public variant databases, such as the Genome Aggregation Database (gnomAD), were screened for their population allele frequencies [15]. Finally, the discovered mutations were confirmed in other relative cases using Sanger sequencing. The details of the sequencing process and variant analysis were described previously [16].

3. Immunohistochemistry

Muscle biopsy was conducted in cases 1-1, 1-8, 2-1, and 3-2. Muscle samples were taken from the quadriceps femoris or rectus femoris and frozen with isopentane cooled in liquid nitrogen. Serial frozen sections of 10 μ m were stained with a set of histochemical stains. Purified mouse anti-caveolin-3 antibody (BD Transduction Laboratories, Lexington, KY, USA) was used for immunohistochemical staining.

Results

1. Clinical presentations and comparison with causal mutations

The clinical and mutational characteristics of the study samples are summarized in Table 1. Each sample with a *CAV3* mutation had hyperCKemia regardless of their age, sex, and symptoms. Four *CAV3* mutations were detected in four different families using NGS technologies, inherited in an autosomal dominant manner. All the mutations were predicted to be pathogenic or likely pathogenic and previously reported in the HGMD. In addition, none of them was listed in the public variant database, gnomAD.

1) Family 1

All the cases genotyped in family 1 shared the same mutation, namely a heterozygous in-frame deletion of *CAV3* c. 307_312del-GTGGTG (p.Val103_Val104del). Case 1-1 visited the clinic after an incidental finding of elevated creatine kinase (CK) concentration (564 to 1,378 IU/L; reference range, 20 to 270 IU/L) at 13 years of age. He was previously healthy and achieved normal motor developmental milestones except for being a slow runner during early childhood. While showing Achilles tendon tightness and calf hypertrophy on physical examination, he had normal motor power until the last follow-up (at 17 years old).

Case 1-2 was a younger brother of case 1-1. Likewise, while he had hyperCKemia, ankle contractures, and muscle cramping during exercise at 8 years of age, calf hypertrophy was not observed, and his motor performance was almost normal. Case 1-5, a maternal cousin of case 1-1, had hyperCKemia and muscle cramping without ankle contracture at 9 years of age. In addition, a 39-year-old maternal uncle (case 1-7) had been clinically diag-

Table 1. Clinical and genetic information of the study subjects

	Sex	Initial symptom (yr)	Current age (yr)	CAV3 mutation	CK (IU/L)	Ankle contracture	Calf hypertrophy	Exercise intolerance	Tiptoeing	Muscle cramping	Other medical conditions
Case 1-1	M	9	17	p.Val103_Val104del	564–1,378	Yes	Yes	No	No	No	-
Case 1-2	M	8	12	p.Val103_Val104del	1,445–2,279	Yes	No	No	No	Yes	-
Case 1-3	F	-	46	p.Val103_Val104del	1,132	No	No	No	No	No	-
Case 1-4	F	-	15	p.Val103_Val104del	1,430	No	No	No	No	No	-
Case 1-5	M	8-9	13	p.Val103_Val104del	1,500–2,000	No	No	No	No	Yes	-
Case 1-6	F	-	42	p.Val103_Val104del	982	No	No	No	No	No	-
Case 1-8	M	3	18	p.Val103_Val104del	265–2,695	Yes	Yes	Yes, mild	No	No	Epilepsy
Case 1-9	F	-	45	p.Val103_Val104del	972	No	No	No	No	No	-
Case 2-1	M	4-5	21	p.Asp28Glu	1,286–1,886	Yes	Yes	Yes	Yes	Yes	Depression
Case 3-1	M	-	2	p.Pro105Leu	-	No	No	No	No	No	Birth asphyxia
Case 3-2	M	-	40	p.Pro105Leu	1,713–1,847	No	No	No	No	No	-
Case 4-1	F	-	6	p.Arg27Gln	320–527	No	No	No	No	No	-
Case 4-2	M	-	36	p.Arg27Gln	813	No	No	No	No	No	-

CAV3, caveolin-3; CK, creatine kinase.

nosed with muscular dystrophy and showed tiptoeing gait, although symptom progression was not definite. However, his DNA sample was not available and the presence of a mutation could not be confirmed. Other female family members (cases 1-3, 1-4, and 1-6) had no symptoms or signs, except for elevated CK levels.

Case 1-8 visited the clinic with an isolated hyperCKemia at 7 months of age, and calf hypertrophy and ankle contracture developed during follow-up. Despite showing normal development and tolerable motor performance, he started to have mild and intermittent exercise intolerance at the age of 16 years. Additionally, he started to show seizures at age 15 years, and there were four episodes of sudden collapse with loss of consciousness followed by generalized tonic-clonic seizures. He was treated with an antiepileptic medication (divalproex sodium) with a clinical diagnosis of idiopathic generalized epilepsy. Electroencephalography and 24-hour Holter monitoring showed no definite abnormal findings, and no further seizures were detected since the start of treatment. His mother, case 1-9, had isolated hyperCKemia without any motor symptoms or signs until the fourth decade.

2) Family 2

Case 2-1 had a missense mutation, CAV3 c.84C > A (p.Asp28Glu). He was the only mutation-proven patient with definite exercise intolerance and tiptoeing gait in this study. He started to show tiptoeing gait from 4 to 5 years old, and visited the clinic for a further evaluation at 16 years old. He had hyperCKemia, calf hypertrophy, exercise intolerance, and muscle cramping at that time, but his mo-

tor power was not definitely impaired. His mother (case 2-2) was shown to have isolated hyperCKemia (694 IU/L), and his maternal uncle (case 2-3), whose mutational status was unknown, also had similar muscle symptoms and was discharged early from military service due to the symptoms. Additionally, case 2-1 had major depressive disorder with suicidal ideation and was treated with antidepressants.

3) Family 3

The members of family 3 shared a CAV3 c.314C > T (p.Pro105Leu) mutation. Case 3-1 had a history of birth asphyxia and suspicious muscle hypotonia at birth and remained in a neonatal intensive care unit for 1 month. At that time, CK elevation was incidentally found and his father brought him to the clinic for further evaluation at 2 months of age. He had no abnormal symptoms or signs and started to control his head, and he showed a social smile at the initial visit. He showed normal development until the last follow-up (at 30 months of age), walking well without support. His father and grandmother (cases 3-2 and 3-3) had no definite exercise intolerance or other muscle symptoms, but their CK levels were elevated (1,713 to 1,847 and 1,386 IU/L, respectively).

4) Family 4

Case 4-1 inherited a missense variant, CAV3 c.80G > A (p.Arg27Gln), from Case 4-2. Case 4-1 visited the clinic due to hyperCKemia at 4 years of age (320 to 527 IU/L), which was incidentally found during treatment for Kawasaki disease at an outside hospital. CK levels were measured in both parents, and her father (case

4-2, 36 years old) was also shown to have CK elevation (813 IU/L). However, neither case 4-1 nor 4-2 had any muscle symptoms or signs at the time of the study.

We also performed electrocardiography (cases 1-1, 1-8, 2-1, and 3-2) and echocardiography (cases 1-1, 1-5, 1-8, and 2-1) for cardiac evaluation. The results showed no abnormal findings and normal heart function, indicating no definite cardiac muscle involvement at that time. Four patients (cases 1-1, 1-2, 1-5, and 2-1) underwent electromyography and nerve conduction studies, and the results were compatible with myopathy, showing increased insertional activity, short duration, reduced-to-complete interference pattern, and an early recruitment pattern.

2. Muscle pathology

We conducted muscle biopsy for four cases (cases 1-1, 1-8, 2-1, and 3-2). All findings were consistent with muscular dystrophy, showing several myopathic changes such as size variation, degeneration and regeneration of myofibers, and endomysial/perimysial fibrosis with fatty changes. Although case 3-2 was 39 years old at the time of biopsy, the oldest among them, his muscle pathology was the least severe, showing minimal size variation of myofibers and minimal endomysial fibrosis.

While immunohistochemical staining for dystrophin 1, 2, and 3 showed no definite abnormalities, the signal activities of dysferlin were weakly positive and negative in cases 1-1 and 2-1, respectively. Therefore, those cases were initially suspected of having dysferlinopathy before genetic diagnosis. We also performed immunohistochemical staining using antibody to caveolin,

which revealed a definitively low expression compared with normal controls (Fig. 3).

Discussion

This study reported 13 caveolinopathy cases in four Korean families. We found four *CAV3* mutations and summarized their clinical presentations in association with their causal mutations. To our knowledge, this is one of the largest clinical studies on caveolinopathy, especially in an East Asian population. Although all mutations were previously reported and listed in HGMD, the clinical features differed from each other even when the same mutations were involved.

Except for case 2-1, who visited for a further evaluation of tiptoeing gait, each patient visited the clinic with a chief complaint of hyperCKemia, and muscle symptoms were mostly mild or absent during follow-up. Although caveolinopathy patients can have diverse clinical presentations, including LGMD, RMD, and long QT syndrome [2], the mutations in our cases (p.Val103_Val104del, p.Pro105Leu, and p.Arg27Gln) might be more associated with mild types of caveolinopathy. The p.Asp28Glu mutation of family 2 was previously reported in a large German family with RMD and LGMD [9]. That study showed that the affected patients did not have muscle weakness, except for ankle dorsiflexion, until their late 30s. Even when the disease progressed to muscle weakness, the study patients had muscle power of no less than grade 4 until the sixth decade of life. The p.Pro105Leu missense mutation of family 3 was also previously found in two Italian families with LGMD [17]. In that study, while disease onset usually occurred at 5 years of age, patients showed normal motor development, mild-to-moderate proximal muscle weakness, and slow disease progression. Likewise, case 3-3, the grandmother of case 3-1, had no definite exercise intolerance at the time of the study, and the muscle biopsy of case 3-2 showed relatively favorable pathology compared with that of the other mutations. Regarding the p.Arg27Gln mutation, there is one case report in a woman who had no neuromuscular symptoms until the age of 70 years [18].

Case 2-1, with the p.Asp28Glu mutation, had more severe symptoms than the other cases in this study; we assume that the difference in mutation position resulted in different clinical features. However, it is early to draw clear conclusions because of the small sample size and short follow-up period. In addition, considering the intrafamilial phenotypic variability of caveolinopathy [2,9], it is difficult to predict the clinical course of each patient according to the type of causal mutation.

Some patients suffered from neuropsychiatric disorders: case 1-8 with epilepsy and case 2-1 with depression. Although the re-

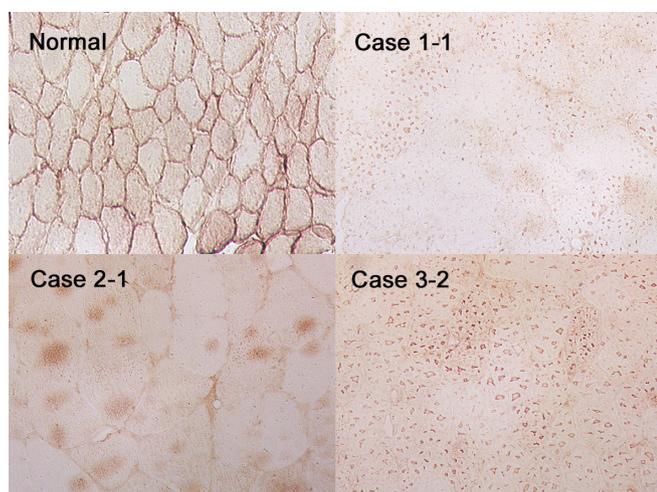


Fig. 3. Immunohistochemical staining of caveolin-3 in muscle biopsies. Sarcolemmal labeling of caveolin-3 (1:1,000 dilution) showed reduced staining in all three unrelated patients (Case 1-1, Case 2-1, and Case 3-2) compared with normal controls.

relationship between *CAV3* and neuropsychiatric disorders has not been well established, one recent study found an association between *CAV3* variants and epilepsy [19]. This group also reported that *CAV3* mutations could alter the current of hyperpolarization-activated cyclic nucleotide-gated channels and may develop cardiac arrhythmias [20]. However, given the limited available data and the absence of additional genetic or molecular studies conducted for these patients, more evidence is needed to support any association between *CAV3* and neuropsychiatric disorders.

Interestingly, in this study, the cases with myopathic features, such as ankle contracture, muscle cramping, exercise intolerance, and tiptoeing gait, were all males, and we speculate that male caveolinopathy patients might have earlier onset and more severe symptoms compared with females. The p.Val103_Val104del mutation has also been reported in a male patient with RMD who showed muscle weakness and atrophy with symptom onset at 17 to 18 years of age [13]. While his mother and elder sister had the same mutations, they had no definite muscle weakness or atrophy. This pattern agrees with our findings showing more severe clinical features in male patients. However, further studies are required to confirm this difference by sex, which might originate from other genetic or environmental factors such as differences in the level of physical activity.

Two patients, cases 1-1 and 2-1, were initially suspected of having dysferlinopathy because of decreased signal activities of dysferlin on immunohistochemical staining. One study noted that two sarcolemmal proteins, dysferlin and *CAV3*, interact with each other in skeletal muscle, and *CAV3* mutations can result in the deterioration of dysferlin [21]. In contrast, dysferlin impairment may also result in decreased *CAV3* expression [22].

We previously reported the p.Val103_Val104del mutation of cases 1-8 and suggested its ethnic specificity and the possibility of asymptomatic or minimally affected carriers in the Korean population [10]. Since none of the four mutations in this study were listed in either gnomAD or our in-house database of around 1,000 Korean genomes, further sequencing is required to reveal the allele frequencies of these rare variants.

In conclusion, this study showed the clinical spectrum of caveolinopathy in patients with four different mutations. Because all the patients in this study had mild myopathic features, caveolinopathy needs to be considered in patients with no or mild muscle symptoms with hyperCKemia. However, considering the phenotypic variability, each patient needs to be checked regularly regardless of the presence and severity of clinical symptoms. Other genetic and environmental factors may determine the clinical presentation of caveolinopathy in addition to *CAV3*

mutations, and further research is needed to elucidate the genotype–phenotype correlation in more detail and predict the clinical course of each patient.

Conflicts of interest

Ki Joong Kim, Jong-Hee Chae and Anna Cho are the editorial board members of the journal, but They 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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Frontal Lobe Epilepsy in a Pediatric Population: Characterization of Clinical Manifestations and Semiology

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Purpose: Frontal lobe epilepsy (FLE) has various clinical presentations depending on the anatomy involved. Seizures are brief and can mimic psychiatric conditions, and patients often cannot describe the aura. Therefore, it is difficult to characterize the semiology, especially in pediatric patients. This study investigated the characteristics of pediatric FLE.

Methods: We retrospectively reviewed the data of pediatric patients with FLE who underwent long-term video-electroencephalography (EEG) monitoring between January 2010 and June 2020. Patients' demographic data, seizure-related clinical presentations, semiology, brain magnetic resonance imaging (MRI), and EEG data were analyzed.

Results: Fifty-six patients were included (31 males, 25 females). The age of seizure onset varied from 1 month to 14 years (mean±standard deviation, 6.1±4.4 years). Seizures were classified into nine categories, including focal tonic (30/56), aura (22/56), hypermotor (17/56), focal clonic (15/56), versive (13/56), and bilateral asymmetric tonic (4/56). Seventeen patients (30.4%) had abnormal MRI results, including focal cortical dysplasia, heterotopic gray matter, and neuroepithelial tumors. Ictal EEG changes were commonly observed in the dorsolateral premotor and central cortices. In focal tonic seizures, EEG changes often originated in the premotor cortex. The location of the lesions on MRI and EEG coincided in six cases.

Conclusion: In pediatric FLE, various seizure types occur depending on the ictal anatomic origin, and individual patients had multiple semiologies. Brain MRI was normal in two-thirds of patients, and interictal EEG did not reveal epileptiform discharges in approximately 25%. Semiology reported on the basis of home videos and interictal EEG will help localize the ictal onset zone.

Keywords: Epilepsy, frontal lobe; Seizures; Electroencephalography; Magnetic resonance imaging

Introduction

Frontal lobe epilepsy (FLE) has a difficult-to-understand and complex seizure semiology, which depends on the location of the epileptogenic area [1-6]. The frontal lobe is large and has several combined functions [1,3]. The epileptic propagation of FLE is complex and rapid; therefore, it is often difficult to predict the localiza-

tion of ictal onset in clinical practice [4-8].

Several previous studies involving adult patients have described the semiological seizure characteristics of FLE as follows: very short duration, sudden onset and offset, stereotypic form, a tendency for clustering, nocturnal disposition, and rapid secondary generalization [2,9-15]. Because the frontal lobe is involved in not only motor function, but also in language and emotional control,

the ictal appearance of FLE can present as psychiatric symptoms such as fearful feelings or paranoid delusions [1,7,16]. FLE is often misdiagnosed as pseudoseizures related to psychiatric conditions [7,9,16-20].

The few studies focusing on FLE in the pediatric population have revealed that frontal lobe seizures in children differ from those of adults [1,2,9,21-23]. Therefore, the semiological characteristics of pediatric FLE require definition using ictal data, which could provide much-needed information for clinicians in terms of diagnosis and treatment. The aim of this study was to identify the semiological characteristics of FLE in pediatric patients using long-term video electroencephalography (EEG) data.

Materials and Methods

1. Patients

We retrospectively reviewed the medical records of pediatric patients admitted to the Samsung Medical Center, Seoul between January 2010 and June 2020. During that period, 71 patients were diagnosed with FLE. Among them, patients with ictal events were included as subjects of this study. The inclusion criteria were as follows: (1) patients who underwent long-term video EEG monitoring; (2) patients under 18 years of age at the time of the long-term video EEG recordings; (3) patients who had clinical seizures during monitoring; and (4) patients with a final diagnosis of FLE. Autosomal dominant nocturnal FLE and genetic FLE were not clinically suspected in any patients. No patients underwent genetic studies, such as a next-generation sequencing-based gene panel study.

2. Data collection

Data on clinical variables, such as demographics, age of onset, seizure semiology, EEG, brain magnetic resonance imaging (MRI) findings, and anti-seizure medications used were collected. The seizure types were described using the semiological seizure classification proposed by Luders et al. [24] and Dugan et al. [25]. When describing patient seizure types, we included both the medical history and the seizure semiology confirmed during long-term video EEG monitoring at the time of admission.

In this study, we summarized the characteristics, including demographics, medical history, EEG, and neuroimaging findings, of pediatric FLE patients who underwent video EEG monitoring, with a focus on describing the seizure semiology. Furthermore, we analyzed the clinical manifestations of 10 patients with psychic auras to identify any common aspects.

3. Ethics

This study was approved by the Institutional Review Board of Samsung Medical Center (IRB file no. 2022-04-183). The requirement for informed consent from patients was waived because of the retrospective nature of this study.

4. Localization of EEG

We classified the frontal lobes into the dorsolateral, mesial, and basal frontal lobes based on the anatomical locations, and the dorsolateral cortex was subdivided into the prefrontal, premotor, and central cortices. When electrodes were attached according to the international 10 to 20 system, the corresponding position of each electrode was checked. Based on this criterion, the Fp1 and Fp2 electrodes were classified as corresponding to the prefrontal cortex, the F3 and F4 electrodes as being placed on the premotor cortex, the C3 and C4 electrodes as corresponding to the central cortex, and the Fz and Cz electrodes as being placed on the mesial frontal cortex.

Results

1. Clinical manifestations

In total, 56 patients (25 females, 31 males) met the inclusion criteria. The age of onset ranged from 1 month to 14 years (mean, 6.1 ± 4.4 years) (Table 1). Seventeen patients (30.4%) had a medical history of neurological conditions such as febrile seizures (5/56, 8.9%), developmental delay (4/56, 7.1%), intellectual disability (4/56, 7.1%), encephalitis (2/56, 3.6%), and cerebral hemorrhage (2/56, 3.6%). The number of patients who underwent monotherapy and polytherapy was 12 (21.4%) and 34 (60.7%), respectively.

2. Seizure semiology features

Based on patients' medical records and ictal events during the long-term video EEG monitoring, we categorized seizures into nine categories according to the semiological seizure classification: focal clonic, focal tonic, asymmetric tonic, hypermotor, versive, dialeptic, automotor, generalized tonic clonic, and aura (Table 2). Auras were further classified as somatosensory, psychic, autonomic, visual, abdominal, or vertiginous. If there was more than one semiology in a single patient, each seizure type was counted. The 56 patients showed 102 seizure types in nine categories. Simple motor seizure (specifically, focal tonic seizure) was the most common type (45/56, 80.4%), followed by aura (21/56, 37.5%). Nine patients had somatosensory auras (9/56, 16.1%), and nine had psychic auras (9/56, 16.1%).

Table 1. Demographic characteristics (n=56)

Clinical characteristic	Value
Sex	
Male	31 (55.4)
Female	25 (44.6)
Age of onset (yr)	6.1 ± 4.4 (0.08–14)
Past medical history	
No	39 (69.6)
Yes	17 (30.4)
Febrile seizure	5 (8.9)
Developmental delay	4 (7.1)
Intellectual disability	4 (7.1)
Encephalitis ^a	2 (3.6)
Cerebral hemorrhage	2 (3.6)
Family history	
No	51 (91.1)
Yes ^b	5 (8.9)
Epilepsy	3 (5.4)
Febrile seizure	2 (3.6)
No. of anti-seizure medications	
1	12 (21.4)
2	13 (23.2)
3	12 (21.4)
4	6 (10.7)
5	1 (1.8)
6	2 (3.6)
None	10 (17.9)

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

^aThe patient was diagnosed with encephalitis based on clinical features such as altered mentality and seizure, and no pathogen was detected;

^bOf the three patients with a family history of epilepsy, two had a sibling and one had a father with epilepsy. The fathers of two patients had febrile seizures.

3. EEG

Interictal EEG was normal in 10 patients (17.9%) (Table 2). Twenty patients had interictal epileptiform discharges with normal background activity (35.7%); three patients had only non-epileptiform discharges, such as regional slow waves or generalized slow waves (5.4%) without any epileptiform discharges. Both epileptiform and non-epileptiform discharges were observed in 23 patients (41.0%). The ictal rhythm showed various forms such as repetitive spikes or sharp waves, fast activity, or rhythmic alpha or theta activity. The ictal onset zones were the left, right, both, or midline frontal or central areas.

4. Neuroimaging

Thirty-nine patients (69.6%) had normal structural brain MRI findings. Abnormal structural lesions were observed in 17 patients (30.4%) (Table 2). Focal cortical dysplasia was detected in five pa-

Table 2. Semiology, EEG, and brain MRI features of the 56 patients diagnosed with FLE

Variable	Value
Seizure semiology	102 (44.1)
Simple motor seizure	45 (44.1)
Focal tonic	28 (27.5)
Focal clonic	13 (12.7)
Bilateral asymmetric tonic	4 (3.9)
Aura	21 (20.6)
Somatosensory	9 (8.8)
Psychic	9 (8.8)
Autonomic	3 (2.9)
Visual	3 (2.9)
Abdominal	1 (1.0)
Vertiginous	1 (1.0)
Hypermotor seizure	17 (16.7)
Versive seizure	12 (11.8)
Focal seizure with impaired awareness	4 (3.9)
Generalized tonic clonic seizure	2 (2.0)
Automotor seizure	1 (1.0)
Interictal EEG	56 (100)
Normal	10 (17.9)
Non-epileptiform discharges ^a	30 (53.6)
Epileptiform discharges	43 (76.8)
Brain MRI findings	56 (100)
Normal	39 (69.6)
Abnormal	17 (30.4)
Focal cortical dysplasia	5 (29.4)
Heterotopic gray matter	3 (17.6)
Neuroepithelial tumor	2 (11.8)
Tuberous sclerosis	1 (5.9)
Hypoxic ischemic encephalopathy	1 (5.9)
Tissue defect due to previous epilepsy surgery	1 (5.9)
Porencephalic cyst	1 (5.9)
Hypothalamic hamartoma	1 (5.9)
Cortical gliosis	1 (5.9)
Neonatal intracranial hemorrhage	1 (5.9)

Values are presented as number (%).

EEG, electroencephalography; MRI, magnetic resonance imaging; FLE, frontal lobe epilepsy.

^aBackground slow or diffuse or regional slow waves.

tients (5/17, 29.4%) (Fig. 1). Other abnormal brain MRI findings were heterotopic gray matter (n = 3, 17.6%), neuroepithelial tumor (n = 2, 11.8%), tuberous sclerosis complex (n = 1), hypoxic ischemic encephalopathy (n = 1), tissue defect due to previous epilepsy surgery (n = 1), porencephalic cyst (n = 1), hypothalamic hamartoma (n = 1), cortical gliosis (n = 1), and neonatal intracranial hemorrhage (n = 1).

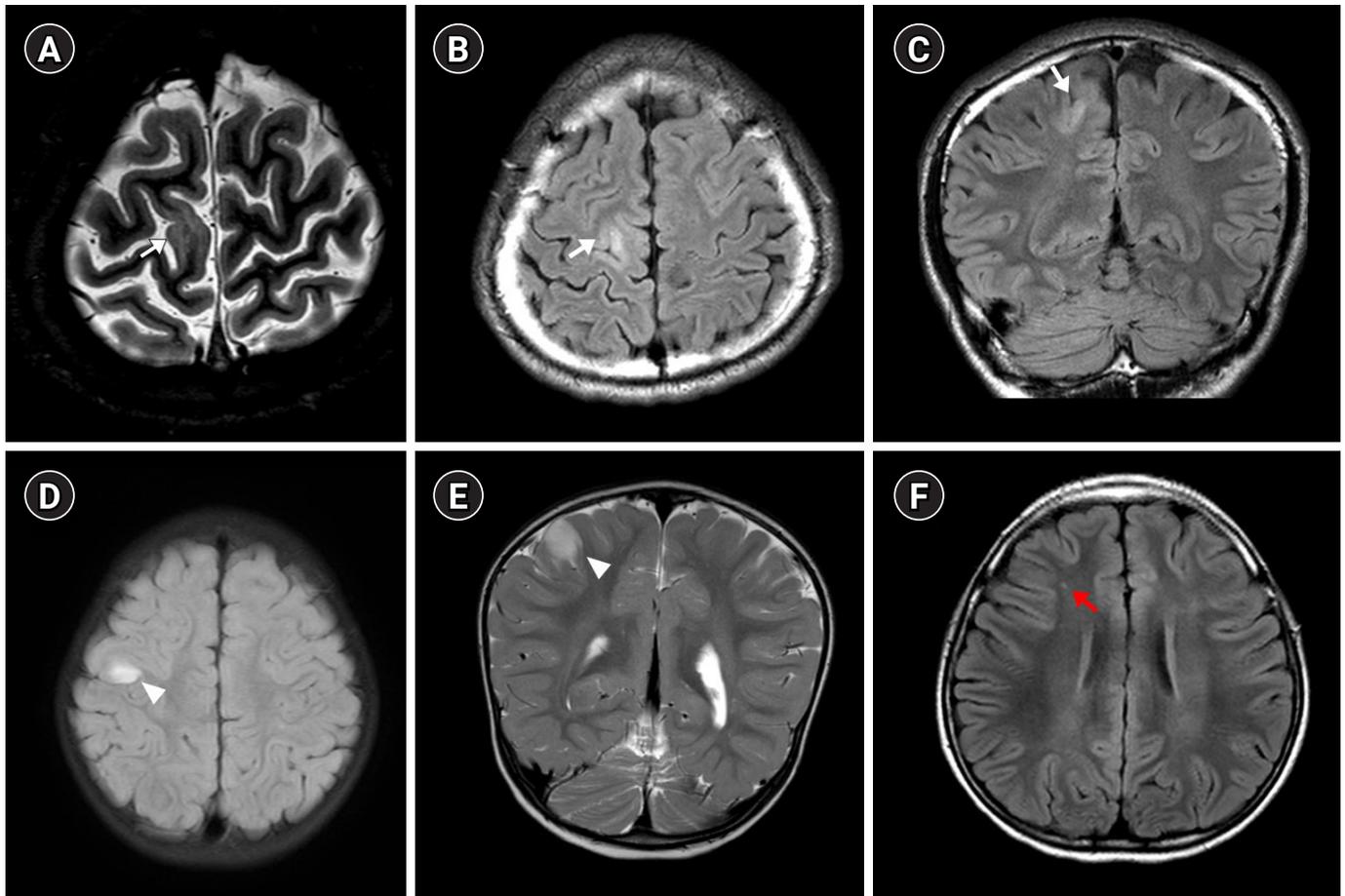


Fig. 1. Axial T2-weighted (A) and fluid attenuated inversion recovery (FLAIR) (B) images and a coronal FLAIR (C) image demonstrate high signal intensity (white arrow) in the right frontal lobe, suggesting focal cortical dysplasia. Axial FLAIR (D) and coronal T2-weighted (E) images show high signal intensity (white arrowheads) in the right precentral gyrus, suggesting a neuroepithelial tumor. An axial FLAIR image (F) shows high signal intensity (red arrow) in the right frontal lobe, suggesting cortical gliosis.

5. Analysis of seizure semiology

1) Seizure frequency and types

Based on the medical records, 39 patients had daily seizures, nine had weekly seizures, and eighth had monthly seizures. The average period of hospitalization for the 56 patients was approximately 2.6 days. The patients experienced 102 types of seizures in nine categories during hospitalization, and a total of 641 seizures were observed. About 1.8 types of seizures were observed per patient, and an average of 4.3 seizures were observed per day. Ten patients had nocturnal seizures, and none had clustering seizures.

2) Ictal onset zone and seizure semiology

Considering the division of the frontal lobe into the dorsolateral, mesial, and basal frontal lobes and further the subdivision of the dorsolateral cortex into the prefrontal, premotor, and central cortices, we classified the ictal onset zone observed for each patient

(Table 3). The dorsolateral cortex was the most common (28/56, 50.0%) onset zone (premotor cortex: 19/56, 33.9%; central cortex: 4/56, 7.1%; and prefrontal cortex: 2/56, 3.6%). Eleven patients (19.6%) had seizures originating in the mesial frontal lobe. Six patients (10.7%) had diffuse frontal lobe seizure origins (in the dorsolateral and mesial frontal lobes).

The ictal onset zones of the 102 types of seizures were also analyzed based on the four types of semiology commonly observed based on ictal EEG (Table 4). Focal tonic seizures commonly originated in the premotor cortex (6/28 patients). In addition, mesial frontal cortex (5/28) and diffuse/non-localized onset (4/28) were frequently observed. When focal clonic seizures occurred ($n = 13$), the EEG changes were diffuse/non-localized in six patients and localized to the premotor area in three patients. Hypermotor seizures ($n = 17$) were accompanied by EEG changes in the prefrontal cortex (4/17) and premotor cortex (3/17). Although auras revealed no EEG changes in most cases (8/21), psychic auras often showed

EEG changes. The ictal onset zone for psychic auras was the prefrontal cortex in one case, the premotor cortex in two cases, the mesial frontal lobe in three cases, and an undetermined location in one case; there was no change in two cases.

3) Correlation between anatomic abnormalities and the ictal onset zone

In the analysis of the ictal onset zones of the 17 patients with anatomical abnormalities on brain MRI, the most common localization of onset was observed to be in the dorsolateral frontal lobe (8/17) (Table 5). The ictal onset zone was in the mesial frontal

lobe (n = 2), diffuse in the frontal lobe (n = 1), and diffuse/non-localized (n = 1). Three cases showed no EEG changes, and in two cases, the ictal onset zone could not be determined (diffuse background attenuation). The location of the lesion on brain MRI coincided with the ictal onset zone on EEG in six cases. The lesions were focal cortical dysplasia in two cases, neuroepithelial tumor in two cases, heterotopic gray matter in one case, and tissue defect due to previous epilepsy surgery in one case. In the six patients with concordance between the location of the anatomic lesion on MRI and the EEG changes, the ictal onset zone was the dorsolateral frontal lobe in five cases and the mesial frontal lobe in one case.

Table 3. Anatomic location of the representative ictal onset zone for each patient based on EEG changes

Location	Value
Dorsolateral	28 (50.0)
Prefrontal	2 (3.6)
Premotor	19 (33.9)
Central	4 (7.1)
Prefrontal and premotor	1 (1.8)
Premotor and central	2 (3.6)
Mesial frontal	11 (19.6)
Diffuse frontal	6 (10.7)
Generalized ^a	3 (5.4)
Multifocal	1 (1.8)
Undetermined ^b	3 (5.4)
Unknown ^c	4 (7.1)

Values are presented as number (%).

EEG, electroencephalography.

^aThere were ictal EEG patterns such as generalized repetitive spikes or sharp waves or generalized spike and slow waves; ^bBackground attenuation/suppression or no EEG changes; ^cArtifact-obscured EEG.

4) Patients with psychic auras

There were 10 patients with psychic auras (Table 6). Nine of these patients showed psychic auras during long-term video EEG monitoring. One patient did not have this type of seizure during hospitalization, but had them habitually otherwise (listed as patient number 10 in Table 3). Patients complained of fears such as a feeling of being squeezed in the throat, being somewhere else, being sucked into something, or everyone disappearing. EEG changes were observed in eight of the nine patients, mainly in the frontal or central areas, when they had a psychic aura. Only one patient showed no EEG changes. Patients with psychic auras had several types of motor seizures and other types of auras as well, such as autonomic or visual auras. One patient (number 6) showed a structural abnormality on brain MRI (heterotopic gray matter in the right periventricular white matter) (Fig. 2). In this patient, ictal changes on EEG were observed in the midline frontocentral areas.

Table 4. Anatomic location of the ictal onset zone based on electroencephalography in four commonly observed types of semiology

Variable	Focal tonic (n = 28)	Focal clonic (n = 13)	Hypermotor (n = 17)	Aura (n = 21)
Dorsolateral				
Prefrontal	2		4	1
Premotor	6	3	3	2
Central	3	1	1	2
Mesial frontal	5	1	2	4
Diffuse frontal		1	1	1
Hemisphere	1			
Generalized ^a	4	6	3	
Multifocal				
Undetermined ^b	3		2	8
Unknown ^c	2		1	3
Others except frontal lobe	2	1		

^aThere were ictal electroencephalography (EEG) patterns such as generalized repetitive spikes or sharp waves or generalized spike and slow waves;

^bBackground attenuation/suppression or no EEG changes; ^cArtifact-obscured EEG.

Table 5. Concordance between the location of the ictal onset zone based on EEG and MRI lesions in 17 patients with structural abnormalities

Patient	Sex	Age (yr)	Ictal onset zone in EEG	MRI findings		Concordance ^a
				Etiology	Location of the lesion	
1	F	3	Dorsolateral	HGM	Lt. postcentral gyrus	Yes
2	F	9	Diffuse frontal	Cortical gliosis	Lt. basal ganglia	No
3	M	15	Dorsolateral	FCD	Rt. superior frontal gyrus	Yes
4	M	5	Dorsolateral	HIE	Basal ganglia, thalamus, multiple white matter	No
5	M	15	General	Neonatal ICH	Unknown (no image)	No
6	M	16	Dorsolateral	FCD	Rt. postcentral gyrus	Yes
7	F	12	Dorsolateral	TSC	Sporadic	No
8	M	16	Undetermined	Porencephalic cyst	Rt. parietal lobe	No
9	F	14	Dorsolateral	Tissue defect ^b	Lt. superior frontal gyrus	Yes
10	F	10	Mesial	HGM	Rt. postcentral gyrus	No
11	M	19	Undetermined	FCD	Lt. superior frontal gyrus	No
12	M	8	Dorsolateral	FCD	Rt. basal frontal lobe	No
13	M	17	Unknown	FCD	Rt. middle frontal gyrus	No
14	M	1	Dorsolateral	Neuroepithelial tumor	Rt. precentral gyrus	Yes
15	F	10	Mesial	Neuroepithelial tumor	Lt. paracentral gyrus	Yes
16	F	11	Unknown	HGM	Rt. middle frontal gyrus	No
17	F	2	Unknown	HH	Hypothalamus	No

EEG, electroencephalography; MRI, magnetic resonance imaging; HGM, heterotopic gray matter; Lt., left; FCD, focal cortical dysplasia; Rt., right; HIE, hypoxic ischemic encephalopathy; ICH, intracranial hemorrhage; TSC, tuberous sclerosis; HH, hypothalamic hamartoma.

^aConcordance between the ictal onset zone and the location of the MRI lesion; ^bTissue defect due to previous epilepsy surgery.

Discussion

This retrospective study describes the characteristics of pediatric patients with FLE based on an analysis of their actual seizures using long-term video EEG monitoring. The seizure semiology observed was diverse, and included simple motor seizures, hypermotor seizures, and auras. Only 17.5% (10/57) of patients had nocturnal seizures and no patient had clustering seizures in long-term video EEG monitoring, although many of them had nocturnal and clustering seizures in their history. The dorsolateral cortex was the most common ictal onset zone (28/56, 50.0%; premotor cortex: 19/56, 33.9%; central cortex: 4/56, 7.1%; prefrontal cortex: 2/56, 3.6%). Focal tonic seizures often began with EEG changes in the premotor or mesial frontal cortices. The lesion location on brain MRI matched the ictal onset zone on EEG in six patients.

In this study, we classified semiology into nine categories. Focal tonic seizures (50.0%), psychic auras (16.1%), and hypermotor seizures (30.4%) were the most frequent categories. Interictal EEG was mostly normal, and was well-localized in 80.4% (45/56) and poorly localized in 19.6% (11/56) of patients (Table 3). In a previous study that included 22 pediatric patients with FLE, the semiology was as follows: focal tonic, focal clonic, hypermotor, versive, dialeptic, and psychic aura [1]. They reported that frontal lobe sei-

zures were frequent and stereotypic and had rapid onset and offset. In their study, semiology was classified into six categories, among which hypermotor seizures (20/22) and psychic auras (15/22) were the most common. However, in our study, where semiology was classified into nine categories, simple motor seizures were the most common type (45/56, 80.4%), and hypermotor seizures were the third most common type (17/56, 30.4%). Auras were the second most common type (21/56, 37.5%), and psychic auras were found in only nine patients (16.1%). Compared with previous studies, this study was conducted with a larger number of patients. It is significant in that it analyzed all the ictal onset zones of 641 ictal events and correlated them with the semiology.

In our study, MRI revealed that 30.4% of patients (17/56) had various abnormalities, including focal cortical dysplasia (n = 5), heterotopic gray matter (n = 3), and neuroepithelial tumor (n = 2). The abnormal findings of brain MRI in our study were different from those found in adult FLE patients. In a previous study including 36 adult patients, encephalomalacia (8/17, 47.1%) was the most common etiology, followed by traumatic brain injury, post-operative changes, malformation of cortical development (6/17, 35.3%), and vascular malformations (2/17, 11.8%) [9]. In a pediatric study, 14.3% to 100.0% of patients had focal cortical dysplasia [1,2], whereas in our study, only 8.9% (5/56) had focal cortical

Table 6. Clinical features, EEG findings, neuroimaging, and ASM history of 10 patients presenting with psychic auras (patient 10 did not show psychic auras during hospitalization)

Patient	Sex	Age at video recording (yr)	Age at onset of seizure (yr)	Past medical history	Family history	Other sz semiology		Aura	Interictal	Scalp EEG		MRI	ASM
						Motor sz	Other sz			Psychic aura	Ictal onset zone		
1	F	16	5	N	N	Hypermotor sz	N	Autonomic	Normal	Bi-Fr	Bi-Fr, gen	Normal	CBZ
2	F	14	5	N	N	Rt. facial tonic, hemi-clonic, frontal absence, and versive sz	N	Autonomic	Lt. Fr or hemi	Lt. Fr	No ictal events	Normal	OXC
3	M	8	8	N	N	Rt. arm tonic sz	N	N	Rt. or both Fr	Lt. Fr	No ictal events	Normal	CLN, LEV
4	M	17	13	Subependymal hemorrhage and neonatal seizure	N	None	N	N	Normal	Midline or bi Fr, Lt. Fr, or gen	No ictal events	Normal	LMT, OXC
5	M	1	1.3	Febrile seizure	N	GTC sz	N	N	Lt., Rt., or midline C	Lt. C	Lt. or midline C	Normal	DPH, LEV, PB
6	F	10	4	N	N	Lt. arm tonic, and versive sz	N	N	Midline FrC	Midline FrC	Midline FrC	Heterotopic gray matter in the Rt. periventricular white matter	CBZ, LEV, TPM, VPA
7	M	8	7	N	N	GTC sz	N	Visual	Lt. C, Rt. CT, or midline P	No EEG change	Rt. C, gen	Normal	OXC
8	M	5	3	N	N	Hypermotor, and versive sz	N	Visual	Lt. and/or Rt. or midline Fr	Midline Fr or FrC	Midline Fr or FrC	Normal	OXC
9	M	18	1	N	N	None	N	N	Normal	Gen	No ictal events	Normal	CBZ, CLB
10	M	17	13	N	N	Rt. facial, arm, leg, or hemi tonic or clonic, Lt. hemi tonic, and versive sz	N	N	Normal	No ictal events	Lt. Fr	Normal	CLB, LMT, TPM, VPA

EEG, electroencephalography; ASM, anti-seizure medication; sz, seizure; MRI, magnetic resonance imaging; F, female; N, none; bi, bilateral; Fr, frontal; gen, generalized; CBZ, carbamazepine; Rt., right; Lt., left; OXC, oxcarbazepine; M, male; CLN, clonazepam; LEV, levetiracetam; T, temporal; LMT, lamotrigine; GTC, generalized tonic-clonic; C, central; DPH, diphenylhydantoin; PB, phenobarbital; TPM, topiramate; VPA, valproate; CT, computed tomography; P, parietal; CLB, clobazam.

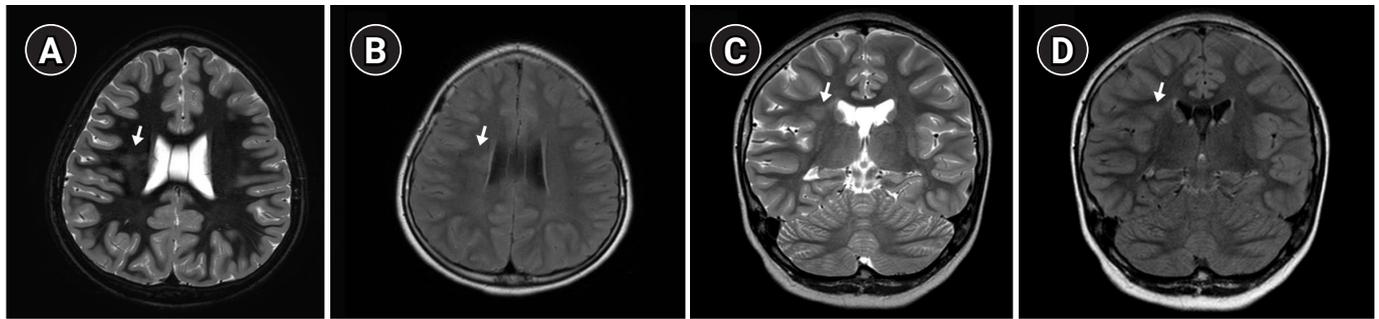


Fig. 2. Axial and coronal T2-weighted (A, C) and fluid attenuated inversion recovery (B, D) images show high signal intensity (arrows) with an irregular margin in the right periventricular white matter, suggesting heterotopic gray matter.

dysplasia. The abnormal brain MRI findings observed in the previous pediatric studies were only focal cortical dysplasia.

Long-term video EEG monitoring was mainly conducted to differentiate semiology in this study, but it has been used in previous studies for the purpose of presurgical evaluations of patients with anatomic abnormalities on MRI. Therefore, the proportion of focal cortical dysplasia among the patients was different.

Lesions located deep in the brain (9/17, 52.9%) had poor EEG correlations, whereas lesions near the cortex (8/17, 47.1%) tended to be well correlated (6/8). The reason for the poor correlation is thought to be that the epileptiform discharges become rapid and widely-propagating because of the rich connectivity between the frontal lobe and other lobes [9]. This proposal is supported by reports that the introduction of stereoelectroencephalography (sEEG) is helpful for confirming ictal spreading [4,8]. A study conducted by Bonini et al. [4] on 54 patients with adult FLE showed the process of clear localization using sEEG; seizures were categorized based on 31 ictal signs, and the electrode at which each seizure started was confirmed, leading to the finding of meaningful correlations between semiology and anatomy. Because our study was based on scalp EEG, there was a limitation in determining the precise ictal onset zone. In routine clinical practice, if the ictal onset zone cannot be precisely localized using scalp EEG, sEEG could be considered for surgical planning.

This study has several limitations. Because this study was focused on pediatric FLE patients who had ictal events during hospitalization, it did not represent all pediatric FLE patients. In addition, because the patients in this study showed various clinical characteristics, seizure semiology, EEG, and brain MRI findings, a limitation is that it was not possible to perform a statistical analysis because the number of patients in the groups to be compared was small.

In conclusion, this study summarizes the characteristics of pediatric patients admitted to our epilepsy monitoring unit and diag-

nosed with FLE. Brain MRI findings were normal in two-thirds of patients, and interictal EEG did not reveal epileptiform discharges in 23.2% of the patients. Because interictal EEG and brain MRI are often normal in patients with FLE, it is important to understand the characteristics of frontal lobe seizures in order to diagnose FLE and differentiate these seizures clinically. To determine the ictal onset zone in the patients already diagnosed with FLE, it is necessary to understand the functional anatomy of the frontal lobe and seizure semiology and to confirm the ictal EEG through long-term video EEG. Reports of semiology on the basis of home videos obtained by parents and interictal EEG will be the first steps in localizing the ictal onset zone. Thereafter, long-term video EEG may be helpful for further localization. For FLE with a nonlocalized structural etiology, sEEG will be helpful for defining the ictal onset zone.

Conflicts of interest

Jeehun 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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Neurological Symptoms of SARS-CoV-2 Infection in Pediatric Patients

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Purpose: Coronavirus disease 2019 (COVID-19) causes various neurological symptoms in children, as well as respiratory symptoms, and the number of reported cases is increasing with the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants. This study aimed to investigate the neurological symptoms and incidence in pediatric patients hospitalized with COVID-19.

Methods: We retrospectively analyzed the medical records of patients under the age of 18 diagnosed with COVID-19 and admitted to National Health Insurance Service Ilsan Hospital using real-time reverse transcription-polymerase chain reaction from December 2020 to March 2022. We reviewed data on the age of confirmed COVID-19 patients, fever, and respiratory, gastrointestinal, and neurological symptoms. We evaluated the chief complaints of hospitalization and classified them as non-neurological or neurological, according to the chief complaints that caused the most discomfort.

Results: Among 376 patients, 63 (16.8%) and 313 (83.2%) patients were classified as having neurological and non-neurological symptoms, respectively. The most common neurological symptoms were headache (49, 13.0%), followed by seizures (39, 10.4%), myalgia (24, 6.4%), and dizziness (14, 3.7%). Additionally, there were patients with anosmia (nine, 2.4%), ageusia (four, 1.1%), and visual disturbance (two, 0.5%). Of the 39 patients who experienced seizures, 15 (15/39, 51.7%) had no symptoms except fever, and seizures were the only main presenting symptom of SARS-CoV-2 infection.

Conclusion: Neurological symptoms are common in pediatric COVID-19 patients. Seizures can be an early symptom of SARS-CoV-2 infection and should not be underestimated during the COVID-19 pandemic.

Keywords: COVID-19; SARS-CoV-2; Neurologic manifestations; Seizures; Pediatrics

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2),

which causes coronavirus disease 2019 (COVID-19), has infected more than 500 million people worldwide. By May 2022, more than 17 million patients had been reported in the Republic of Ko-

rea, with children comprising 25.3% of reported cases [1].

Previous studies in adults have reported that COVID-19, which mainly shows respiratory symptoms, can also cause neurological symptoms. Neurological symptoms, which have mainly been reported in adults, range from headache and dizziness to seizures, and the neurological manifestations of COVID-19 range from neuropathy to stroke and encephalopathy [2]. In a study of 873 adults, the most common non-neurological symptoms were fever and cough, and common neurological symptoms included myalgia, headache, and dizziness. More than 60% of patients had hyposmia and dysgeusia, and these symptoms improved approximately 8 weeks later [3]. In another adult study, of 6,147 COVID-19 patients, 110 died and five had seizures (seizure rate, 0.08%) [4].

Many studies have been conducted to obtain more information on the clinical characteristics of COVID-19; however, most of them were for adults, and studies on children remain limited [5]. Most studies on pediatric patients have focused on respiratory symptoms and multisystem inflammatory syndrome in children (MIS-C); however, several studies on neurological manifestations in children have been reported [2]. There have been cases of pediatric patients with various clinical manifestations, from Guillain-Barre syndrome to sensor and motor polyradiculopathy, epileptic seizures, encephalopathy, and abnormal eye movements [6,7]. In a study of 90 pediatric patients with COVID-19, 13 (14.4%) showed neurological symptoms, which ranged from mild (headache, muscle weakness, anosmia, ageusia) to severe (status epilepticus, encephalopathy, and demyelinating events) [8].

After an 11-year-old boy in the United States visited the hospital for a new-onset seizure and was diagnosed with COVID-19 [9], cases of seizures have been reported in pediatric patients infected with SARS-CoV-2 [10]. Therefore, unlike in adults, it has been suggested that seizures may be a major sign of acute COVID-19 in children [11]. In particular, after the emergence of the Omicron (B.1.1.529) variant in South Africa, the increased infection rate was consistent with a rapid increase in COVID-19-related hospitalizations in children, and 19 out of 61 patients showed seizures [12]. Therefore, there is a need for further research on the clinical symptoms of pediatric patients in Korea, where the Omicron variant is prevalent.

This study aimed to investigate the neurological symptoms and incidence compared with non-neurological symptoms in pediatric patients hospitalized with COVID-19 at a single center in Korea. We also evaluated the age, presence of fever, and severity of seizures in patients infected with COVID-19 in the context of other neurological symptoms.

Materials and Methods

1. Study population

This retrospective study was performed at the National Health Insurance Service Ilsan Hospital from December 2020 to March 2022. Patients aged < 18 years diagnosed with COVID-19 were included in the study. A sterile nasopharyngeal swab was inserted into the patient's nasopharynx. COVID-19 was confirmed by detecting SARS-CoV-2 RNA in the nasopharyngeal swabs using real-time reverse transcription–polymerase chain reaction (RT-PCR). RT-PCR for SARS-CoV-2 detection was conducted at various hospitals or the Research Institute of Public Health using COVID-19 test kits. The inclusion criteria were as follows: (1) patients diagnosed with COVID-19 and (2) patients admitted to the National Health Insurance Service Ilsan Hospital. The exclusion criteria were as follows: (1) confirmed other respiratory infections and (2) voluntary discharge before the end of the isolation period. This study was approved by the Institutional Review Board of the National Health Insurance Service Ilsan Hospital (IRB 2022-03-016). Written informed consent by the patients was waived due to a retrospective nature of our study.

2. Data collection

We retrospectively reviewed the patients' medical records and collected data on the age upon COVID-19 confirmation, the duration of hospitalization, the presence of underlying diseases, vaccination history, fever, respiratory symptoms, gastrointestinal symptoms, neurological symptoms, chest X-ray findings, brain magnetic resonance imaging (MRI) findings, laboratory findings, and electroencephalography (EEG) findings.

First, we evaluated patients' chief complaints during hospitalization. Children infected with COVID-19 were hospitalized through the emergency room at the hospital or entered the Residential Treatment Center and then admitted to the National Health Insurance Service Ilsan Hospital if their symptoms worsened. When a patient was hospitalized through the Residential Treatment Center, symptoms requiring hospitalization were evaluated as the chief complaint rather than the first symptom. We divided patients into non-neurological and neurological groups according to the chief complaint that caused the most discomfort. Chief complaints such as headache, dizziness, seizure, and anosmia were classified as neurological, and asymptomatic, fever-only, respiratory symptoms, gastrointestinal symptoms, chest pain, and rash were classified as non-neurological. In addition to the chief complaint, new symptoms that appeared after hospitalization were also recorded daily. If the patient complained of both non-neurological and neurological symptoms, they were classified according to which symptoms

were most uncomfortable during hospitalization.

Based on the International Classification of Headache Disorders, headache was evaluated to be related to COVID-19 if it met the criteria for “headache attributed to systemic viral infection” [13]. A patient with a headache who was hospitalized because of a high fever was classified into the non-neurological group, whereas a patient with febrile seizure was classified into the neurological group. In addition to the chief complaints, we evaluated multiple respiratory, gastrointestinal, and neurological symptoms of each individual. Underlying diseases, including prematurity and epilepsy, were evaluated. For allergic rhinitis and asthma, only patients currently taking drugs were included.

The onset date was based on the date of RT-PCR examination; the number of patients was evaluated monthly, and the number of patients who had seizures in the neurological group was evaluated separately. EEG and brain MRI were performed on the day of discharge when the patient was released from isolation if the patient's condition was not status epilepticus.

3. Statistical analysis

Statistical analyses were performed using MedCalc version 19.2 (MedCalc Software bvba, Ostend, Belgium). Variables with a normal distribution were represented as mean and standard deviation (SD), while variables without a normal distribution were represented as medians and interquartile ranges. For comparisons of the two groups, the independent t-test or Mann-Whitney U test was used for continuous variables, and the chi-square test or Fisher exact test was used for categorical variables. All tests were considered statistically significant when *P* values were less than 0.05.

Results

1. Baseline characteristics

Between December 2020 and March 2022, 380 patients were diagnosed with COVID-19 and admitted to the National Health Insurance Service Ilsan Hospital. Four patients who were voluntarily discharged owing to the COVID-19 ward environment were excluded from the study. A total of 376 patients were enrolled in this study.

The mean \pm SD age at diagnosis of COVID-19 for all patients was 6.0 ± 4.7 years and 218 patients (58.0%) were male. Patients aged 5 to 12 were hospitalized more frequently ($n = 159$; 42.3%) than other age groups, and the mean hospitalization period was 8.4 ± 2.3 days. Most patients were not vaccinated against COVID-19 (98.9%) (Table 1).

We evaluated the chief complaints during patients' hospitalization. There were 313 patients in the non-neurological group (83.2%) and 63 patients in the neurological group (16.8%). There was no significant difference between the two groups in baseline characteristics, except for the proportion of patients under the age of 1 year and the hospitalization period. There was no significant difference in the ratio of obesity, defined as a body mass index of 25 kg/m^2 or higher, between the two groups. The number of patients per month tended to increase over time, and 242 patients were hospitalized between October 2021 and March 2022 (64.4%). In March 2022, 31 patients in the neurological group were hospitalized, which was the highest number among all months (31/63, 49.2%) (Fig. 1).

Table 1. Baseline characteristics of patients according to their chief complaint

Variable	Total (n = 376)	Non-neurological group (n = 313)	Neurological group (n = 63)	<i>P</i> value
Male sex	218 (58.0)	183 (58.5)	35 (55.6)	0.669
Age at COVID-19 confirmation (yr)	6.0 ± 4.7	5.6 ± 4.6	7.7 ± 4.6	0.001
< 1	65 (17.3)	64 (20.4)	1 (1.6)	<0.001
1–5	111 (29.5)	92 (29.4)	19 (30.2)	0.903
5–12	159 (42.3)	126 (40.3)	33 (52.4)	0.075
> 12	41 (10.9)	31 (9.9)	10 (15.9)	0.166
Duration of symptoms before hospitalization (day)	3.0 (2.0–5.0)	3.0 (2.0–5.0)	1.0 (1.0–4.0)	<0.001
Duration of hospitalization (day)	8.4 ± 2.3	8.6 ± 2.3	7.8 ± 2.2	0.009
Obesity (BMI $\geq 25 \text{ kg/m}^2$)	22 (5.9)	18 (5.8)	4 (6.3)	0.773
Underlying disease	64 (17.0)	49 (15.7)	15 (23.8)	0.116
Cardiac	8 (2.1)	8 (2.6)	0	0.362
Asthma or allergic rhinitis	11 (2.9)	10 (3.2)	1 (1.6)	0.699
Epilepsy	8 (2.1)	5 (1.6)	3 (4.8)	0.134
Vaccination	4 (1.1)	4 (1.3)	0	1.000

Values are presented as number (%), mean \pm standard deviation, or median (interquartile range). COVID-19, coronavirus disease 2019; BMI, body mass index.

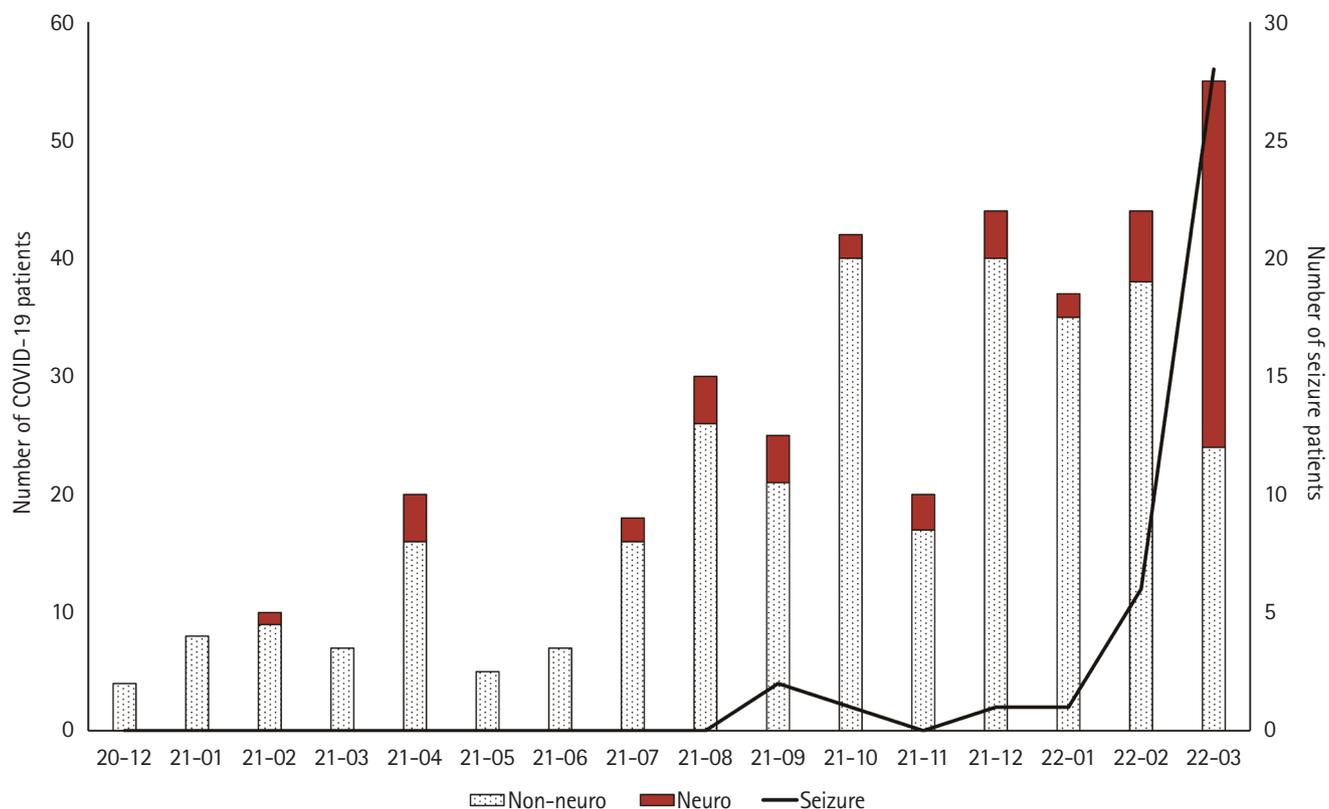


Fig. 1. Incidence of coronavirus disease 2019 (COVID-19) patients according to the symptoms that caused them the most discomfort, including patients with seizures. Neuro, neurological.

2. Non-neurological symptoms: overall and comparison between the groups

Twenty-nine patients were asymptomatic from before hospitalization to discharge, accounting for 7.7%. Of the total patients, 86 patients (22.9%) had no fever. There were 85 patients (22.6%) with a high fever above 39.0°C, and high fever was more frequent in the neurological group ($n = 23$, 36.5%) ($P = 0.004$). The most common non-neurological symptom in both groups was fever ($n = 290$, 77.1%), followed by cough, rhinorrhea, and sore throat. However, cough (65.8%) and rhinorrhea (31.3%) were significantly more common in the non-neurological group than in the neurological group (36.5%, $P < 0.001$; 17.5%, $P < 0.027$, respectively). Of the total patients, 67 (17.8%) showed symptoms of the gastrointestinal tract, and these symptoms often appeared a few days after hospitalization, rather than on the first day of hospitalization. The most common gastrointestinal symptom was diarrhea (9.6%), followed by vomiting (7.4%) and abdominal pain (5.6%). There was no significant difference in gastrointestinal symptoms between the two groups ($P = 0.522$). Twenty-two patients showed irregular erythematous rashes, which usually appeared throughout the body approximately 5 days after hospitalization. Twenty-eight patients were diagnosed with pneumonia, five with acute bronchiolitis,

three with croup, and one with MIS-C (Table 2).

3. Neurological symptoms: overall and comparison between the groups

The most common neurological symptom among all patients was headache ($n = 49$, 13.0%), followed by seizures ($n = 39$, 10.4%), myalgia ($n = 24$, 6.4%), and dizziness ($n = 14$, 3.7%). However, the most common symptom in the neurological group was seizures (39/63, 61.9%) (Table 3). In the non-neurological group, 35 (11.2%) and nine (2.9%) patients had symptoms of headache and dizziness, respectively. Most patients did not present with neurological symptoms.

Nine patients (14.3%) had loss of smell, and four patients (6.3%) had loss of taste. Anosmia and ageusia only mildly improved until discharge, and did not completely resolve. Four patients (6.3%) had rhabdomyolysis, with creatine kinase levels increasing by more than 400 IU/L, and no patient had worsening symptoms after intravenous hydration. Patients with cerebellar ataxia had normal brain MRI findings; however, the symptoms persisted. Following oral administration of methylprednisolone for 7 days, the symptoms improved. A 16-year-old female patient suddenly developed left homonymous hemianopsia on the seventh

Table 2. Non-neurological symptoms according to the chief complaint

Variable	Total (n = 376)	Non-neurological group (n = 313)	Neurological group (n = 63)	P value
Asymptomatic	29 (7.7)	29 (9.3)	0	0.008
Fever (°C)	290 (77.1)	236 (75.4)	54 (85.7)	0.075
37.5–38.0	60 (16.0)	55 (17.6)	5 (7.9)	0.057
38.0–39.0	145 (38.6)	119 (38.0)	26 (41.3)	0.629
≥ 39.0	85 (22.6)	62 (19.8)	23 (36.5)	0.004
Respiratory symptoms	277 (73.7)	247 (78.9)	30 (47.6)	<0.001
Cough	229 (60.9)	206 (65.8)	23 (36.5)	<0.001
Sputum	105 (27.9)	96 (30.7)	9 (14.3)	0.008
Rhinorrhea	109 (29.0)	98 (31.3)	11 (17.5)	0.027
Nasal stuffiness	45 (12.0)	38 (12.1)	7 (11.1)	0.818
Sore throat	109 (29.0)	97 (31.0)	12 (19.0)	0.057
Respiratory difficulty	21 (5.6)	20 (6.4)	1 (1.6)	0.224
Gastrointestinal symptoms	67 (17.8)	54 (17.3)	13 (20.6)	0.522
Abdominal pain	21 (5.6)	17 (5.4)	4 (6.3)	0.764
Vomiting	28 (7.4)	21 (6.7)	7 (11.1)	0.288
Diarrhea	36 (9.6)	32 (10.2)	4 (6.3)	0.340
Chest pain	8 (2.1)	7 (2.2)	1 (1.6)	1.000
Rash	22 (5.9)	21 (6.7)	1 (1.6)	0.114
Clinical diagnosis				
Pneumonia	28 (7.4)	24 (7.7)	4 (6.3)	0.716
Acute bronchiolitis	5 (1.3)	5 (1.6)	0	0.313
Croup	3 (0.8)	3 (1.0)	0	0.435
MIS-C	1 (0.3)	1 (0.3)	0	0.653

Values are presented as number (%). Patients often showed multiple symptoms. MIS-C, multisystem inflammatory syndrome in children.

Table 3. Neurological symptoms of patients in the neurological group

Variable	Neurological group (n = 63)
Seizure	39 (61.9)
Headache	14 (22.2)
Anosmia	9 (14.3)
Myalgia	7 (11.1)
Dizziness	5 (7.9)
Ageusia	4 (6.3)
Visual disturbance	2 (3.2)
Ataxia	1 (1.6)
Clinical diagnosis	
Rhabdomyolysis	4 (6.3)
Status epilepticus	2 (3.2)
Stroke	1 (1.6)

Values are presented as number (%). Patients often showed multiple symptoms.

day of hospitalization, and posterior cerebral artery infarction was confirmed on brain MRI. She recovered from visual loss 5 days after taking aspirin.

4. Seizures as the main presenting characteristics of COVID-19

The most common symptom in the neurological group was seizures (39 patients). Two of these patients did not have a fever. Fifteen patients (15/39, 51.7%) had no symptoms other than fever throughout hospitalization, and seizures were the only main presenting symptoms of COVID-19.

Ten patients (10/39, 25.6%) had a history of febrile seizures, and three patients with epilepsy had Dravet syndrome, Landau-Kleffner syndrome, and focal epilepsy. There were 15 patients (15/39, 38.5%) aged > 7, four of whom had no previous history of febrile seizures (10.2%). Most of the seizures were generalized tonic-clonic seizures (n = 29, 74.4%); however, 10 patients showed focal impaired-awareness seizures (10/39, 25.6%). Of the 10 patients who had focal seizures, six patients underwent EEG and four showed abnormal findings (respectively, left hemisphere slowing, right central and parietal slowing, bilateral frontal slowing, and left occipital sharp wave discharges). Of the 29 patients who showed generalized seizures, 13 patients underwent EEG, with normal findings except for one who showed generalized spike and wave discharges. Three patients with focal seizures underwent brain

MRI; one showed left ventriculomegaly, while another showed developmental venous anomalies in right occipital area. Thirty-five patients (35/39, 89.7%) experienced a seizure on the first day of fever, and there were no cases of seizures on the second day of hospitalization (Table 4). Since January 2022, when the Omicron variant became predominant in Korea, the number of patients with seizures has increased rapidly (Fig. 1).

Discussion

This study investigated various neurological symptoms and the incidence rates thereof in pediatric patients hospitalized with COVID-19. The most common neurological symptoms among all patients was headache (13.0%), followed by seizures, myalgia, and dizziness. The most common symptom in the neurological group was seizures (39/63, 61.9%), followed by headache and anosmia. Seizures were the only major presenting symptoms of SARS-CoV-2 infection, and more than half of the patients with seizures had no symptoms other than fever. In this study, the proportion of patients under the age of 1 was higher in the non-neurological group. It is possible that fewer patients in this age group were hospitalized because they could not express subjective ex-

pressions such as headache, dizziness, anosmia, and ageusia, whereas seizures could be more directly observed. In addition, the guardians of younger patients more frequently requested hospitalization, rather than treatment in a Residential Treatment Center, even if the patient had mild respiratory symptoms. The duration of symptoms before hospitalization was shorter in the neurological group, which seems to have been because patients with seizures and severe headaches were usually hospitalized on the same day. However, patients with fever and respiratory symptoms in the Residential Treatment Center were hospitalized later if the symptoms did not improve.

In previous studies on COVID-19, children's symptoms were thought to be milder than those of adults, and pediatric symptoms tended to be underestimated because they had lower mortality rates than adults. However, the number of intensive care unit (ICU) hospitalizations has increased since MIS-C has been reported in children [14,15]. In our study, three patients were admitted to the ICU for respiratory difficulty; however, no patient was admitted to the ICU due to neurological symptoms. In another large cohort study, 1,278 children were diagnosed with acute SARS-CoV-2, of whom 215 were diagnosed with MIS-C. Forty-four percent of patients in that cohort showed neurological signs, and the most common neurological signs were headache (16%) and acute encephalopathy (15%) [16]. Headaches mainly showed a tension-type-like phenotype and were bifrontal, while 25% of patients had migraine-like characteristics [17]. Encephalopathy was defined as altered mental status and lethargy, with reports of MIS-C-related encephalopathy mainly in pediatric patients [2]. However, that study investigated neurological symptoms associated with MIS-C, whereas in our study, it was difficult to compare the proportion of neurological symptoms because only one case could be diagnosed as typical MIS-C. In another meta-analysis with 3,707 children, myalgia (14.3%), headache (3.7%), seizures (3.1%), and encephalopathy (12.6%) were the main neurological symptoms. Approximately 1% of children were reported as having definite neurological complications, with rare cases of intracranial hemorrhage, Guillain-Barre syndrome, and visual disturbance [18]. For comparison, in a multi-center study of 873 adults, the most common neurological symptoms were smell and taste dysfunction (64.3%), myalgia (24.8%), headache (12.6%), and dizziness (11.9%). In rare cases, there were patients with cerebrovascular events (n = 10), demyelinating myelitis (n = 1), and Guillain-Barre syndrome (n = 1) [3]. Seizures and encephalopathy were common in children, whereas loss of smell and taste were common in adults. However, it is possible that the frequency of anosmia and ageusia was underestimated because children cannot express these symptoms. In our study, 13% of patients showed headaches; 6.4% had myalgia, which was

Table 4. Clinical characteristics of patients with seizures

Variable	Total (n = 39)
Seizure with fever, without any other symptoms	15 (51.7)
Afebrile seizure	2 (5.1)
History of febrile seizure	10 (25.6)
History of epilepsy	3 (7.7)
Age of seizure with COVID-19 (yr)	5.5 ± 3.3
< 5	18 (46.2)
5–7	6 (15.4)
> 7	15 (38.5)
First seizure at the age > 7 years	4 (10.2)
Semiology	
Generalized tonic-clonic seizure	29 (74.4)
Focal impaired awareness seizure	10 (25.6)
Seizure on the first day of symptoms	35 (89.7)
EEG findings	23 ^a
Focal slowing	3 (13.0)
Sharp wave discharges	1 (4.3)
Generalized spike and wave discharges	1 (4.3)
Brain MRI	9 ^a
Ventriculomegaly	1 (11.1)
Developmental venous anomalies	1 (11.1)

Values are presented as number (%) or mean ± standard deviation. COVID-19, coronavirus disease 2019; EEG, electroencephalography; MRI, magnetic resonance imaging.

^aNumber indicates the number of patients examined.

similar to a previous study; and 39 patients had seizures, more than in the previous study. In particular, based on a study reporting that headache attacks in pediatric patients with migraines occur more frequently during the COVID-19 pandemic, the association between COVID-19 and headaches is thought to be significant [19]. In this study, there were no cases of facial paralysis, but a previous study reported some cases of facial paralysis as a neurological symptom of COVID-19 [20]. In addition, as in the previously reported case of cerebellar ataxia, our patient responded well to methylprednisolone treatment [21]. However, in our study, only a few patients lost their senses of taste and smell. This finding should be interpreted in light of the fact that our cohort had 46.8% of patients under the age of 5, for whom it was difficult to identify these symptoms [22].

In our study, 15 patients had seizures without respiratory symptoms, and in previous studies, new neurological symptoms, including the central and peripheral nervous systems, were present even in the absence of respiratory symptoms [23]. It is now well known that SARS-CoV-2 infection exacerbates epileptic seizures in children [24]. However, in our study, two epilepsy patients showed status epilepticus and responded well to antiepileptic drugs. In particular, the rapid increase in seizure cases since January 2022, when the Omicron variant became predominant in Korea, is thought to be correlated with the Omicron variant.

The mechanism of SARS-CoV-2 infection was initially known to involve cell entry through angiotensin-converting enzyme 2 (ACE2), thereby causing vascular damage, such as Kawasaki disease, as well as damage to the lung. SARS-CoV-2 has also been found to cause specific neurological symptoms such as seizures, headaches, anosmia, and ageusia, and these symptoms seem to occur in patients without neurological disorders. Several pathological hypotheses have been proposed after it was found that specific neurological symptoms may occur due to the effects of ACE2 and the central nervous system on the renin-angiotensin-aldosterone system [25]. Currently, four major hypotheses have been proposed to explain the neurological symptoms of SARS-CoV-2 infection: (1) direct injury to neural cells; (2) vascular endothelial injury; (3) post-infectious inflammation; and (4) para-infectious inflammation [2,24]. The virus is known to directly invade the olfactory nerve and affect the central nervous system through the cribriform plate [22,26]. Endothelial damage can trigger thrombotic events such as stroke, as well as SARS-CoV-2 release. In addition, post-infectious and para-infectious inflammation can cause specific neurological symptoms through innate and adaptive immune activation. These excessive inflammatory reactions are thought to cause neurological symptoms such as headaches, seizures, central nerve palsy, and encephalopathy [27]. Therefore, children infected with

SARS-CoV-2 frequently present with neurological symptoms, and pediatric patients experienced more epileptic seizures during the period of the pandemic characterized by the predominance of the Omicron variant, which mainly caused symptoms of upper respiratory tract infections [28].

The present study had several limitations. First, since there were many young patients under the age of 5 years, the frequency of neurological symptoms such as anosmia and ageusia may have been underestimated. Second, due to the need for negative pressure facilities for COVID-19, for most patients, EEG and brain MRI were not performed on the first day of hospitalization. Third, since the isolation period for COVID-19 has been reduced from 10 days to 7 days in Korea, it is possible that the change in the isolation policy, rather than the severity of the disease, affected the hospitalization duration.

In conclusion, our results identified the neurological symptoms and incidence of pediatric patients hospitalized with SARS-CoV-2 infection. Common neurological symptoms were headache, seizures, myalgia, and dizziness. In addition, neurological symptoms of anosmia, ageusia, ataxia, and visual disturbance were also observed in a small number of patients. We suggest that seizures may be an early symptom of SARS-CoV-2 infection and should not be underestimated during the COVID-19 pandemic.

Conflicts of interest

Hoon-Chul Kang is an associate editor 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: HCK. Data curation: DY. Formal analysis: DY and HCK. Methodology: HCK. Project administration: HCK. Visualization: DY. Writing-original draft: DY. Writing-review & editing: DY and HCK.

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Variable Phenotypes of *ZC4H2*-Associated Rare Disease in Six Patients

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Purpose: Wieacker-Wolff syndrome is a rare disease caused by X-linked zinc finger C4H2-type containing (*ZC4H2*) mutations. It is characterized by arthrogyriposis multiplex congenita (AMC) and intellectual disability (ID), including impairment of central and peripheral synaptic plasticity. Currently, it is named "ZC4H2-associated rare disease" (ZARD) due to various clinical features other than AMC and ID. Here, we report six cases of ZARD, and describe their variable clinical phenotypes.

Methods: We analyzed the detailed clinical features and genotypes of six patients diagnosed by whole-exome sequencing or a chromosomal microarray.

Results: In the four male patients, hemizygous mutations were found (c. 245A>C in two patients, c. 610C>A in one patient, and c.637C>T in one patient), and all variants were identified by Sanger sequencing. In the female patients, a 1.16-Mb deletion in Xq11.2, including *ZC4H2*, was identified by chromosomal microarray. All patients had heterogeneous phenotypes with variable severities. Motor delay was observed in all patients, four of whom could not walk independently. Other neurological features included ID, spasticity, and seizures. The craniofacial features included microcephaly, low-set ears, strabismus, ptosis, ocular motor apraxia, a U-shaped upper lip vermilion, short neck, and microretrognathia. The most common musculoskeletal symptoms were multiple arthrogyriposis: metacarpophalangeal joint contracture, clubfoot, distal muscle weakness, Achilles tendon contracture, knee flexion contracture, camptodactyly, elbow flexion contracture, and hip subluxation.

Conclusion: The ZARD phenotypes were prominent in male patients, and female patients with loss of function showed more severe symptoms. Further research is needed to clarify phenotypic variability in this rare disorder.

Keywords: Wieacker syndrome; Intellectual disability; Arthrogyriposis

Introduction

Wieacker-Wolff syndrome (WWS) (WRWF [OMIM #314580]; female restrictive type known as WRWFR [OMIM #301041])

was first reported as being characterized by congenital contracture, slowly progressive distal muscle atrophy, dyspraxia of the eye, face, and tongue muscles, and mild intellectual impairment. This disease was initially described by monitoring disease progression in

six men from three generations in one family, and an X-linked recessive genetic pattern was observed in 1985 [1]. Later, Kloos et al. [2] revealed that the causative gene of WWS was located at Xq11.2. In 2013, Hirata et al. [3] investigated the causative gene of WWS, discovering the zinc-finger gene zinc finger C4H2-type containing (*ZC4H2*) as pathogenic using next-generation sequencing (NGS) and array comparative genomic hybridization in patients with X-linked intellectual disability (ID) and arthrogryposis multiplex congenita (AMC). In 1991, exotropia, microcephaly, distal muscle wasting, and digital arch were observed in one family, which was reported at the time as having Miles-Carpenter syndrome [4]. In 2015, May et al. [5] revealed this to be a case of *ZC4H2* related-disease via genetic evaluation. This familial disease was thereafter classified as the same disease and referred to as “*ZC4H2*-associated rare disease” (ZARD) [6].

The *ZC4H2* protein comprises a zinc finger domain, four cysteine residues, two histidine residues, and a coiled-coil region. The coding gene, *ZC4H2* is located on the long arm of the X chromosome (Xq11.2). To date, various phenotypes of WWS have been reported, with symptoms including facial dysmorphism, skeletal-muscular symptoms involving multiple joint contractures, and neurologic symptoms including ID and motor delay [4-13]. In addition, several recent studies have reported that the female phenotype can be particularly severe [6-10].

Here, we present a summary of various clinical phenotypes associated with *ZC4H2* in six patients.

Materials and Methods

In this study, six patients diagnosed with diseases related to a *ZC4H2* gene variant who visited a pediatric neurologic outpatient clinic at the Seoul National University Hospital Children's Hospital are reported.

For clinical phenotyping, a retrospective chart review was conducted, as well as an analysis of brain magnetic resonance imaging (MRI), and electroencephalography (EEG) findings. Based on NGS analysis, SureSelect Human All Exon V5 (Agilent Technologies, Santa Clara, CA, USA), a capture probe targeting the entire exonic region was used for exome sequencing.

Library preparation was performed in accordance with the manufacturer's instructions. Libraries were sequenced (paired-end) using the HiSeq 2500 sequencing system (Illumina, San Diego, CA, USA). For sequencing, paired-end sequence reads with a read length of 151 base pairs were aligned to the Genome Reference Consortium Human Build 37 (GRCh37) using the Burrows-Wheeler Aligner (v. 0.7.17). Picard software (v. 2.9.0), SAMtools (v. 1.9), and Genome Analysis Toolkit (v. 4.1.2) were used

for deduplication, realignment, and basic recalibration. We used a different haplotype, Caller, to perform the calling. SnpEff, ANNOVAR, and InterVar were used to annotate transformations. In the absence of a specific trait gene, a variant with an alternate allele frequency (AF) of 0 in the gnomAD database was considered a candidate autosomal dominant gene. AF variants of less than 0.01% were considered candidates for autosomal recessive genes. For low-frequency strain detection, we used MuTect2 to search for variants with an AF between 0.05 and 0.25. Only low-frequency variants with an allele depth of 30 or more and 10 or more variant alleles were selected. For copy number variation (CNV) analysis, chromosomal microarray (CMA) assays were performed using the Agilent Human Genome Oligonucleotide Comparative Genome Hybridization 244, 80, or 60 K microarrays (Agilent Technologies), with median total probe spacings of 8.9, 13, or 41 kb, respectively. All CNVs were called based on human GRCh37 (hg19).

Quantitative polymerase chain reaction (qPCR) was performed to differentiate germline mosaics using ribonuclease P (*RNase P*) as the reference gene. All variants were categorized according to the standards and guidelines of the American College of Medical Genetics and Genomics, and pathogenic or likely pathogenic variants were defined as causative variants.

This study was approved by the Institutional Review Board (IRB) of Seoul National University Hospital (IRB No. 1406-081-588) for whole-exome sequencing and a review of medical records. All patients or their legal representatives provided written informed consent.

Results

Four of the enrolled patients were male and two were female. Four patients were diagnosed using NGS through the Korean Undiagnosed Disease Program, and three missense variants were identified (Table 1). None of the variants confirmed by Sanger sequencing were found in the 1,000 Genomes Project or the gnomAD database. All missense mutations, c.245A > C (p. Gln82Pro), c.610C > A (p. Pro204Thr), and c.637C > T (p. Arg213Trp), were inherited from the mother. c.637C > T has been reported previously, and the other two variants are novel [3]. The female patients were dizygotic twins, in whom we identified a 1.16-Mb deletion including the *ZC4H2* gene in Xp11.2 by CMA.

These patients had a wide variety of clinical manifestations (Table 2), with central or peripheral neurological symptoms being the most prominent phenotype. The clinical features were analyzed with a focus on previously reported phenotypes, according to research stating that 30% of ZARD cases were seen in 42 families (Frints et al. [6]). All patients experienced motor delays. Oth-

Table 1. ZC4H2 variants observed in the patient cohort

Index	Age/Sex	Mutation site	Type	Inheritance
1	9 yr/M	c.245A > C:p.G82P	Missense	X-linked recessive
2	4 yr/M	c.245A > C:p.G82P	Missense	X-linked recessive
3	4 yr/M	c.610C > A:p.P204T	Missense	X-linked recessive
4	8 yr/M	c.637C > T:p.A213T	Missense	X-linked recessive
5 ^a	24 mo/F	Xq11.2;1.16Mb deletion (63510019-64671980)	CNV	Mosaic
6 ^a	24 mo/F	Xq11.2;1.16Mb deletion (63510019-64671980)	CNV	Mosaic

All male patients had missense variants in zinc finger C4H2-type containing (ZC4H2) inherited from their mothers. The two dizygotic twin female patients had a 1.16-Mb deletion in chromosome Xq11.2 including ZC4H2.

CNV, copy number variation.

^aDizygotic twin.

Table 2. Clinical phenotypes observed in different ZC4H2 variants

Index case	Growth	Head/neck	Respiratory /abdominal	Skeletal/muscular	Neurologic
1	NA	High forehead, low-set ears, strabismus, long philtrum, carp shaped mouth	Recurrent aspiration pneumonia	Metacarpophalangeal joint contractures	Motor delay, inability to walk, generalized hypotonia, intellectual disability, seizures
2	NA	High forehead, low-set ears, long (flat) philtrum, carp shaped mouth	NA	NA	Motor delay, inability to walk, intellectual disability, seizures
3	NA	Ptosis, strabismus, microretrognathia, long (flat) philtrum, carp shaped mouth	NA	Arthrogryposis multiplex congenita, distal muscle weakness	Motor delay, intellectual disability
4	Growth delay	High arched palate	Poor feeding in infancy	NA	Motor delay, inability to walk, intellectual disability, spasticity, seizures
5	Short stature, growth delay	Microcephaly, low-set ears, ptosis, strabismus, microretrognathia, short neck	Neonatal respiratory distress, recurrent aspiration pneumonia, apnea, poor feeding in infancy	Arthrogryposis multiplex congenita, knee/elbow flexion contracture, camptodactyly, distal muscle weakness/atrophy	Motor delay, inability to walk, intellectual disability, spasticity
6	Short stature, growth delay	Microcephaly, short neck	Recurrent aspiration pneumonia, poor feeding in infancy	Arthrogryposis multiplex congenita, knee flexion contracture, camptodactyly, distal muscle weakness/atrophy	Motor delay, intellectual disability, spasticity

NA, not available.

er findings included ID (6/6), poor speech (6/6), inability to walk (4/6), spasticity (3/6), seizures (3/6), and hyperreflexia (1/6). Brain MRI was performed in five patients, except for index case 6, and only index case 4 showed myelination delay, with no major abnormalities. EEG was performed on three patients who had seizures. In index case 1, generalized spikes and waves were observed, and seizures were controlled by taking two anticonvulsants (valproic acid and levetiracetam) due to frequent recurrence. In index case 2, convulsions occurred only once around 1 year of age and were observed normally on EEG at the time; the patient was observed without taking anticonvulsants. Index case 4 showed intermittent generalized slow activity without definite epileptiform discharges on EEG, but there was no seizure after the first seizure.

Craniofacial symptoms included microcephaly (2/6), low-set ears (3/6), a U-shaped upper lip vermilion (3/6), short neck (2/6), and microretrognathia (2/6). Some patients also exhibited ocular symptoms, such as strabismus (3/6), ptosis (2/6), and ocular motor apraxia (2/6). Five of the six patients had musculoskeletal phenotypes. The symptoms included multiple arthrogryposis: metacarpophalangeal joint contracture (4/6), clubfoot (3/6), distal muscle weakness (3/6), other Achilles tendon contractures (3/6), knee flexion contractures (2/6), camptodactyly (2/6), elbow flexion contractures (1/6), and hip subluxation (2/6). Other clinical symptoms included short stature (2/6), recurrent aspiration pneumonia (3/6), and feeding difficulty during infancy (3/6).

Discussion

The zinc-finger gene *ZC4H2* was first identified by Hirata et al. [3] in 2013, and it was confirmed to be strongly expressed in the brain and spinal cord of both mice and zebrafish. In mice, *Zc4h2* was revealed to play an important role in brain development, being strongly expressed in the embryo stage, with a decrease in the post-natal stage; knockdown of *zc4h2* in zebrafish results in impaired swimming and a reduced number and disorganized pattern of neuromuscular endplates. Based on these findings, it was concluded that pathologic variants of the *ZC4H2* gene cause AMC and ID due to impairment of central and peripheral synaptic plasticity. In 2015, May et al. [5] reported that homozygous *zc4h2* mutants resulted in an abnormal swing capacity, pectoral fin flexion, and eye position in zebrafish.

Although the first known and characteristic clinical features of WWF are AMC and ID, this condition is now referred to as ZARD because of its various clinical expressions. The patients in our cohort often showed one of the features of AMC and ID, or a clinical variation other than those two symptoms. Two female patients showed a global developmental delay of 24 months, but all other patients were diagnosed with ID, and three patients had AMC. In addition, relatively unknown facial dysmorphism, ocular symptoms, and gastrointestinal and respiratory symptoms such as poor

feeding and aspiration pneumonia were also observed.

All four male patients had missense mutations that were transmitted from asymptomatic mothers. Index cases 1 and 2 showed the same mutation, c.245A > C (p.Gln82Pro), and the clinical symptoms also showed similar facial dysmorphisms, including a high forehead, low-set ears, ocular motor apraxia, and a long philtrum in both patients. Musculoskeletal symptoms were not clear, and other neurologic phenotypes, such as inability to walk, motor delay and ID, poor speech, and seizures, were observed. The missense variant c.187G > C (p.Val63Leu), found in the coiled-coil domain, was identified in a family containing six patients, all of whom showed ptosis, contracture of the Achilles tendon, distal muscle weakness, motor delay, and ID [3]. In both these mutations, AMC, the most well-known characteristic of ZARD, was relatively inconspicuous or showed only relatively mild musculoskeletal symptoms such as metacarpophalangeal joint contractures. Although a previous study revealed that a point mutation in the coiled-coil domain can cause destabilization of the *ZC4H2* protein, there has been no study on the exact function of the coiled-coil domain to date [5].

Index case 3 had a c.610C > A (p.Pro204Thr) mutation, which is located in the zinc finger domain (Fig. 1). Unlike the previous two patients, the musculoskeletal symptoms of AMC, clubfoot, Achilles tendon contracture, and distal muscle weakness were

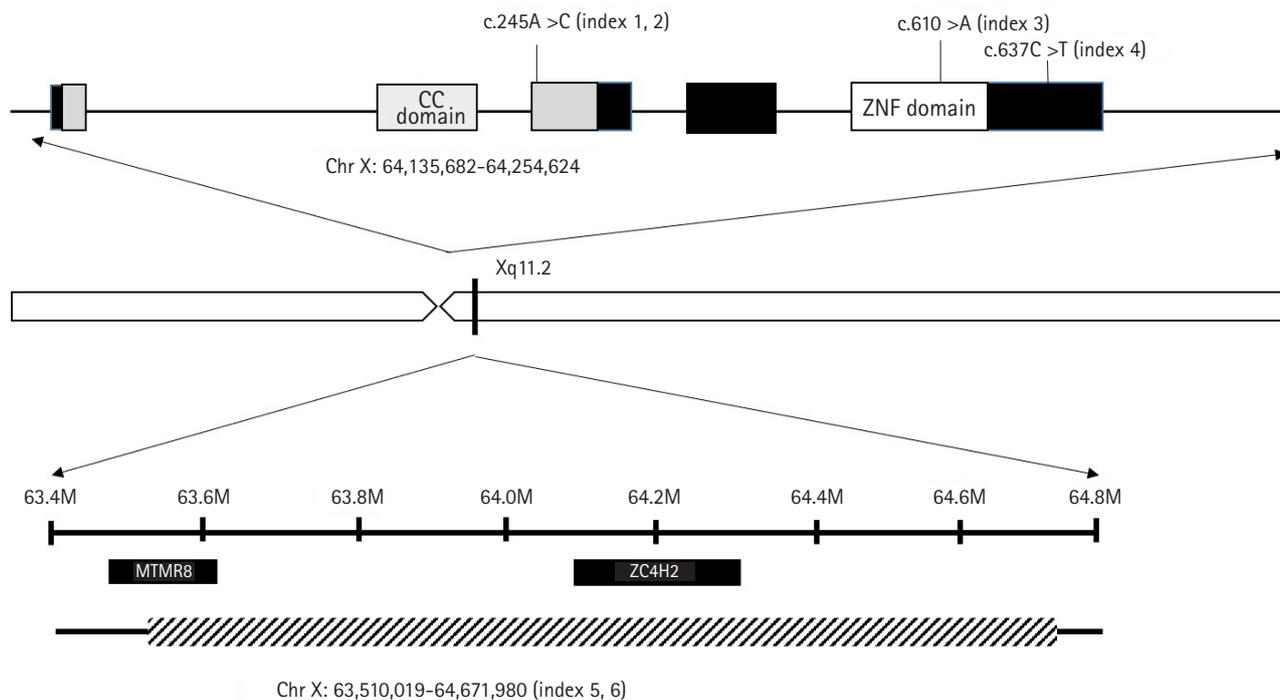


Fig. 1. Structure of the human zinc finger C4H2-type containing (*ZC4H2*) gene in our patients and schematic view of the X chromosome with the location with *ZC4H2* deletion. CC, coiled coil; ZNF, zinc finger; MTMR8, myotubularin related protein 8.

prominent. Many other studies have reported missense mutations in this area and associated characteristic musculoskeletal symptoms and ID, including AMC [3,11].

Index 4 had the c.637C > T (p.Arg213Trp) variant, which is located in the C-terminus, and has been identified as a pathologic variant in previous reports [3]. Similar to a previously reported patient with the same mutation, this patient also did not have musculoskeletal symptoms, and central or peripheral neurologic symptoms such as motor delay, ID, seizure, and spasticity were more definite.

Although the exact function of each domain has not yet been determined, considering our patients and other previously reported patients, missense mutations in the zinc finger domain appear to be prominently associated with musculoskeletal symptoms such as AMC.

However, all previous studies, including large-scale studies that investigated several families, showed several clinical variability even with the same genotype, so the genotype-phenotype (G-P) correlation could not be clearly identified [3,6,11]. However, in our cohort, the phenotypes were different for each protein region. If further studies identify more patients with ZARD, that would be of great help in revealing common G-P correlations.

Previous reports have shown that female carriers can be asymptomatic or almost unaffected, but a missense mutation, frameshift mutation, splicing variant, and Xq11.2 microdeletion caused by *de novo* changes had mild to severe impacts when ZC4H2 was completely or partially lost [6-10,13]. In a study by Frints et al. [6], which analyzed the difference between *de novo* mutations and those transmitted by carrier females in 24 families, 12 of the carrier females (n = 33) showed a normal phenotype, and the remaining

20 had mild ID or joint contractures (phenotype limited to fingers, wrists, etc.). In contrast, more than 90% of the patients with *de novo* pathologic phenotypes showed central and peripheral neurologic symptoms, such as clinical presentation, AMC, motor delay, inability to walk, distal muscle weakness, and more characteristics of ZARD. Our cohort included two female patients. These patients were dizygotic twins harboring the same deletion (X: 63510019-64671980). In the twin female patients, the entirety of the ZC4H2 gene and part of the myotubularin related protein 8 (MTMR8) gene were included in the deletion. The MTMR8 gene is known to be related to X-linked myotubular myopathy, making it difficult to describe the patient's phenotype [14].

Interestingly, in these two patients, other benign CNVs were found in different forms. Parental tests were performed using qPCR, a target-specific detection method, which found that neither the father nor mother harbored this deletion (Fig 2).

The mother of the dizygotic twins had previously selectively aborted her first fetus with fetal hydrops at a gestational age of 20 weeks, and had subsequently given birth to a healthy boy. The known prenatal clinical features of ZARD include clubfoot, hypokinesia/akinesia, rocker-bottom foot contractures, AMC, and edema [6,13]. Since the aborted fetus was not tested for Xp11.2 microdeletion, it is not known whether fetal hydrops was a prenatal manifestation. The second and third children, index cases 5 and 6, had decreased fetal movement, showed facial dysmorphism (e.g., a small mouth and retracted chin), and AMC, and suffered respiratory difficulties due to a short neck and laryngomalacia. Although both patients carried the same deletion, index case 5 showed a more severe phenotype than index case 6, which is consistent with other studies showing variable phenotypes arising from the same

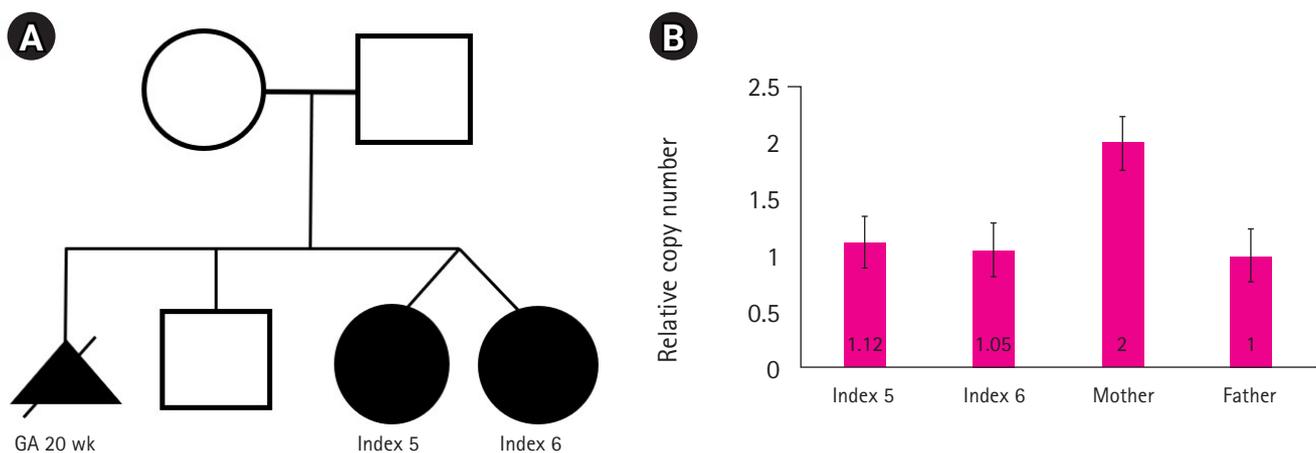


Fig. 2. Pedigree (A) and quantitative polymerase chain reaction (qPCR) results (B) of the dizygotic twins' family. The first fetus had fetal hydrops and was selectively aborted at 20 weeks of gestation. On qPCR, the father and mother showed normal gene expression without the deletion. GA, gestational age.

variant [3,6].

In the paternal test, the parents were normal, but the deletion of the same region in both fraternal twins was observed. Furthermore, the details of the previously aborted fetus gave rise to suspicion of a history of ZARD. Although invasive tests, such as ovarian biopsy, were not performed to confirm this speculation, germline mosaicism rather than a *de novo* mutation was considered. In the absence of such reports previously, it is not possible to compare the severity of *de novo* mutations with expression due to germline mosaicism. Nonetheless, more cases and additional laboratory studies on these germline mutations are required.

Although not implemented in our study, other studies have attempted to prove the severe phenotype in *de novo* females via the X-inactivation test [6-8]. However, since several studies have shown inconsistent results, this cannot be clearly determined as a phenomenon caused by X-inactivation.

In conclusion, ZARD is well known to present with severe AMC and ID, mainly in males, but none of the patients showed the same phenotype. Phenotypic severity can vary. Since ZARD is an X-linked recessive disease, most female patients with conserved carriers are asymptomatic or have mild symptoms; however, female patients with deleterious mutations showing severe symptoms were also observed. Further research is needed to clarify the phenotypic variability and the molecular and cellular mechanisms of this rare disorder.

Conflicts of interest

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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Clinical Characteristics and Neurologic Outcomes of X-Linked Myotubular Myopathy

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Purpose: X-linked myotubular myopathy (XLMTM) is a rare condition of centronuclear myopathy caused by myotubularin 1 (*MTM1*) mutations. Patients with XLMTM show different neurodevelopmental outcomes after the neonatal period depending on age and acquired hypoxic damage. We aim to evaluate the clinical characteristics and neurodevelopmental outcomes of patients with XLMTM who were followed up at a single center. It is essential to understand the volume and conditions to prepare for being a candidate for new therapeutic strategies.

Methods: Patients diagnosed with centronuclear myopathy by muscle pathology and *MTM1* mutation analysis were included. We retrospectively investigated motor milestones, communication skills, and bulbar and respiratory function in the patients. The patients were categorized into two groups: with and without hypoxic insults (HI).

Results: All 13 patients were severely affected by neonatal hypotonia and required respiratory support and a feeding tube during the neonatal period. The follow-up duration was 4.4 years (range, 0.3 to 8.9). In the non-HI group, developmental milestones were delayed but were slowly achieved. Some patients underwent training in oral feeding with thickened foods and weaning from ventilation. Patients with HI showed poor motor function catch-up and communication skills. Three deaths were associated with acute respiratory failure.

Conclusion: Patients with XLMTM without HI can survive long-term with the slow achievement of motor milestones and bulbar and respiratory function. However, hypoxic brain damage following acute respiratory failure negatively influences their developmental potential or even lead to death. Therefore, parental education for proper respiratory management is necessary, especially for young children.

Keywords: Myopathies, structural, congenital; Myotubularin

Introduction

X-linked myotubular myopathy (XLMTM; OMIM 300415) is a neuromuscular disorder pathologically categorized as centronuclear myopathy [1]. *MTM1* encodes myotubularin, which is implicated in the phosphatidylinositol 3-kinase pathway and is required for muscle cell growth, differentiation, and intracellular trafficking [2-4]. Newborns affected by XLMTM usually present severe hypotonia and typically require mechanical ventilators and nutritional support [5]. Although long-term survivors depend on a wheelchair, ventilator, and tube feeding, the disease course of XLMTM is relatively stable [1]. The critical roles of a multidisciplinary unit include encouragement of exercise training to maximize patients' motor function and independence as well as safe maintenance of feeding tubes and home ventilators [6,7]. In a cross-sectional study about a natural history of patients with XLMTM, all observed deaths were associated with respiratory failure [1]. Despite a patient surviving respiratory failure, hypoxic brain damage can cause devastating neurodevelopmental outcomes.

New therapeutic strategies have recently been identified for XLMTM in children, with studies investigating gene transfer (Gene Transfer Clinical Study in X-Linked Myotubular Myopathy, ASPIRO, NCT03199469), an antisense oligonucleotide (ASO) strategy (Early Phase Human Drug Trial to Investigate Dynamin 101 (DYN101) in Patients \geq 16 Years With Centronuclear Myopathies, Unite-CNM, NCT04033159), and tamoxifen therapy (Tamoxifen Therapy for Myotubular Myopathy, TAM4MTM, NCT04915846). To participate in clinical trials, it is essential to understand the volume and their conditions of domestic patients [8-11]. Data regarding patient characteristics must be shared to allow careful monitoring.

This study is the first to review the clinical characteristics and neurological outcomes, including motor milestones, communication skills, and bulbar and respiratory function, in Korean pediatric patients with XLMTM. To evaluate the neurological consequences of hypoxic events, we described the neurological status of patients with XLMTM divided into the following two subgroups: the hypoxic insults (HI) group and the non-HI group.

Materials and Methods

1. Patients and genetic studies

Male patients with a confirmed muscle biopsy result consistent with centronuclear myopathy and an *MTM1* mutation from January 2004 to August 2021 were included. *MTM1* mutations were identified by a single-gene sequencing or next-generation sequencing (NGS) panel of congenital myopathy-related genes. For posi-

tive probands, maternal segregation analyses were performed. The medical records were retrospectively reviewed, including the genotype, prenatal/postnatal history, clinical features of XLMTM, developmental milestones in motor and language, dependency on a ventilator and feeding tube from the neonatal stage to childhood, and mortality. We compared neurodevelopmental outcomes between the HI and non-HI groups. The Institutional Review Board (IRB) of the Seoul National University Hospital approved this study (IRB no. 1101-110-353) and waived the requirement for informed consent.

Results

1. Clinical features

The median age of the 13 patients was 4.4 years (range, 0.3 to 8.9). Muscle biopsies were performed at a median age of 3.7 months (range, 0.9 to 11.3). All patients presented with neonatal hypotonia (Table 1). Among them, preterm birth (before 37⁺⁰ gestational weeks) occurred in nine patients (69.2%), and asphyxia at birth was reported in five patients (38.5%). All patients received nutri-

Table 1. Clinical features of patients with XLMTM (n=13)

Variable	Value
Prenatal and postnatal features	
Polyhydramnios or poor fetal movement	5 (38.5)
Preterm birth	9 (69.2)
Birth asphyxia	5 (38.5)
Physical examination at the first admission	
Hypotonia after birth	13 (100)
Myopathic face	7 (53.8)
Facial weakness or ophthalmoplegia	5 (38.5)
Early bulbar weakness	13 (100)
High-arched palate	5 (38.5)
Club foot/joint contracture	3 (23.1)
Pigeon/funnel chest	5 (38.5)
Undescended testis	10 (76.9)
Creatine kinase (IU/L)	58.7 (25-88)
Bulbar/respiratory support after birth	
Initial tube feeding	13 (100)
Initial respiratory support	13 (100)
IPPV/SIMV	7 (53.8)
CPAP/BiPAP	3 (23.1)
Oxygen supply	2 (15.4)
Unknown	1 (7.7)

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

XLMTM, X-linked myotubular myopathy; IPPV, intermittent positive-pressure ventilation; SIMV, synchronized intermittent mechanical ventilator; CPAP, continuous positive airway pressure; BiPAP, bilevel positive airway pressure.

tional and respiratory support after birth. Respiratory support consisted of intermittent positive-pressure ventilation/synchronized intermittent mechanical ventilator ($n=7$, 53.8%), use of continuous positive airway pressure/bilevel positive airway pressure (BiPAP) ($n=3$, 23.1%), and oxygen supply ($n=2$, 15.4%). The accompanying clinical features were myopathic face ($n=7$, 53.8%), facial weakness or ophthalmoplegia ($n=5$, 38.5%), a high-arched palate ($n=5$, 38.5%), club foot/joint contracture ($n=3$, 23.1%), pigeon/funnel chest ($n=5$, 38.5%), and undescended testis ($n=10$, 76.9%).

2. Genotype

Among the 13 patients, *MTM1* mutation was identified in 11 patients (84.6%) by single-gene sequencing and in two patients (15.4%) by a NGS panel. Maternal inheritance was revealed in nine patients (69.2%) (Table 2). We identified 11 pathogenic *MTM1* variants, including four splice-site mutations, three missense mutations, two deletions, one frameshift mutation, and one nonsense mutation. Three patients (no. 7, no. 9, and no. 11) had the same variant, c.342_342+4del (NM_000252.3).

3. Neurological outcomes

1) Motor and language development

Eight patients (61.5%) in the non-HI group and five (38.5%) in the HI group had different neurodevelopmental outcomes in terms of maximum performance of motor and language function (Table 2). The presented results include the neurological status at the last outpatient visit. No patient showed loss of motor and language functions. In the non-HI group (median follow-up duration, 5.0 years [range, 0.4 to 8.9]), patients slowly achieved motor milestones such as sitting up without assistance and walking alone. Verbal communication was compatible with their age. Three instances of mortality occurred due to acute respiratory failure in the HI group (median follow-up duration, 1.1 years [range, 0.3 to 4.5]). One survivor (no. 13) became bedridden after resuscitation at 2 years of age. Patient no. 12 showed severe developmental delay and HI, which was revealed by brain magnetic resonance imaging, without any prior hypoxic event. The patient showed eye contact, minimal hand use, and non-verbal communication at the age of 4.5 years.

2) Bulbar dysfunction

All patients received nutritional support through a nasogastric tube after birth. Percutaneous endoscopic gastrostomy (PEG) was performed on five patients (38.5%) at a median age of 2.4 years (range, 0.3 to 4.6). According to a videofluoroscopic swallow study,

some patients underwent a challenge with oral feeding with thickened food. Two patients in the non-HI group were able to switch to full oral feeding. Both survivors in the HI group underwent PEG after revealing hypoxic brain insults.

3) Respiratory dysfunction

Eight patients (61.5%) underwent tracheostomy at a median age of 3.8 months (range, 1.6 to 11.5) and in them prolonged intubation was maintained due to failure of ventilator weaning and airway protection from respiratory emergencies was required [12]. In the non-HI group, two patients were discharged without respiratory support on room air. Four patients underwent trained ventilation weaning (weaning time range, 0.5 to 4 hours) through respiratory rehabilitation, but the ventilator support was provided for > 12 hours per day.

Discussion

This study reported the neurodevelopmental outcomes, including motor function, language skills, and swallowing and respiratory function in patients with XLMTM who were followed up at a single center. Similar to our results (age range, 0.4 to 8.9 years) in a prospective study of 45 patients with XLMTM, patients < 10 years (age range, 3.5 months to 56.8 years) presented slow improvements in objective muscle functions [2]. However, older patients with ventilation support for > 12 hours per day showed accelerated loss of motor function. Because the aforementioned study covered a wide variation in patient ages, the measurement tools for muscle strength and motor function had been designed to accommodate very weak patients. These tools included grip and pinch strength tests, the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders scale for patients < 2 years of age, the Motor Function Measure scale for patients > 2 years of age, the MoviPlate device for upper limb motor function testing, and the North Star Ambulatory Assessment scale for ambulant patients. Pulmonary function tests showed results below the normal range, even in patients without ventilator support. In our study, six patients in the non-HI group required ventilator support for > 12 hours per day; therefore, a relatively earlier loss of motor function would be expected in those patients than in the two patients without ventilator support (Table 2).

In our cohort, the leading causes of respiratory failure included T-cannula obstruction, T-cannula omission, or aspiration pneumonia. Because patients with XLMTM are at risk for acute respiratory failure, proper education and techniques, such as chest compression, airway clearance by tracheal suction, T-cannula obstruction response, and Ambu bag ventilation, are essential for the man-

Table 2. Neurodevelopmental outcomes in patients with XLMTM

Variable	Variant	Inheritance	Current age (yr)	Maximum motor performance	Maximum language/cognitive functions	Nutritional support (oral feeding trial, amount)	Respiratory support (ventilation weaning trial, amount)	ARF (yr)	HIE (yr)	Death (yr)
Non-hypoxic insult group (n = 8)										
A. Walking alone without respiratory assistance (n = 2)										
1	c.1786_1795del (p.Met596Cysfs)	De novo	4.4	Walking alone	Sentences	PEG since 3 yr (yes, 10%)	Room air	No	No	No
2	c.679G>A (p.Val227Met)	Maternal	5.5	Walking alone	Sentences	Full oral feeding since 5 mo	Room air	No	No	No
B. Sitting up with BiPAP (n = 4)										
3	exon 3 and 4 deletion	Maternal	4.4	Sitting with assistance	Sentences, reading characters, doing a puzzle	NG tube (yes, spoon)	BiPAP via T-can since 6 mo (yes, 5 hr)	No	No	No
4	c.1558C>T (p.Arg520Ter)	Maternal	5.8	Sitting with assistance	A few words, obeying simple commands	Full oral feeding since 9 mo	BiPAP via T-can since 2 mo (yes, 4 hr)	No	No	No
5	c.1353+2T>G	Maternal	8.2	Sitting with assistance	Counting number	PEG since 5yr (no)	BiPAP via T-can since 2 mo (yes, 2 hr)	0.5	No	No
6	c.678+1G>C	Maternal	8.9	Sitting with assistance	Reading characters	PEG since 4mo (yes, spoon)	BiPAP via T-can since 9 mo (yes, 0.5 hr)	No	No	No
C. Unable to control head with BiPAP (n = 2)										
7	c.342_342+4del	De novo	0.4	Eye contact	-	NG tube (no)	Nasal CPAP (no)	No	No	No
8	c.1353+1G>A	Maternal	0.7	Eye contact	Social smile	NG tube (no)	BiPAP via T-can since 5 mo (no)	No	No	No
Hypoxic insults group (n = 5)										
9	c.342_342+4del	De novo	0.3	Eye contact	-	NG tube (no)	Nasal CPAP (no)	0.3	0.3	0.3
10	c.1261-10A>G	Maternal	1.1	Head control	Speech imitation	NG tube (no)	Mask BiPAP (yes, 1 hr before ARF)	1.1	1.1	1.1
11	c.342_342+4del	Maternal	1.1	Eye contact > bedridden	Unknown	NG tube (no)	BiPAP via T-can since 2 mo (no)	0.5, 1	0.5	1.1
12	c.1262G>A (p.Arg421Gln)	De novo	3.3	Eye contact, hand use	Non-verbal (preference)	PEG since 1 yr (yes)	BiPAP via T-can since 1 yr (no)	No	Unknown	No
13	c.566A>G (p.Asn189Ser)	Maternal	4.5	Sitting with assistance > incomplete head control	Non-verbal > none	PEG since 2 yr (no)	BiPAP via T-can since 2 mo (yes, 1 hr → no after ARF)	2	2	No

NMIM accession numbers: NM_000252.3 and NP_000243.1. XLMTM, X-linked myotubular myopathy; ARF, acute respiratory failure; HIE, hypoxic-ischemic encephalopathy; PEG, percutaneous endoscopic gastrostomy; BiPAP, bilevel positive airway pressure; NG, nasogastric; T-can, T-cannula; CPAP, continuous positive airway pressure.

agement of respiratory emergencies. Emergencies are more likely to occur at a younger age; thus, close attention is required [13]. Repeated training with time intervals might be helpful.

Genotype-phenotype studies have shown that most pathogenic *MTM1* variants, regardless of the mutation type, resulted in loss-of-function effects, leading to the classic and severe phenotype [1,14,15]. Our cohort included all reported pathogenic variants, which were identified as three missense mutations and 10 loss-of-function mutations, including deletion, nonsense, frameshift, and splice-site mutations [16,17]. Additionally, a phosphatase domain in exon 11 is critical for maintaining protein function, but our cases did not include a variant located in the phosphatase domain [14]. In the non-HI group, only one patient (no. 2) with a missense mutation (c.679G > A [p.Val227Met]) walked alone without respiratory assistance, consistent with a mild phenotype. Although patient no. 1 had a frameshift mutation, he presented a mild phenotype. Because the frameshift mutation was located at the end of the *MTM1* gene (exon 15), it would be expected to produce a relatively stable protein. All patients assisted with BiPAP had loss-of-function mutations, and the duration of BiPAP support differed among patients. Because patients no. 7 and no. 8 were < 1 year of age, the serial follow-up will reveal their maximum motor achievement and amount of ventilator support. The HI group had two missense and two splice-site mutations, and the accordance data did not associate survival with these mutation types [1,14].

In the non-HI group, swallowing and self-respiratory function gradually improved. In two patients, the transition to a complete oral diet was made relatively early, at 5 and 9 months. Some patients gradually tried oral intake in small amounts in childhood. Similarly, except for two patients discharged without a ventilator in the neonatal period, other patients were stable only for a few hours on room air without respiratory support. Complete recovery of swallowing and self-respiratory function was particularly difficult. Nevertheless, even a small amount of oral challenge and attempt at short spontaneous breathing without a ventilator yielded positive effects. The challenges were aimed not at achieving complete normal function but at improving the patients' quality of life by being able to tasting food and broadening the scope of daily activity. Therefore, it is crucial to encourage rehabilitation for patients.

Multidisciplinary therapeutic approaches are emphasized, including those related to neurology, neonatology, pulmonology, gastroenterology, rehabilitation medicine, and orthopedic surgery [6,7,18,19]. The decision to conduct a diagnostic workup involving a muscle biopsy for infants with hypotonia is sometimes difficult owing to a few factors, including the general anesthesia required for invasive procedures, the risk of respiratory complications, and occasional non-specific results. Except in cases of spinal

muscular atrophy, which is routinely diagnosed through genetic investigations without the need for histopathological results, the roles of a muscle biopsy are to classify the specific disease category, help clinicians choose a genetic test, and/or modify a previous diagnosis [20]. Patients with congenital myopathy present a higher concordance rate between biopsy and genetic findings than those with congenital muscular dystrophies and metabolic myopathies. Although the genetic era of neuromuscular diseases has conspicuously developed, muscle biopsy remains a valuable tool guided by rational diagnostic algorithms. Regarding treatment, clinicians should provide proper management during serial follow-up, including routine assessments of skeletal and respiratory muscle function, speech therapy for pronunciation, and the application of devices for independent walking and scoliosis depending on the patients' ages.

There have been recent moves towards therapeutic strategies [14]. Medical approaches suggested from *Mtm1* null mouse models include phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 beta (PIK3C2b) inhibition and mammalian target of rapamycin (mTOR) modulation [21,22]. Moreover, in light of proven efficacy in animal models, several clinical trials of gene therapy have been initiated, including gene replacement therapy (ASPIRO, NCT03199469) and ASO-based gene knockdown (Unite-CNM, NCT04033159). The gene transfer study (ASPIRO, NCT03199469) included 26 patients < 6 years of age who required mechanical ventilator support. A clinical study of the ASO-based RNA knockdown (Unite-CNM, NCT04033159) is recruiting patients > 15 years of age with identified *MTM1* or dynamin 2 (*DNM2*) mutations. The study also has an upcoming plan for children 2 to 16 years of age. Another study, focused on tamoxifen therapy for XLMTM patients (TAM4MTM, NCT04915846), is recruiting patients > 1 year of age. Tamoxifen is expected to reduce *DNM2* expression, resulting in changes in the triad structure and improvement of muscle contractility [23,24]. To facilitate enrollment in upcoming clinical trials, it is important to collect patients in Korea and evaluate their conditions.

However, this study had several limitations. First, it did not have a substantial degree of variation in age distribution due to the small size of the cohort. Second, data on developmental milestones were retrospectively collected from the descriptive medical records. Third, for an accurate evaluation of motor function improvement and deterioration, it is necessary to adopt measurement scales suitable for each patient's age.

Patients with XLMTM without HI can be long-term survivors with the slow achievement of motor milestones and bulbar and respiratory function. However, hypoxic brain insults following acute respiratory failure are significant events that negatively influence

the developmental potential or even lead to death. Therefore, parental education for proper respiratory management is necessary, especially when children are at a young age.

Conflicts of interest

Anna Cho, Ki Joong Kim and Jong-Hee Chae are the editorial board members of the journal, but They 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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A *SERPINC1* Mutation in a Patient with Cerebral Venous Thrombosis and Upper-Extremity Deep Vein Thrombosis

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Cerebral venous thrombosis (CVT) in children is rare, with an incidence of 0.67 per 100,000 children, including newborns [1]. Its clinical manifestations may vary, including seizures, papilledema, headache, loss of consciousness, coma, and local neurological defects. The most common manifestation in neonates is seizures, whereas headache predominates in non-neonatal age groups [2]. Hereditary thrombophilia causes 34% to 41% of CVT cases, within which antithrombin (AT) deficiency is rarely reported [3]. Here, we report a case of CVT, pulmonary thromboembolism (PTE), and upper-extremity deep vein thrombosis (DVT) associated with AT deficiency due to a novel missense mutation in *SERPINC1*.

A 16-year-old boy visited our emergency department with a 5-day history of headache, nausea, and vomiting. The patient's height and weight were 178 cm and 90 kg, respectively. His body mass index was 28.4 kg/m², indicating obesity. He had a history of taking psychiatric medication for intermittent explosive disorder at a private hospital 1 year ago. Without his parents, the family history of CVT was unknown. On admission, the patient was conscious and no specific findings were noted. His vital signs were as follows: blood pressure, 121/70 mm Hg; pulse, 72 beats/min; respiratory rate, 20 breaths/min;

body temperature, 36.3°C. A neurological examination revealed cervical rigidity. The hemoglobin level (15.6 g/dL), white blood cell count (12,640/mL), platelet count (280,000/mL), prothrombin time (12.3 seconds), and activated partial thromboplastin time (23.9 seconds) were normal. Cerebrospinal fluid analysis showed 12 red blood cells/mm³ and 8 white blood cells/mm³, with protein and glucose concentrations of 58 and 73 mg/dL, respectively, and an opening pressure of 38 mm Hg. An ophthalmologic examination for papilledema was not performed. Brain magnetic resonance imaging demonstrated bilateral thalamic infarction and multifocal petechial hemorrhages around the bilateral cortical veins, while brain magnetic resonance venography (MRV) detected right transverse/sigmoid sinus thrombosis and superior/inferior sagittal sinus thrombosis (Fig. 1A and B). Chest computed tomography with contrast revealed thrombi in the jugular vein, subclavian vein, brachiocephalic vein, and proximal superior vena cava on the right side and a filling defect in the distal portion of the main pulmonary trunk and bilateral segmental pulmonary arteries (Fig. 1C).

The autoimmune test results were negative, with the following results: factor VIII, 129.9% (60%–140%); factor IX, 151.2% (60%–140%); factor XI, 90% (60%–140%); protein C Ag,

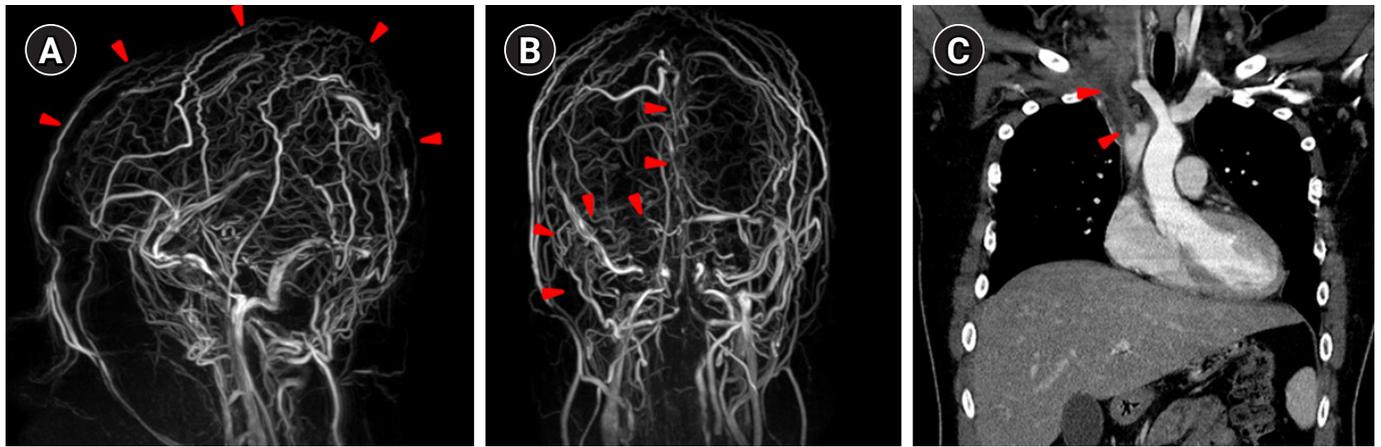


Fig. 1. Extensive cerebral venous thrombosis and upper-extremity deep vein thrombosis. Contrast-enhanced magnetic resonance venography images of the lateral view (A) and posterior view (B) showed extensive filling defects along the superior/inferior sagittal sinuses and right transverse/sigmoid sinuses (arrowheads). (C) Chest computed tomography with contrast revealed thrombi in the jugular vein, subclavian vein, brachiocephalic vein, and proximal superior vena cava on the right side (arrowheads).

131.07% (72%–160%); and protein S Ag, 123.71% (60%–150%). AT III was reduced to 62.2% (75%–125%). The amino acid and urine organic test results were negative. The homocysteine level was elevated to 17.3 $\mu\text{mol/L}$ (0 to 15), whereas ADAMTS13 activity was 83.5% (>40%). No methylenetetrahydrofolate reductase (MTHFR) A1298C or C677T polymorphisms were found. Additionally, no mutation in the *JAK2V617F* gene was detected. Gene panel testing for hereditary blood coagulation disorder was performed, and the patient was found to be heterozygous for a c.409-1G>T mutation in the *SERPINC1* gene. The *SERPINC1* gene is related to thrombophilia seven due to AT III deficiency (OMIM 613118, autosomal dominant or recessive), and a loss-of-function mutation of this gene is established as a mechanism of disease. The c.409-1G>T variant is a canonical –1 splice variant that is assumed to cause aberrant splicing and has not been detected in the general population (gnomAD, Korean Reference Genome Database [KRGDB]). The same variant has been reported in a family with AT deficiency [4], and the clinical phenotype and laboratory findings of our patient are consistent with AT deficiency. Although it was not possible to determine whether this mutation was inherited or de novo due to the lack of a parental test, this c.409-1G>T variant was interpreted as a pathogenic variant according to the American College of Medical Genetics and Genomics guideline (2015) on the basis of the above findings [5].

Starting on the first day of hospitalization, the patient was treated with subcutaneous low-molecular-weight heparin (1 mg/kg) for anticoagulation and osmotic agents (mannitol and hypertonic saline) for increased intracranial pressure. The patient's headache improved on the 10th day of hospitalization. A direct oral anticoagulant (apixaban) was administered on the 25th day of hospitaliza-

tion. Brain MRV on the 24th day after admission demonstrated resolution of almost all previously observed multifocal cerebral thrombi.

To our knowledge, this is the first case report in Korea to describe CVT, PTE, and extensive upper-extremity DVT associated with a *SERPINC1* gene mutation. The glycoprotein, AT (58 kDa), belongs to the serine protease superfamily and is a potent inhibitor of many coagulation proteases, including thrombin and factor Xa. Deficiency of AT is a risk factor for venous thromboembolism and has a prevalence of 1% to 5% in patients with venous thrombosis and 0.2% in the general population [6]. Congenital AT deficiency is an uncommon autosomal dominant disorder, while acquired AT deficiency can be attributed to decreased AT production, increased protein consumption, or enhanced clearance [7]. The major clinical manifestations of AT III deficiency are young age at onset, idiopathic venous thrombosis, family history, recurrent venous thromboembolism, and thrombosis in an unusual site (cerebral or mesenteric veins) [8]. In our case, the patient had extensive idiopathic thrombosis, including CVT and DVT, but he had no typical risk factors of age of onset and thrombotic events, such as surgery, immobility, or trauma. In pediatric cases in Korea, one study reported venous thrombosis in the lower extremities caused by simultaneous AT III and protein S deficiencies, while another study reported neonatal cardiac thrombosis and CVT due to AT III deficiency [9,10]. In our patient, CVT, PTE, and upper extremity DVT were observed in the absence of specific risk factors, and these conditions were associated with AT deficiency that was genetically confirmed. Therefore, genetic testing should be considered even when there are no risk factors for patients with extensive thrombosis.

This case was reviewed and approved by the Institutional Review Board of Keimyung University Dongsan Hospital (IRB No. 2022-02-096). Informed consent was waived by the board.

Conflicts of interest

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

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X-Linked Cerebral Creatine Deficiency Syndrome with Prolonged QT Interval: A Case Report

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Creatine is a metabolite that plays an important role in maintaining brain, heart, and muscle function [1]. It is synthesized in the kidney, liver, and pancreas by arginine glycine acyltransferase (AGAT, chromosomal location 15q15.1) and guanidinoacetic acid methyl transferase (GAMT, chromosomal location 19p13.3), and it is transported to the brain and muscle by the creatine transporter SLC6A8 [1]. Creatine is metabolized by creatine kinase to produce adenosine triphosphate, which maintains organ function [1]. Cerebral creatine deficiency syndromes (CCDS) are classified into three types: two autosomal recessive types, in which mutations in the peptide sequence of either AGAT or GAMT disrupt creatine synthesis, and an X-linked type, in which the creatine transporter SLC6A8 is deficient [2]. Intellectual disability, seizures, and speech delays are commonly observed in CCDS-affected individuals [3]. Intractable epilepsy and early global developmental delay with autistic behavior have also been observed in severe cases [3]. The diagnosis of CCDS is based on the measurement of guanidinoacetate, creatine, and creatinine in plasma and urine, as well as genetic testing [1]. Creatine deficiency in the brain detected by proton magnetic resonance spectroscopy (¹H-MRS) is a characteristic finding of CCDS [1]. Here, we describe a case of CCDS caused by creatine

transporter deficiency presenting with long QT syndrome and a large arachnoid cyst in the frontal lobe.

A 7-year-old boy presenting with bilateral tonic-clonic seizures, intellectual disability, and hyperactivity was referred to our pediatric clinic. He had been delivered at 40 weeks of gestation without pre- or post-partum complications. The birth weight was small for the gestational age (2,600 g, third percentile). The parents were healthy and nonconsanguineous, with no history of neurological or genetic disorders. The patient had a history of complex febrile seizures starting at 14 months of age with cognitive, speech, and motor delays. He could not speak any words until the age of 7 years. A physical examination revealed a short stature (114.6 cm, second percentile), low body weight (15.8 kg, less than the first percentile), and microcephaly (48 cm, less than the first percentile). The patient had dysmorphic facial features, including a triangular face, flat forehead, downward slanting palpebral fissures, and protruding mouth. Laboratory findings (complete blood count, serum electrolyte and glucose concentrations, hepatic and renal function tests, and routine urinalysis) were normal. Metabolic tests for lactic acid, creatine kinase, ammonia, plasma amino acids, and urine organic acids were normal; but a test for thyroid function

revealed hypothyroidism. A large arachnoid cyst in the right frontal lobe was identified via brain magnetic resonance imaging (MRI) (Fig. 1A); however, electroencephalography (EEG) results were normal. Peripheral blood chromosome analysis and a chromosomal microarray test revealed no abnormalities. Whole-exome sequencing (WES) was performed to identify potential genetic abnormalities that could cause intellectual disability, seizures, and dysmorphic facial features. Written informed consent was obtained from the parents, and the study was approved by the Institutional Review Board of Busan Paik Hospital (Busan, Korea; approval number: 2021-07-002). The results of WES revealed the presence of a hemizygous frameshift *SLC6A8* variant, NM_005629: c.626_627delCT (p.Pro209ArgFs*87) that was also found to be carried by the patient's mother. This result was confirmed by direct sequencing (Supplementary Fig. 1). Since there was no specific family history in the maternal family and no family members showed phenotypes such as intellectual disability or seizures, additional genetic testing of the maternal family was not performed. The patient's brain ¹H-MRS revealed a decreased creatine peak in the corona radiata and basal ganglia (Fig. 1B). He was therefore diagnosed with an X-linked creatine transport disorder caused by a pathogenic variant of *SLC6A8* and was started on drug therapy comprising creatine monohydrate (100 mg/kg/day), L-arginine (400 mg/kg/day), and L-glycine (150 mg/kg/day). These supplements failed to improve his cognitive or behavioral problems and were eventually discontinued.

At age 9, the patient experienced repetitive bilateral tonic-clonic seizures that were well controlled with oxcarbazepine treatment. A new brain MRI revealed that the arachnoid cyst had increased in size and displaced the right optic nerve, but an ophthalmologic examination was normal, and an additional brain imaging session was scheduled for a year in advance as follow-up, without any neurosurgical treatment. EEG results identified intermittent spikes in the right frontal area. Unexpectedly, a prolonged QT interval (QTc time 488 ms; reference range, 350 to 440) was detected on an electrocardiogram (ECG) (Fig. 2). The patient had never been treated with any QT-prolonging drug. Long QT syndrome was finally diagnosed following a test with a Holter monitor. The patient was placed under close observation, but no beta-blocker medication was prescribed due to his young age.

The CCDS diagnosis in this patient presented difficulties because the clinical manifestations, such as seizures, intellectual disability, and facial dysmorphism, were non-specific. As we were unable to find an etiology for our patient using conventional cytogenetic methods, chromosomal microarray, or metabolic tests, we resorted to WES and were able to make a diagnosis of creatine transporter deficiency caused by a frameshift variant in *SLC6A8*. His mother was a carrier of this variant. After genetic diagnosis, brain ¹H-MRS demonstrated a specific decrease in creatine levels in the brain of the patient. Analyses of urinary guanidinoacetate and the creatine/creatinine ratio are important screening tests for all CCDSs [1]. In patients with *SLC6A8* deficiency, the increase in

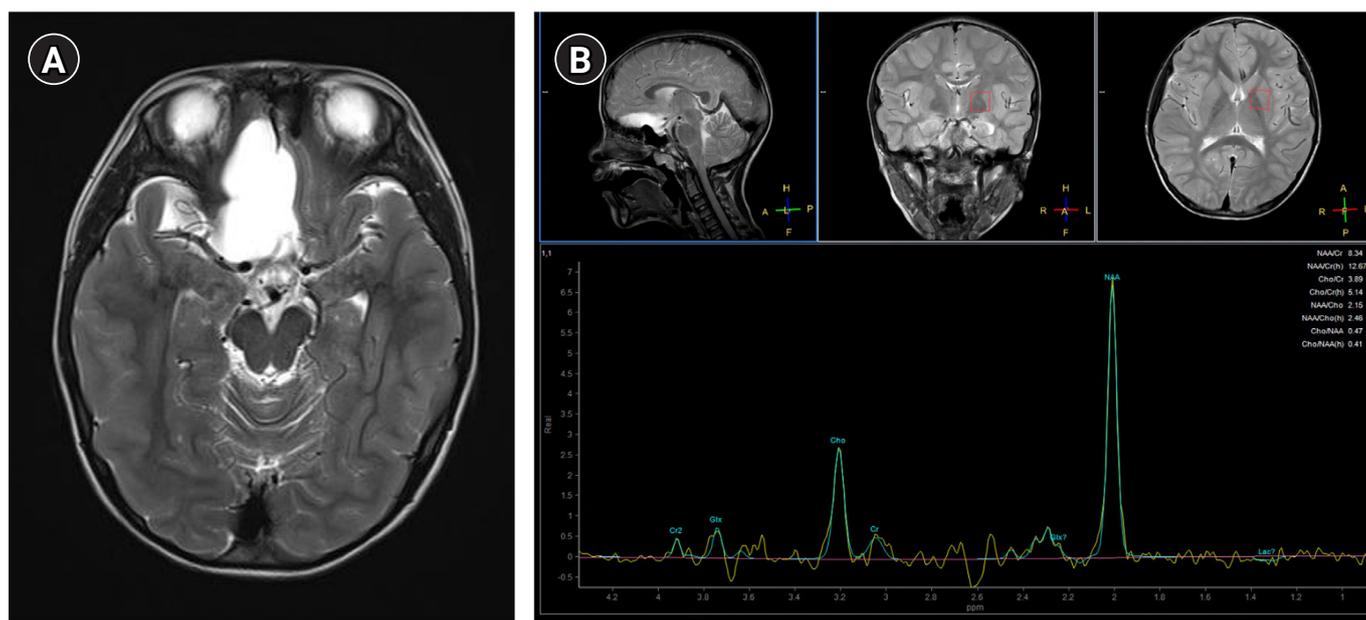


Fig. 1. (A) T2-weighted axial brain magnetic resonance imaging showing a focally enlarged arachnoid cyst located in the right inferomedial frontal area. (B) Proton magnetic resonance spectroscopy results showing a decreased creatine peak in the regions of interest of the corona radiata and basal ganglia.

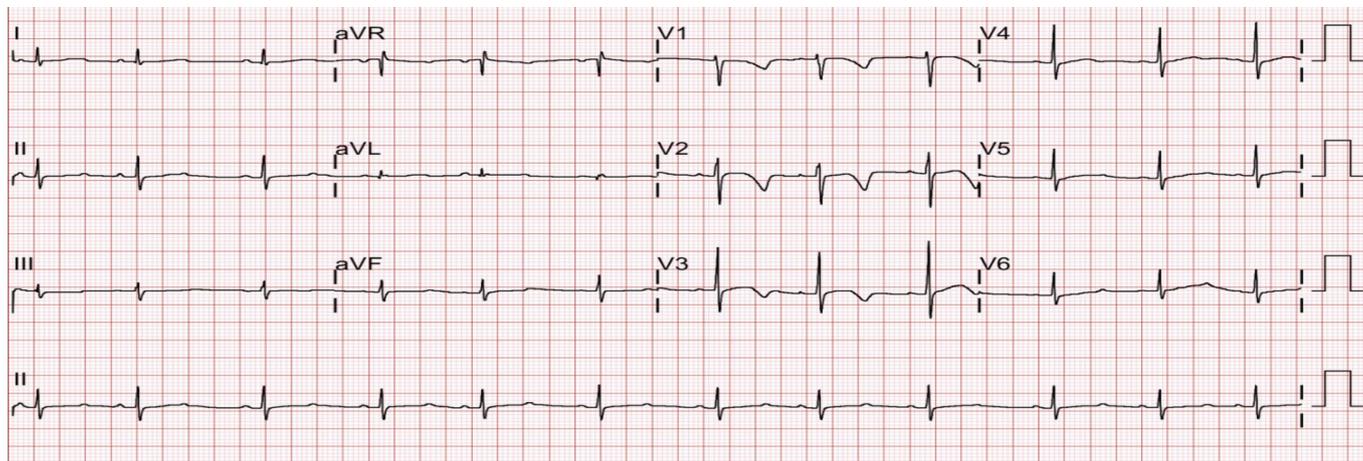


Fig. 2. Electrocardiogram results showing a prolonged QT interval (QTc 488 ms).

urinary creatine excretion together with the inherently low urinary creatinine excretion results in an elevation of the urinary creatine/creatinine ratio, which serves as a valuable diagnostic marker in male patients [4,5]. We could not measure urinary creatine levels in our patient because this test was not available at our institution. However, the decreased creatine level in the brain and genetic testing revealing a mutation in *SLC6A8* were sufficient to confirm the diagnosis of creatine transporter deficiency.

Long QT syndrome was also detected from the prolongation of the QTc interval in our patient's ECG results. Several genes (*KCNQ1*, *KCNH2*, *SCNSA*, *KCNE1*, and *KCNE2*, among others) are associated with long QT syndrome [6]. However, none of these genetic mutations were detected in our patient. A few studies have reported cardiac syndromes in patients with creatine transporter deficiency. van de Kamp et al. [7] described one patient with long QT syndrome, two patients with mild cardiomyopathy, and one patient with multiple premature ventricular contractions in their retrospective study of 101 male individuals with X-linked creatine transporter deficiency. Levin et al. [8] recently reported seven patients with QTc prolongation in a sample of 18 patients with creatine transporter deficiency. The same authors also demonstrated that long QTc was associated with cardiac dysfunction and sudden death in an animal model for creatine transporter deficiency (*Slc6a8*^{-/-} mice). Because expression of the *SLC6A8* gene is high in skeletal muscle, heart, and kidney, cardiac problems could be expected in patients with creatine transporter deficiency [9]. Although the exact mechanism is currently unknown, it can be hypothesized that the *SLC6A8* gene is somehow involved in the physiology of heart muscle. Therefore, cardiac evaluations such as ECG and echocardiography are necessary in children with creatine transporter deficiency to detect potential cardiac involvement.

CCDS treatment can correct cerebral creatine depletion [2].

Oral supplementation of creatine can be administered to maximize the delivery of creatine into the brain, together with creatine precursors such as L-arginine and L-glycine [2]. However, the value of creatine supplementation in patients with creatine transporter deficiency is unclear because delivery into the brain is not efficient enough to fully compensate for the creatine depletion [2]. Our patient failed to show any clinical improvement in behavior, attention, or cognitive problems associated with the syndrome in response to creatine, L-arginine, and L-glycine supplementation. Since there may be some residual transporter activity, creatine supplementation is generally recommended for all new patients, but definite success has not been reported [1].

To our knowledge, this is the first case of creatine transporter deficiency reported in Korea. Based on our experience, we conclude that the urinary creatine/creatinine ratio and brain MRI scanning with spectroscopy can be useful tools to screen male patients with intellectual disability and seizures of unknown origin for creatine transporter deficiency. Furthermore, it is to be expected that advances in next-generation sequencing will facilitate the diagnosis of creatine transporter deficiency. Cardiac evaluation and monitoring are necessary because these patients are likely to have cardiac problems such as long QT syndrome and cardiomyopathy.

Supplementary materials

Supplementary materials related to this article can be found online at <https://doi.org/10.26815/acn.2022.00031>.

Conflicts of interest

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

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A Case of Intellectual Disability without Epilepsy Associated with a Pathogenic Variant of *STXBP1*

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Syntaxin-binding protein 1 (*STXBP1*) is a representative gene related to intractable epilepsy. Pathogenic variants of *STXBP1* cause a phenotype of developmental and epileptic encephalopathy 4 [1-4]. Diagnostic tools for pathogenic variants of *STXBP1* have recently been developed using genetic tests such as targeted gene panels or whole-exome sequencing [5]. The disease phenotype comprises early-onset seizures, including tonic spasms, a suppression-burst pattern on electroencephalography (EEG), and profoundly impaired intellectual development. Subsequently, most patients progress to intractable epilepsy, such as West syndrome or Lennox-Gastaut syndrome [1,2]. Progression to Dravet syndrome, as well as classic or atypical Rett syndrome, has also been reported [1,3]. Various anti-seizure medications (ASMs) have been used to control seizures in patients with *STXBP1* mutations, but most become refractory to conventional ASMs [2,4]. However, patients with *STXBP1* mutations who have intellectual disabilities in the absence of epilepsy have rarely been reported [6]. This report presents a case of intellectual disability without epilepsy associated with an *STXBP1* mutation. This study was approved by the Institutional Review Board of the Gangnam Severance Hospital, Yonsei University College of Medicine (3-2017-0168). Informed

consent for this retrospective study was waived by the board.

The case concerns a 14-year-old boy with no specific history at birth, but with global developmental delays during childhood. He was unable to walk independently at 12 months and to say “mama” and “dada” until 2 years of age. Brain magnetic resonance imaging findings revealed minimal corpus callosum hypoplasia, and genetic tests for Fragile X syndrome and Prader-Willi syndrome were negative. There was no seizure history, and no epileptogenic foci were observed on EEG. In the metabolic work-up, mitochondrial dysfunction was suspected from a urine organic acid test due to the presence of elevated levels of citric acid cycle intermediates such as ethylmalonic acid, succinate, and citrate, and no pathologic findings were found on muscle biopsy. Complex I deficiency was diagnosed in a biochemical analysis. The patient was diagnosed with severe intellectual disability with autistic features and mitochondrial dysfunction; he was administered psychiatric medication along with multiple vitamins, such as vitamin B, vitamin C, ubiquinone, thiamine, and L-carnitine, and received cognitive development therapy.

With recent advances in genetic diagnostic tools, next-generation sequencing of the patient’s blood sample revealed a pathogenic variant of

STXBP1 (nucleotide NM_003165.3:c.325+2_325+3delTG), which was confirmed as a *de novo* variant in the Trio test. *De novo* pathogenic variants of *STXBP1* cause early-onset neurocognitive conditions, early infantile epileptic encephalopathy type 4 (EIEE4, also known as *STXBP1* encephalopathy), epilepsy, developmental delay, and intellectual disability [7]. The patient recently had symptoms of seizure-like movements, such as vacant staring, brief motion arrests, and involuntary tremor-like behaviors. In a long-term video EEG, none of the abnormal behaviors reported by the parents were associated with epileptic discharges. In addition, no other seizure-related findings or epileptogenic EEG findings were observed. Only slow waves with a disorganized background rhythm were observed, mainly in both frontal areas; this finding was very different from most phenotypes of the pathogenic variant of *STXBP1* (Fig. 1). The typical EEG findings of *STXBP1* encephalopathy are characterized by focal epileptic activity, burst suppression, hypsarrhythmia, or generalized spike and slow waves. Most pathogenic variants of *STXBP1* are accompanied by intractable epilepsy, and the gene was first described in patients with severe epileptic encephalopathy [1,8]. The review by Stamberger et al. [6] indicated that 140 of 147 patients (95%) with *STXBP1* mutations had intractable seizures.

Intractable epilepsy caused by a pathogenic variant of *STXBP1* not only has a negative developmental impact on patients, but is also sometimes life-threatening. Therefore, many pediatric neu-

rologists treat seizures by attempting a ketogenic diet, palliative care, or resective surgery, as well as using various ASMs [1,8].

A phenotype in which the *STXBP1* mutation is associated with severe intellectual disability, but not associated with epilepsy, has rarely been reported. Hamdan et al. [9] reported the first case of a patient with a truncating mutation in *STXBP1* who did not show epilepsy. Gburek-Augustat et al. [10] also reported three female patients with ataxia-tremor-retardation syndromes caused by a *de novo* *STXBP1* mutation. Their reports are similar to the case presented here, showing that the spectrum of *STXBP1* presentation can be broad.

As in this case, the *STXBP1* pathogenic variant can be diagnosed in cases of intellectual disability, autistic spectrum disorder, ataxia, and dystonia. Therefore, there is a need to consider the *STXBP1* pathogenic variant in the differential diagnosis for patients with moderate to severe developmental delay. Early genetic diagnosis and targeted treatment plans will become more important in the future, with further developments in technology. The correlation between various pathogenic variants of *STXBP1* and the epilepsy phenotype has not been established, and further studies and observations are needed in the future [1]. The observation of this phenomenon expands the clinical spectrum associated with pathogenic variants of *STXBP1*, and further genetic functional studies on *STXBP1* are needed.



Fig. 1. Electroencephalography (EEG) of the patient with a pathogenic syntaxin-binding protein 1 (*STXBP1*) variant. The background of EEG was mildly slow and disorganized mainly in both frontal areas, without any epileptic discharges, and normal sleep tracing was recorded. In addition, there were no seizure-related findings or epileptogenic findings on EEG.

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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SARS-CoV-2 Neurotropism in a 12-Year-Old Filipino Boy with Focal Encephalitis

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Since the onset of the coronavirus disease 2019 (COVID-19) pandemic, there have been reports of neurological manifestations of COVID-19, mostly in adults, albeit with a few cases described in children [1-3]. Several mechanisms have been proposed to explain how severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may induce neurological damage. There is emerging evidence supporting neurotropism and neuroinvasion in COVID-19, with polymerase chain reaction (PCR) testing of the cerebrospinal fluid (CSF) for SARS-CoV-2 yielding positive results in 6% to 13% of adult and pediatric patients according to a review of the literature [1,4,5].

This report discusses a case of focal encephalitis presenting with seizure and altered mental status following fever and mild respiratory illness associated with COVID-19. A notable aspect of this case was the detection of SARS-CoV-2 RNA in the CSF, despite its absence in two consecutive nasopharyngeal swab specimens. Written informed consent for publication was obtained from the patient's parents.

A previously well 12-year-old Filipino boy, presenting with a 2-day history of chills and undocumented fever, developed seizures and an altered sensorium on the 3rd day of illness. The seizures were described as focal motor right versive seizures with secondary generalization. He

arrived at a nearby hospital in active seizure and was given two doses of diazepam intravenously. Post-ictus, the patient was drowsy but arousable, able to follow commands, and oriented to place and person. The neurological examination was generally unremarkable, except for manual muscle testing of 3/5 on all extremities. A complete blood count was normal, and COVID-19 nasopharyngeal rapid antigen and dengue immunoglobulin G (IgG) and IgM tests were negative. A chest radiograph showed bilateral pneumonia. An axial helical non-contrast multi-detector computed tomography scan of the brain done on day 3 of symptoms revealed edema of the right hemisphere (Fig. 1).

Upon transfer to our institution, the patient was highly febrile with fine crackles on both lower lung fields. Further probing revealed exposure to his father, who had a recent fever and respiratory symptoms but was untested for COVID-19. The patient was drowsy but arousable and was able to follow commands, albeit with slowed responses. He had intact remote memory, good recall, and fair attention, with left central facial palsy, mild left pronator drift, and a positive Oppenheim test on the left. He did not present with sensory deficits or signs of meningeal irritation. Laboratory tests revealed elevated high-sensitivity C-reactive protein (9.94 mg/L) and procalcitonin.

tonin levels (30.96 µg/L). Ceftriaxone was started for pneumonia and levetiracetam to manage seizures. Two SARS-CoV-2 reverse-transcription polymerase chain reaction (RT-PCR) tests of nasopharyngeal swab specimens performed 48 hours apart were negative.

CSF analysis done on the 4th day of illness revealed increased opening and closing pressures, no pleocytosis, and normal protein and glucose levels. RT-PCR testing of the CSF for SARS-CoV-2 was positive. Aerobic culture, latex agglutination test, and viral studies for herpes simplex virus and Japanese encephalitis were negative. During the succeeding hospital days, the patient was more alert, with no new neurological deficits; however, his fever and respiratory symptoms did not improve. The management of COVID-19 was escalated according to local guidelines.

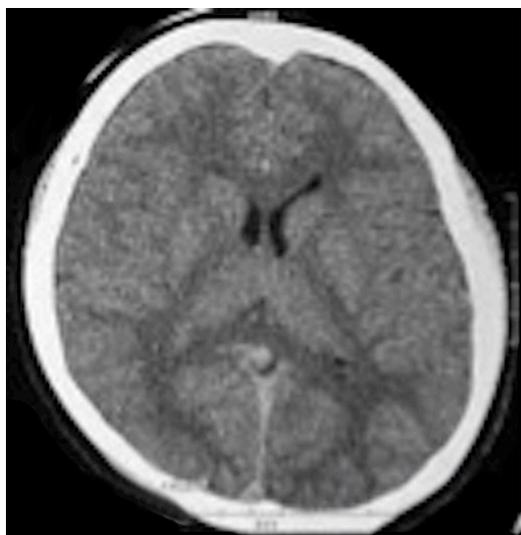


Fig. 1. Axial helical multi-detector computed tomography scan of the brain obtained on day 3 after symptom onset. There is subtle effacement of cortical sulci in the right cerebral hemisphere and mild compression of the right lateral ventricle. There was no hydrocephalus or cerebral herniation.

Electroencephalography showed slower background activity on the right. There was 2 to 3 Hz rhythmic delta activity seen in the right fronto-temporal head region, as well as occasional admixed spike discharges, with the highest electronegativity at right prefrontal electrode (Fp2). Occasional intermittent spikes, spike, and slow-wave discharges were also seen over the left frontal head region, with the highest electronegativity at left prefrontal electrode (Fp1).

Magnetic resonance imaging of the brain (Fig. 2) obtained on day 8 of symptoms showed findings consistent with meningoencephalitis commonly seen in viral diseases. The patient was discharged for home isolation with clinical improvement and afebrile status on the seventh hospital day. A neurological examination revealed more prominent facial asymmetry, spasticity of left-sided extremities, and, aside from left pronator drift, mild weakness of the left lower extremity. Physical rehabilitation was done after completing isolation, 3 weeks after admission. A quantitative COVID-19 antibody test via electrochemiluminescence immunoassay performed during follow-up in the 3rd week after illness onset was reactive for IgG anti-SARS-CoV-2 and non-reactive for IgM anti-SARS-CoV-2. A neurological examination 8 months after discharge showed resolution of motor deficits. The patient has since returned to school.

Although primarily considered a virus impacting the respiratory system, several case studies have highlighted substantial neurological sequelae of SARS-CoV-2 infection [1,2]. The most frequently reported neurological symptoms in pediatric patients were headache, altered mental status, seizures, muscular weakness, and meningism [1]. In a study by LaRovere et al. [3], neurological symptoms were broad and varied by age—seizures/status epilepticus were noted in younger patients, while anosmia, ageusia, headache, fatigue, and weakness were more frequent in older patients. Across all pediatric age groups, approximately one in four patients presented with confusion or altered sensorium. In the

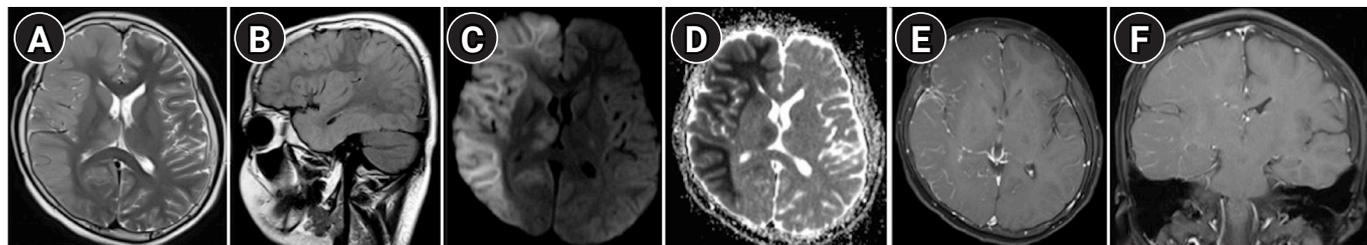


Fig. 2. Magnetic resonance (MR) imaging of the brain obtained on day 8 after symptom onset. (A) Axial T2-weighted and (B) sagittal T2-Fluid-attenuated inversion recovery MR images show abnormal hyperintense signals in the cortex-subcortex of the right cerebral hemisphere, head of the right caudate nucleus, and right thalamus. (C) Axial diffusion-weighted imaging and corresponding (D) apparent diffusion coefficient maps at the same level revealed restricted diffusion in the subcortical white matter of the right cerebral hemisphere. (E) Axial and (F) coronal post-gadolinium fat-suppressed T1-weighted imaging showed diffuse leptomeningeal enhancement in the right cerebral hemisphere.

Philippines, the most frequently reported symptoms are seizures, anosmia, and increased sleeping time. Patients are susceptible to seizures by various mechanisms. New-onset seizures can be triggered by metabolic derangements from systemic complications, while breakthrough seizures can occur in established epilepsy. Ischemic, inflammatory, or direct viral encephalitis can cause a structural insult to the brain, resulting in seizures, as seen in our patient.

Despite numerous reports of neurological manifestations in pediatric COVID-19, few studies have provided evidence to support the neurotropism of COVID-19 by detecting SARS-CoV-2 RNA in CSF. In a literature review by Carroll et al. [4], 13% of patients with COVID-19 and seizures had positive CSF SARS-CoV-2 PCR results, and in a review by Siracusa et al. [1], SARS-CoV-2 RNA in the CSF was only observed in two out of 26 (7.7%) pediatric COVID-19 patients with neurological manifestations, with one case associated with a negative RT-PCR nasopharyngeal swab. In a literature review by Lewis et al. [5] on CSF findings in the setting of COVID-19, CSF SARS-CoV-2 PCR was positive for 17 out of 303 patients (6%), all of whom had symptoms localized to the central nervous system (CNS).

The presence of SARS-CoV-2 RNA in the CSF, despite two negative nasopharyngeal swabs done 48 hours apart, is evidence of acute COVID-19 infection. The first nasopharyngeal swab was done on the 3rd day of symptoms. Kucirka et al. [6] reported that the sensitivity of RT-PCR was highest (80%) 3 days after symptom onset. The patient's second swab was done on the 5th day, with sensitivity still presumed to be high. It is still unclear why both swabs had negative results, despite the patient's respiratory symptoms. In the study by Lewis et al. [5], five out of 16 CSF-positive patients (31%) did not have a positive nasopharyngeal/oropharyngeal SARS-CoV-2 PCR test, but one had positive SARS-CoV-2 serum antibodies. It has been postulated that SARS-CoV-2 may be more persistent in the CNS because it is an immunoprivileged site [7].

In the Philippines, there have been no reports documenting SARS-CoV-2 in the CSF. The notable absence of SARS-CoV-2 in the CSF of most patients with neurological manifestations casts doubt on neurological manifestations resulting from direct invasion of the CNS. However, Lewis et al. [5] emphasized that CSF results change over the course of the patient's illness, as evidenced by patients who had more than one lumbar puncture performed. It has been suggested that the detection of viral neuroinvasion via a positive CSF PCR test is highest when the CSF is obtained 5 days after the onset of neurological symptoms [8]. Additionally, the sensitivity of viral detection via PCR testing is affected by the

virus's genetic variability and technical factors. Rapid CSF clearance, low titers, and the timing of collection also contribute to the challenge of isolating SARS-CoV-2 RNA from the CSF. Regardless, there must be a high index of suspicion for COVID-19 in patients with respiratory symptoms and neurological manifestations.

In our patient, aside from increased pressures, the CSF findings were otherwise normal. The absence of pleocytosis in general, and acellular CSF in particular, is atypical in the setting of viral encephalitis; however, Lewis et al. [5] reported that 10 out of 17 (59%) CSF SARS-CoV-2 positive patients lacked pleocytosis. There is the possibility of false-positive CSF SARS-CoV-2 PCR results in patients with acellular CSF. However, reports have described patients with positive CSF PCR for viruses such as enterovirus, echovirus, adenovirus, and herpes simplex virus in the absence of pleocytosis [9]. How the SARS-CoV-2 virus crosses the blood-brain barrier (BBB) is unclear. In a study by Zubair et al. [10], potential mechanisms of neuroinvasion included transsynaptic transfer across infected neurons, entry via the olfactory nerve, infection of the vascular endothelium, and leukocyte migration across the BBB. It has yet to be determined whether SARS-CoV-2 is transmitted directly to the CNS or carried to the CNS by infected circulating immune cells. It is also unclear whether its neurological manifestations are caused by direct replication in the CNS or neuronal injury promoted by virus-induced inflammation.

Previous reports have shown that positive findings for CSF SARS-CoV-2 PCR are infrequent, leading to the conclusion that neuroinvasion by SARS-CoV-2 may be rare. To the best of our knowledge, this is the first report in the Philippines of COVID-19-associated focal encephalitis in a child, supported by a positive result of SARS-CoV-2 RT-PCR in the CSF. The new-onset focal seizure and acute encephalopathy accompanied by definitive evidence of SARS-CoV-2 in the CSF highlights the neurotropism of SARS-CoV-2. The electroencephalographic and neuroimaging abnormalities found in our patient will necessitate follow-up to understand the impact of COVID-19 encephalitis on the neurodevelopment of affected children. More studies on pediatric COVID-19 patients with neurological symptoms are encouraged to further characterize SARS-CoV-2 as a potential emerging neurotropic virus.

Conflicts of interest

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

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A Rare Case of Anti-Ma2 Antibody-Mediated Autoimmune Encephalomyelitis in Childhood

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A 9-year-old boy with no relevant previous medical history was admitted to our clinic following 9 days of persistent fever and headache, followed by generalized motor weakness and an altered state of mentality. The patient was initially evaluated by a primary care physician and treated with antibiotics, although no improvement was observed. On his first visit to our hospital, he displayed a confused mental state, signs of meningeal irritation, and decreased motor power (grades I–II) in all extremities on neurologic examinations. Laboratory tests, including thyroid function tests, level of immunoglobulin, and complements, were unremarkable. Brain and spine magnetic resonance imaging (MRI) showed T2 fluid-attenuated inversion recovery (FLAIR) high signal changes in the basal ganglia, thalami, corpus callosum, brainstem, cerebellum, and entire spinal cord with leptomeningeal enhancement (Fig. 1A). Electroencephalography revealed excessive delta slow-wave discharges throughout the entire record (Fig. 1B). Cerebrospinal fluid (CSF) analysis presented lymphocytic pleocytosis (360/ μ L, lymphocytes 94%, opening pressure 17 cmH₂O), a high protein level (185 mg/dL), and a low glucose level (32 mg/dL). CSF bacterial culture was negative, as was a panel for herpes simplex virus, Epstein-Barr virus, and a FilmArray panel for meningitis/encephalitis. Due to a suspicion of infectious meningoenceph-

alitis, antibiotics with acyclovir were used until negative confirmation in the infectious workup.

This research was approved by the Medical Sciences Ethics Committee of Asan Medical Center (IRB file No. 2020-1331). Written informed consent by the patients was waived due to a retrospective nature of our study.

During testing, his mental status progressed to a stupor without response to pain. According to the neurologic examination and tests, we empirically diagnosed autoimmune encephalitis (AE) and initiated 1 g of high-dose methylprednisolone (mPD) pulse therapy within 24 hours of the patient's visit, and mannitol was used to control the increased intracranial pressure. On the third day of mPD pulse therapy, anti-Ma2 antibodies were detected in the serum, but not in the CSF. In contrast, other paraneoplastic antibodies, rheumatoid factor, antinuclear antibodies (ANA), dsDNA, anti-neutrophil cytoplasm antibodies (ANCA), aquaporin-4 (AQP4), anti-myelin oligodendrocyte glycoprotein (MOG), N-methyl-D-aspartate (NMDA), and anti-ganglioside antibodies were all negative. Considering the high risk of underlying malignancy in patients with anti-Ma2-associated AE, extensive tumor screening, including chest and abdomen computed tomography scans, testis ultrasonography, whole-body positron emission tomography-computed tomography, an ophthalmologic ex-

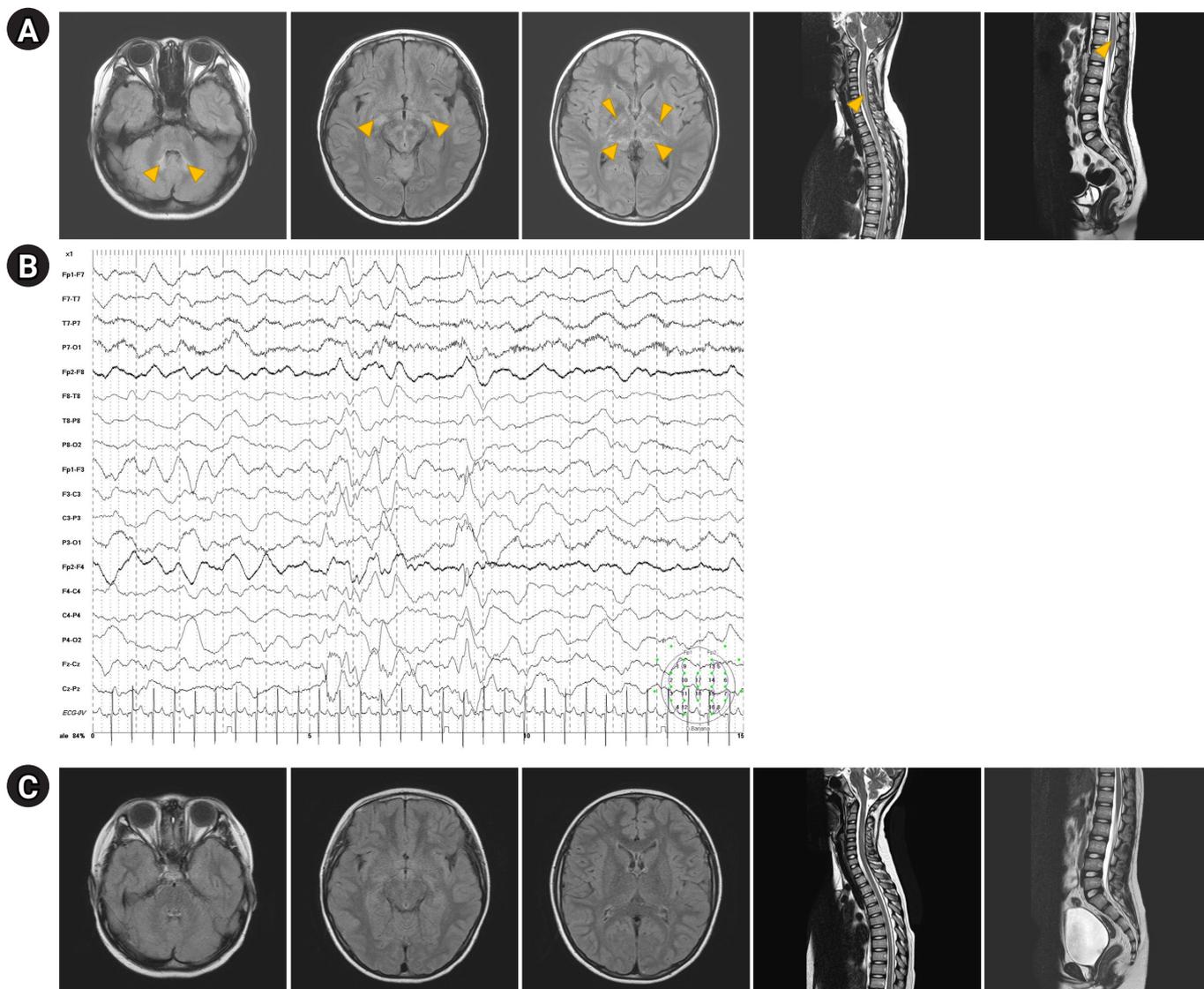


Fig. 1. (A) Brain and spine magnetic resonance imaging (MRI) (T2 fluid-attenuated inversion recovery [FLAIR]) at diagnosis show multifocal high signal intensity in both basal ganglia, thalami, cerebellum and brainstem, and diffuse longitudinally extensive hyperintensity involving the almost entire cord (yellow arrows in A). (B) Electroencephalography at diagnosis. (C) Brain and spine MRI (T2 FLAIR) at 5 months after onset.

amination, and echocardiography were performed; all showed no evidence of a tumor.

After consecutive treatment with intravenous immunoglobulin (IVIg, 2 g/kg, 4 days) and rituximab, the patient displayed some alert mentality with partial response. However, his quadriplegia present remained during day 7 of hospitalization. Additional plasmapheresis was performed, and his motor power in all extremities recovered to grades IV–V. After additional rehabilitation for 2 weeks, he could sit without assistance, stand with minimal support, and eat on his own.

Although bladder dysfunction and mild sleep disturbances remained, he was discharged from the hospital on day 25 with an an-

nual follow-up schedule of neuroimaging and tumor screening. At a 5-month follow-up visit following disease onset, the boy could walk and lead a normal life without any significant neurologic disorders. Follow-up brain and spine MRI also showed a markedly decreased extent of T2 FLAIR high signal changes (Fig 1C), despite persistently positive serum anti-Ma2 antibodies.

Here, we report a 9-year-old boy diagnosed with anti-Ma2-associated encephalomyelitis by a systemic screening for various auto-antibodies who was successfully treated with effective immunomodulation for this rare disease.

Anti-Ma2-associated encephalitis is a paraneoplastic immune-mediated disorder with preferential involvement of the lim-

Table 1. Summary of the reported pediatric anti-Ma2-associated encephalitis in pediatric patients

Study	Sex/Age	Initial symptoms	MRI findings	CSF	Tumor	Treatment	Outcome
Mirabet et al. (2015) [2]	F/2 yr	Fever/seizure	Increased T2 signal in left frontoparietal areas	Normal	(-)	mPD IVIg	Partial recovery
Douma et al. (2021) [3]	F/6 yr	Behavioral/speech disorder	Normal	Normal	(-)	mPD IVIg	Partial recovery
Douma et al. (2021) [3]	M/8 yr	Behavioral disorder, decreased mentality	Increased T2 signal in the external capsule	Pleocytosis	(-)	mPD IVIg	Full recovery
Douma et al. (2021) [3]	F/23 mo	Behavioral/speech disorder, decreased mentality	Increased T2 signal in left temporo-parietal, basifrontal, occipital areas	Normal	(-)	Azathioprine mPD	Partial recovery
Douma et al. (2021) [3]	M/2.5 yr	Behavioral/speech disorder, decreased mentality, swallowing disorder	Increased T2 signal in right frontal areas	Normal	(-)	mPD IVIg	Partial recovery
Boniell et al. (2018) [4]	F/4 yr	Myoclonus, chorea, ataxia	Normal	Normal	(-)	Azathioprine ACTH mPD IVIg	Partial recovery
Hu et al. (2015) [5]	M/8 yr	Seizure	Slight enlargement of the left lateral ventricle		(-)	Azathioprine mPD IVIg	Partial recovery

MRI, magnetic resonance imaging; CSF, cerebrospinal fluid; mPD, methylprednisolone pulse therapy; IVIG, intravenous immunoglobulin; ACTH, adrenocorticotropic hormone.

bic, diencephalic, and upper brainstem caused by antibodies for cytoplasmic proteins, Ma2, in the neurons and testis. Owing to its strong association with cancer, which mainly presents as testicular germ cell tumors in adults, anti-Ma2 antibody is described as a “paraneoplastic” or “onconeural” antibody [1]. However, reports of anti-Ma2-associated encephalitis in childhood are extremely rare, and an autoimmune origin appears to have been more likely in this case than a paraneoplastic mechanism [2]. To our knowledge, only seven pediatric cases of anti-Ma2-associated neurological symptoms have been reported to date (Table 1) [2-5]. There were three male and four female patients, with a median age of 4 years. Brain MRI showed an increased T2 FLAIR signal in four patients, whereas mild enlargement of the lateral ventricle was observed in one patient. CSF analysis was performed in six patients, and pleocytosis was observed in one case. Tumor screening was performed in all patients, and there was no evidence of tumors. Six patients were treated with mPD pulse therapy and IVIG, whereas one received mPD alone. Subsequently, azathioprine was administered to three patients, and only one patient achieved full recovery. The remaining six patients had a partial recovery.

Consistent with our case, the initial reported symptoms of patients with anti-Ma2-associated encephalomyelitis are mainly changes in behavior and mental status, with or without a seizure. When there are only neurological symptoms and signs in children, it is difficult to differentiate between other inflammatory brain diseases; a strong clinical suspicion with extensive screening can provide an accurate diagnosis in our case and others. Although no evidence-based treatment protocols are currently available for pediatric AE, the first-line treatment often consists of high-dose steroids, IVIG, and/or plasmapheresis. Clinicians can escalate to second-line immunotherapy, including treatment with rituximab and cyclophosphamide, when first-line therapies have failed [6]. Anti-Ma2 antibody is not directly pathogenic, but indicates a T-cell-mediated immune response against neurons. High-dose steroid treatment is a favored option for immunosuppression over plasmapheresis in patients with classical onco-neuronal antibodies associated with AE due to primarily T-cell mediated inflammation [7,8]. Considering the pathomechanism of anti-Ma2-associated encephalomyelitis, we administered high-dose steroids and IVIG as a first-line treatment. However, there was no response to these first-line therapies. Rituximab is a representative B-cell-depleting agent that can suppress T-cell activity by reducing B-cell-induced activation of T-cells. Unfortunately, we achieved only a partial response after administering rituximab as second-line therapy. Finally, we performed plasmapheresis to remove putative disease mediators, including toxic macromolecules and pathogenic antibodies [7,9]. Consequently, a significant improvement was achieved fol-

lowing 10 cycles of plasmapheresis.

We did not observe any evidence of an underlying paraneoplastic tumor with anti-Ma2-associated encephalomyelitis in our and other pediatric cases. There are no clear guidelines regarding how frequently or how long tumor screening should occur. However, according to other studies in adult patients, paraneoplastic neurological disorders can be diagnosed before the detection of a tumor by up to 4 or 5 years. Therefore, we recommended annual tumor screening for at least 5 years with the imaging modalities, tumor markers, and clinical examinations due to the high-risk of malignancy in adult patients with anti-Ma2 encephalitis, despite the lack of specific evidence [2,10]. In our patient, at least 5 years of neurological follow-up and periodic screening have been initiated. A high index of suspicion and extensive autoantibody screening in a child with acute-onset encephalomyelitis could lead to an accurate diagnosis of anti-Ma2 encephalitis and prevent irreversible neurological sequelae with early appropriate therapeutic intervention. However, a standardized immunotherapy protocol and guidelines for tumor screening in pediatric patients are still under discussion. Additional large cohort studies are required to support evidence-based treatment protocols.

Conflicts of interest

Tae-Sung Ko 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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TSEN54 Gene-Related Pontocerebellar Hypoplasia Type 2 in a Newborn with Refractory Myoclonic Seizures

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Neonates are vulnerable to epileptic seizures. Eighty percent of neonatal seizures occur in the first 1 or 2 days of life. The most common cause of neonatal seizures is hypoxic ischemic encephalopathy. Brain malformations can also be an important cause of seizures. Herein, we present a case of an infant who experienced severe myoclonic seizures with little response to anti-seizure medications from the first day of life. The infant had refractory myoclonic seizures associated with pontocerebellar hypoplasia (PCH) and a mutation in transfer ribonucleic acid splicing endonuclease 54 (*TSEN54*). This case was reviewed and approved by the Institutional Review Board of Keimyung University Dongsan Hospital (IRB No. 2022-04-017). The requirement for informed consent was waived by the board.

A female infant was born at 38 weeks of gestation by cesarean section due to breech presentation at a local hospital. She had a birth weight of 2,840 g (25th to 50th percentile), length of 45 cm (10th to 25th percentile), and head circumference of 31 cm (< 10th percentile). Although her Apgar scores were 7 and 9 at 1 and 5 minutes, respectively, the baby suffered from severe seizures and respiratory distress from the first day of life. Therefore, she was transferred to a university hospital. A neurological examination revealed gener-

alized myoclonic seizures with respiratory distress, hypertonia of the extremities, joint stiffness of both elbows, and increased deep tendon reflexes. Initial electroencephalography (EEG) showed frequent ictal EEG patterns with 6 to 7 Hz rhythmic activities beginning at P3, P4, and T6 independently. Radiologic studies (Fig. 1) showed a flat ventral pons and a small cerebellum. This baby had no specific findings in studies for metabolic disorders or genetic screening tests associated with early myoclonic seizures in infancy. To determine the genetic cause of PCH, targeted exome sequencing was performed when she was 1 month of age. Finally, two variants of the *TSEN54* gene, c.919G > T and c. 623G > A, were found, representing compound heterozygosity. These two variants were confirmed by Sanger sequencing (Fig. 2), and no other copy number variation was found in the diagnostic exome sequencing study. The c.919G > T mutation (NM_207346.2:c.919G > T, p.Ala307Ser, rs113994152) has been already reported as the most common *TSEN54* variant found in PCH patients [1,2], and has been classified as a pathogenic variant in the ClinVar database. It is very rare in large population databases, such as gnomAD (<https://gnomad.broadinstitute.org/>), where it has a minor allele frequency (MAF) of 0.09%, and Kore-

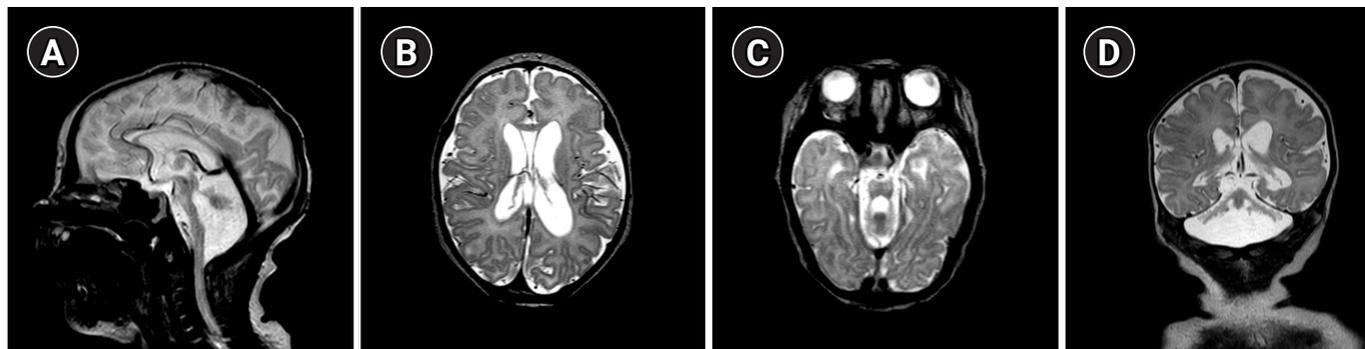


Fig. 1. Contrast brain magnetic resonance imaging findings of the patient. (A) Sagittal T2-weighted imaging showing a markedly small cerebellum, a small flattened anterior pons, diffuse widening of the cerebrospinal fluid space in the entire posterior fossa, and thinning of the corpus callosum. (B) Axial T2-weighted imaging revealing diffuse high signal intensity of the entire cerebral white matter, loss of the normal low signal in the posterior limb of the internal capsule at birth, and a diffuse prominent lateral ventricle. (C) Axial T2-weighted imaging revealing a markedly small vermis. (D) Coronal T2-weighted imaging showing flat and thin cerebellar hemispheres.

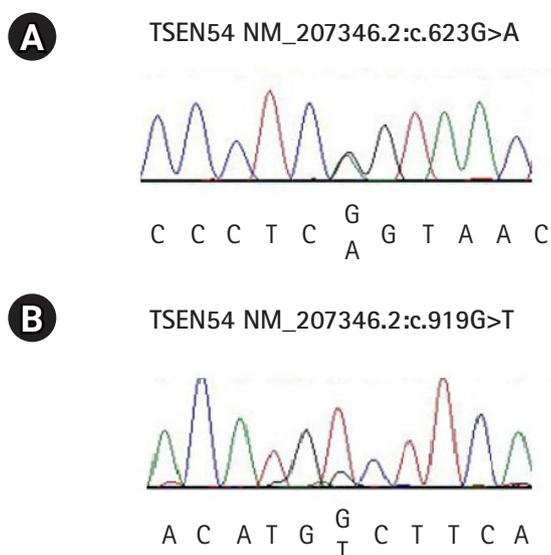


Fig. 2. Sanger sequencing results performed as a confirmatory test, showing two variants of the transfer ribonucleic acid splicing endonuclease 54 (*TSEN54*) gene. (A) *TSEN54* NM_207346.2:c.919G>T, (B) *TSEN54* NM_207346.2:c.623G>A (p.Arg208Gln).

an Reference Genome Database (KRGDB; <http://coda.nih.gov/coda/KRGDB/>), where it has a MAF of 0%. This variant was also predicted to be deleterious in an *in silico* analysis using Sorting Intolerant From Tolerant (SIFT; <https://sift.bii.a-star.edu.sg/>) and Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>). Based on this evidence, the c.919G>T variant was classified as a likely pathogenic variant. The other mutation, c.623G>A (NM_207346.2:c.623G>A, Arg208Gln, rs85291542), is also a very rare variant (GnomAD, MAF = 0.0008% and KRGDB, MAF = 0.00%), and has been not reported in any articles or databases. An *in silico* prediction suggested that this variant is likely to have a splicing effect by splice site analysis, and it was identified as a dis-

ease-causing mutation using MutationTaster (<http://mutationassessor.org/>). According to the American College of Medical Genetics guideline, c.623G>A was classified as a variant of unknown significance. However, based on its rarity in the general population and consistent clinical findings, this variant has a high probability of being a pathogenic cause in this patient, although there is a limitation in interpretation since the patient's family was not tested.

After two and a half months of hospitalization, the patient had brief myoclonic seizures that persisted several times a day, with no noticeable improvement. At discharge, neurological examination revealed that hypertonia of the extremities, joint stiffness, and abnormal ankle myoclonus were still present. The baby was discharged from the hospital with assisted ventilation, gavage, and anti-seizure medications, including carbamazepine and levetiracetam.

PCH is a rare genetic neurodevelopmental disorder mostly characterized by prenatal developmental disorders and deterioration of cerebral structures [3]. Of the 13 known subtypes, PCH2 is the most common form of autosomal recessive PCH [4,5]. The age at onset in PCH2 varies from prenatal to early infancy. Most patients present with this condition in the first months of life. Patients with PCH2 have symptoms including microcephaly, abnormal muscle tone, epilepsy, and severe psychomotor retardation [6]. In our case, the patient had microcephaly, hypertonia of extremities, joint stiffness of both elbows, and generalized myoclonic seizures with respiratory distress. Both PCH2 and 4 are caused by mutations in the *TSEN54* gene, and both exhibit an absence of transverse pontine fibers and underdevelopment of the cerebellar folia. Early-onset infantile PCH has numerous causes, including chromosomal disorders (e.g., trisomy 13, 18, or 21), metabolic disorders (e.g., a congenital disorder of glycosylation type 1a or glutaric aciduria), exposure to teratogens (e.g., alcohol, anticonvulsant therapy, or infection with cytomegalovirus), syndromic associations (e.g., Bow-

en-Conradi syndrome or Marden-Walker syndrome), and an emerging group of autosomal recessive single-gene disorders [7,8]. In our patient, the cerebellar hemispheres were flat and very small, whereas the cerebellar vermis was relatively conserved. In simple cerebellar atrophy, the pons volume is maintained relatively well. Joubert syndrome shows characteristic molar tooth signs. Typical PCH2 brain magnetic resonance imaging (MRI) findings include a characteristic dragonfly shape in the coronal plane [1,3,9]. Supratentorial involvement is also possible. It is reflected by variable neocortical atrophy, ventriculomegaly, and microcephaly. The MRI findings of the present case were consistent with PCH. Causative mutations in genes that encode 3 of the 4 subunits of the *TSEN* complex, namely *TSEN54* (MIM 277470), *TSEN2* (MIM 612389), and *TSEN34* (MIM 612390), have been found in most cases with PCH2 [3,8]. Of these, the “common” c.919G > T/p. A307S variant, *TSEN54*, accounts for approximately 70% of mutated PCH alleles and more than 85% of all PCH2 cases [10]. Our case also showed the most common mutation of *TSEN54*. To the best of our knowledge, this is the first case report in Korea of PCH2 that was confirmed by genetic testing.

Conflicts of interest

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

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Author contribution

Conceptualization: CSK. Data curation: JCB, JSH, and CSK. Formal analysis: JCB, JSH, and CSK. Methodology: JCB, JSH, and CSK. Project administration: JCB. Visualization: JSH and CSK.

Writing-original draft: JCB and JSH. Writing-review & editing: JCB and CSK.

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Instructions to authors

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- 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 orderin

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) Brief communications

Brief communications should include a concise and timely original research, a preliminary study, and significant material for rapid dissemination. It should contain a structured abstract (no more than 150 words), main text (less than 2,000 words), no more than 3 illustrations (figure or table), and no more than 20 references. The main text should be written under the heading "Findings". Subheadings should not be used but the text should chronologically consist of a short background, aim, materials and methods, a short and focused discussion and a brief conclusion (but without including subheadings). Supplementary materials can be published online.

2) Reviews

Reviews may be written by invitation by the editorial board and provide concise reviews of important subjects to medical researchers. 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.

3) Letters to the editor

Letters to the editor should be a brief description with short discussions 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 tables or figures, and less than 5 authors.

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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 on 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.

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Author's checklist

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.

2. Cover page

- 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.
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- Compliance with quotation styles should be observed.
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6. Table

- Each table must have its own title and be given a separate page.
- All abbreviations used in the table should be spelled out.

- All superscript numbers used in the table should comply with the contribution rules.

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.
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