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Original article

# Developmental outcomes and prevalence of *SLC2A1* variants in young infants with hypoglycorrhachia

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**Abstract**

*Introduction:* The neurodevelopmental outcomes of young infants with hypoglycorrhachia that is comparable to glucose transporter 1 deficiency syndrome (GLUT1DS), i.e. cerebrospinal fluid (CSF) glucose ≤40 mg/dL and CSF lactate <2.2 mM without causes of secondary hypoglycorrhachia are unknown. This study investigated the developmental outcomes and possibility of GLUT1DS in infants with hypoglycorrhachia, or low CSF glucose concentration.

*Material and methods:* 1655 neurologically asymptomatic infants aged <4 months had CSF examinations for fever workup from 2006 to 2016. Among the infants with normal CSF cell counts and without isolated pathogens, there were hypoglycorrhachia group who had CSF glucose levels that were comparable to GLUT1DS, and age- and gender-matched non-hypoglycorrhachia group. Both groups were at a mean age of 5.9 ± 2.4 years (ranged 1–10 years) at neurodevelopmental evaluation in 2017. Mutational analysis of solute-carrier-family 2, which facilitated the glucose transporter member 1 (*SLC2A1*) gene was performed.

*Results:* Among the 722 infants with normal CSF cell counts and without isolated pathogens, 30 (4.2%) had hypoglycorrhachia that was comparable to GLUT1DS. In the 25 infants with hypoglycorrhachia available for follow-up, 4 (16%) had abnormal outcomes, of which 3 (12%) had the history of mixed-type developmental delay before age 6 and 1 (4%) had type 1 diabetes mellitus. In the non-hypoglycorrhachia control group (n = 50), 2 patients (4%) showed abnormal outcomes, both with the history of pure speech delay. The hypoglycorrhachia group had a higher rate of the history of mixed-type of developmental delay than the control group (12% vs. 0%, $P = 0.034$). No *SLC2A1* pathogenic variants were observed in the hypoglycorrhachia group.

*Conclusion:* Hypoglycorrhachia may be a potential biomarker for neurodevelopmental delay instead of for GLUT1DS in neurologically asymptomatic young infants.


*Keywords:* Hypoglycorrhachia; Infant; Glucose transporter 1 deficiency syndrome; *SLC2A1*

*Abbreviations:* GLUT1DS, glucose transporter 1 deficiency syndrome; CSF, cerebrospinal fluid; *SLC2A1*, solute carrier family 2, facilitated glucose transporter member 1; T1DM, Type 1 diabetes mellitus

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## 1. Introduction

Glucose is an important energy source for the brain and its tight regulation is critical for the physiology and pathology of the brain. The cerebrospinal fluid (CSF) glucose concentration is controlled by the glucose transported into and out of CSF via the glucose transporter 1 (GLUT1) and the glucose utilization by cells [1,2]. Hypoglycorrhachia, or low CSF glucose concentration, has more adverse effects in the developing brain than in the mature brain [2]. Hypoglycorrhachia is commonly associated with central nervous system (CNS) microbial infections, neoplastic causes or impaired function of GLUT1 [3–5]. Patients with hypoglycorrhachia caused by impaired function of GLUT1, or glucose transporter 1 deficiency syndrome (GLUT1DS), have diverse clinical symptoms which occur at any age, especially in early infancy [6,7].

The classic phenotypes of GLUT1DS include epileptic encephalopathy, infantile seizures, developmental delay, acquired microcephaly, spasticity, and complex movement disorders [6–10]. Milder phenotypes, namely paroxysmal exertion-induced dyskinesia, hemiplegic migraine, and early-onset absence epilepsy have been recognized [11–18]. The diagnosis of GLUT1DS depends on neurological manifestations and hypoglycorrhachia [7,10,17,18], which is further confirmed by detecting the pathogenic variants of the corresponding gene, solute carrier family 2, facilitated glucose transporter member 1 (*SLC2A1*) [6,7]. Early diagnosis is pertinent because early treatment with ketogenic diets may attenuate seizure frequency and improve cognitive function [19,20].

A recent systemic review study using the CSF results of the patients with genetically proven GLUT1DS showed that if age-specific reference values are applied, the CSF glucose levels below the 10th percentile is a useful tool in the diagnostic workup of GLUT1DS including patients with the mildest phenotypes [17]. CSF examinations using lumbar puncture have been performed routinely in young infants who are presented with fever because of their vulnerability to CNS infections [21,22]. However, after excluding the possibility of CNS infections, hypoglycorrhachia is usually not emphasized in clinical practice. The neurodevelopmental outcomes of young infants with hypoglycorrhachia that is comparable to GLUT1DS, i.e. CSF glucose $\leq$40 mg/dL and CSF lactate $<$2.2 mM without causes of secondary hypoglycorrhachia are unknown. Moreover, the genetic diagnosis of GLUT1DS in such young infants remains unclear. Therefore, using the data from febrile young infants who were neurologically asymptomatic at the time of CSF analysis, this study examined: (1) the prevalence rate of hypoglycorrhachia, (2) the type of neurodevelopmental disorders at follow-up, and (3) the genetic diagnosis of GLUT1DS in young infants with hypoglycorrhachia.

## 2. Material and methods

### 2.1. Patients

Overall, 6574 patients had CSF samples, which were collected through lumbar punctures and analyzed from 2006 to 2016 in a tertiary medical center in Southern Taiwan. Among them, CSF examinations were performed as a routine fever workup in 1655 infants younger than 4 months. Among the 1655 infants, 722 young infants remained after excluding those with CSF analysis that met any of the following conditions: (1) an erythrocyte count $>$100/μL; (2) abnormal leukocyte count and total protein concentration based on age-specific reference values; (3) lactate $\geq$2.2 mM; (4) pathogenic organisms isolated from the CSF or blood samples; and (5) patients with abnormal neurological presentations at CSF study [23]. 2 patients who had abnormal neurological presentations were excluded from analysis, which included 1 patient with pre-existing infantile seizure, and the other with brain malformation.

The GLUT1DS-CSF criteria defined as CSF glucose $\leq$40 mg/dL and lactate $<$2.2 mM without causes of secondary hypoglycorrhachia [17]. In this study, infants who had CSF glucose levels $\leq$40 mg/dL were categorized as the hypoglycorrhachia group [7,23,24]. Another group of infants with normal CSF glucose levels that were age- and gender-matched with the hypoglycorrhachia group was served as the non-hypoglycorrhachia control group (Fig. 1).

Neurodevelopmental evaluations were undertaken in both groups in 2017. This study was approved by the Institutional Review Board of National Cheng Kung University Hospital.

## 3. Variant analysis

A variant analysis of the *SLC2A1* gene was performed in the hypoglycorrhachia group. Genomic DNA was extracted from peripheral blood mononuclear cells. Automated Sanger sequencing was performed to study all exons and exon–intron boundaries of *SLC2A1* using an autonomic sequencer (ABI 377; Advanced Biotechnologies, Columbia, MD, USA) in the hypoglycorrhachia group and multiplex ligation-dependent probe amplification (MLPA) using probemix P138-B1 (MRC Holland, Amsterdam) was performed to detect deletions or duplications of *SLC2A1* in the patients with mixed-type neurodevelopmental disorder [11].

## 4. Long-term neurodevelopmental outcome evaluations

In the hypoglycorrhachia group, 25 patients were interviewed and completed a questionnaire on the developmental history of their infants, and the symptoms and

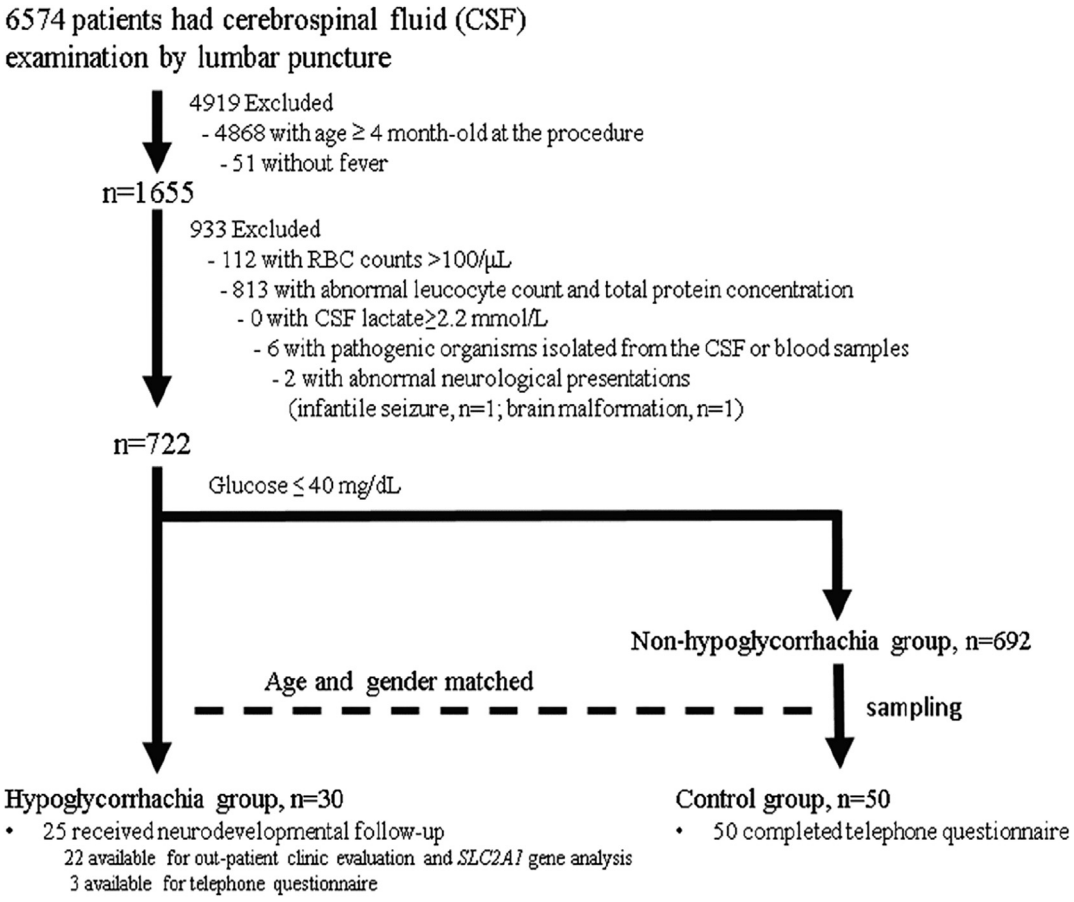


Fig. 1. A flowchart of the patient recruitment for hypoglycorrhachia among young infants who had CSF analysis as a part of routine fever workup from 2006 to 2016.

signs that related to GLUT1DS, namely seizures, movement, and learning problems in the outpatient clinics or via telephone questionnaire in 2017. [8–15]. For the 22 patients available for follow-up in the outpatient clinic, physical and neurological examinations were performed by a pediatric neurologist in the outpatient clinic. In the non-hypoglycorrhachia group, all parents completed telephone questionnaire on the developmental history of their infants, and the symptoms and signs that related to GLUT1DS.

Developmental delay was considered for children under 6 years old whose performance was 2 standard deviations below their age-matched peers in development using the instruments of the Chinese Child Development Inventory, Bayley scales of Infant Development-III, Wechsler Preschool and Primary Scale of Intelligence (Revised Edition or Fourth edition, WPPSI-R or WPPSI-IV), or Movement Assessment Battery for Children (Second Edition). For patients who were older than 6 years of age at neurodevelopmental evaluation, the history of developmental delay was confirmed by the evaluation reports they had from the Center of Team Evaluation for Children's Development across Taiwan before they turned 6 years old. The need of intelligence quotient test was judged by the pediatric neurologist after the follow-up assessment.

## 5. Statistical analysis

Differences in demographic factors, neurodevelopmental outcomes, and genotype frequencies of the *SLC2A1* gene polymorphism between the hypoglycorrhachia group and the control group were determined using the Fisher's exact test for categorical variables and the independent samples *t* test for numerical variables. CSF data within the hypoglycorrhachia group was presented in median and interquartile range (IQR) and the difference was determined using the Mann–Whitney *U* test. All analyses were performed using SPSS version 17 (IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA).

## 6. Results

Among the 722 young infants with normal CSF cell counts and protein levels and without any isolated pathogens, 30 infants (a prevalence rate of 4.2%) had hypoglycorrhachia that was comparable to GLUT1DS.

The mean age of CSF examinations was comparable between the hypoglycorrhachia (4.2 ± 3.3 weeks, ranged 0–12 weeks) and the non-hypoglycorrhachia control group (5.5 ± 4.1 weeks, ranged 0–13 weeks). The mean CSF glucose levels in the hypoglycorrhachia group were significantly lower than that in the non-hypoglycorrhachia group (37.0 ± 3.0 mg/dL vs. 53.0 ± 9.9 mg/dL, $P < 0.001$). In contrast, the CSF to blood glucose ratios were similar between the two groups (Tables 1 and 2). The most common causes of young infantile fever in the two groups were urinary tract infection, acute bronchiolitis, and acute pharyngitis.

Both groups were at a mean age of 5.9 ± 2.4 years (ranged 1–10 years) at neurodevelopmental evaluation in 2017. Among the 30 infants in the hypoglycorrhachia group, 25 patients (83.3%) completed the follow-up evaluation. In the 25 children with infantile hypoglycorrhachia, 4 (16%) had abnormal outcomes, which included 3 patients (12%) with the history of mixed-type of developmental delay before age 6 (motor and speech delay in 1, motor and cognitive delay in 1, and global delay with autism in 1), and 1 patient with type 1 diabetes mellitus (T1DM) diagnosed at the age 3 years. In the 50 children without infantile hypoglycorrhachia (the non-hypoglycorrhachia control group), only 2 patients (4%) reported abnormal outcomes, both with the history of developmental delay in speech only. While the hypoglycorrhachia group had a significantly higher rate of the history of mixed-type developmental delays than the non-hypoglycorrhachia control group (12% vs. 0%, $P = 0.034$; Table 2).

Among the 3 patients with the history of mixed-type developmental delay before age 6 in the hypoglycorrhachia group, none of them had weakness, spasticity or hypotonia at our neurological evaluation at age 6, 7 and 8 respectively. 2 of them attended regular elementary school after early intervention, even though they were frequently reported being clumsy by their parents. As for the other patient with global delay and autism, he still needed much assistance in coping with daily life activities, and his full scale intelligence quotient using WPPSI–IV was 71. In the non-hypoglycorrhchia group, the 2 patients who had the history of simple speech delay had made progress and showed catching up in development when evaluated at school age after early intervention.

In the hypoglycorrhachia group, there was no difference in the CSF glucose levels between the infants with adverse outcomes (median 38.5 mg/dL, IQR 6 mg/dL, ranged 33–40 mg/dL) and those without adverse outcomes (median 37 mg/dL, IQR 3.5 mg/dL, ranged 27–40 mg/dL, $P = 0.625$) (Fig. 2). In contrast, the CSF to blood glucose ratio was higher in the patients with adverse outcomes (median 0.52, IQR 0.10, ranged 0.49–0.61) than those without adverse outcomes (median 0.47, IQR 0.12, ranged 0.35–0.57, $P = 0.041$) (Fig. 3).

A total of 22 hypoglycorrhachia patients received Sanger sequencing of *SLC2A1* gene, and none of them had the *SLC2A1* pathogenic variants. No deletion or duplication of *SLC2A1* gene was detected in 3 infants with mixed-type developmental disorder in the hypoglycorrhachia group. Moreover, the frequency of *SLC2A1* gene polymorphisms in the hypoglycorrhachia group showed no difference compared with the *SLC2A1* database in the Taiwan Biobank [25], which contains the genomic data of 1517 Taiwanese people (Table 3).

## 7. Discussion

This study is the first to address the prevalence rate of hypoglycorrhachia in young infants, and the long-term neurodevelopmental outcome and genetic diagnosis of GLUT1DS in the children with infantile hypoglycorrhachia. The prevalence rate of hypoglycorrhachia that was comparable to GLUT1DS was 4.2% in neurologically asymptomatic infants. At follow-up assessment, the hypoglycorrhachia group had a significantly higher rate of the history of mixed-type neurodevelopmental delays (including autism) than the non-hypoglycorrhachia group. None of the hypoglycorrhachia patients had *SLC2A1* pathogenic variants of GLUT1DS. Furthermore, the frequency of *SLC2A1* polymorphisms in the hypoglycorrhachia group was also comparable with the *SLC2A1* information from the Taiwanese genomic data. This study suggests hypoglycorrhachia detected in infants through routine CSF examinations for fever workup may be an early biomarker for neurodevelopmental delay instead of for GLUT1DS.

Leen et al and Thomson et al published the age-specific reference values for CSF glucose concentrations [23,24]. Leen et al proposed the 10th percentile CSF glucose concentration was the cutoff level for the diagnosis of GLUT1DS [18]. The CSF glucose level in the 10th percentile for infants less than 4 months old ranged from 36 to 40 mg/dL [23,24]. We used the CSF glucose level of ≤40 mg/dL as the inclusion criterion for hypoglycorrhachia because GLUT1DS patients with milder phenotypes may have higher CSF glucose levels and develop neurological symptoms at an older age [7]. We revealed that for the febrile infants, the prevalence of hypoglycorrhachia was 4.2% (30/722) using the criterion of a CSF glucose level of ≤40 mg/dL, and 2.5% (18 of 722) using the age-specific 10th percentile criteria for CSF glucose levels. Nevertheless, the rate of adverse outcomes was similar whether hypoglycorrhachia was defined as having a CSF glucose level of ≤40 mg/dL or using the age-specific 10th percentile criteria (16% vs. 16.7%).

Glucose is a vital energy source for the human brain, and especially for the developing brain. The cerebral metabolic rate for glucose increases linearly after birth until age 3 years old [26]. Most patients with GLUT1DS

Table 1
CSF characteristics, outcome and genetic testing of the children with young infantile hypoglycorrhachia.

| Demographic profile | | CSF analysis | | | Adverse outcomes | | | | Molecular genetic testing |
|---|---|---|---|---|---|---|---|---|---|
| Patient | Gender/Age at follow-up (y) | Age at CSF exam (wk) | CSF glucose (mg/dL) | CSF:blood glucose ratio | Seizure | Movement disorder | History of DD | Others | Pathogenic variant |
| 1 | M/7y | 2 | 37 | 0.54[a] | – | – | M/S[b] | – | Neg[c] |
| 2 | M/6y | 3 | 33 | 0.61[a] | – | – | C/M/S | Autism | Neg[c] |
| 3 | M/8y | 4 | 40 | 0.49 | FS | – | C/M[b] | – | Neg[c] |
| 4 | M/5y | 2 | 27 | 0.47 | – | – | – | – | Neg |
| 5 | F/5y | 1 | 40 | 0.57[a] | – | – | – | – | Neg |
| 6 | M/6y | 3 | 35 | 0.47 | – | – | – | – | Neg |
| 7 | F/6y | 1 | 39 | 0.48 | – | – | – | – | Neg |
| 8 | F/6y | 4 | 36 | 0.46 | – | – | – | – | Neg |
| 9 | F/7y | 4 | 38 | 0.39 | – | – | – | – | Neg |
| 10 | F/7y | 2 | 36 | N/A | – | – | – | – | Neg |
| 11 | M/8y | 5 | 40 | 0.50[a] | – | – | – | – | Neg |
| 12 | F/10y | 8 | 40 | 0.49 | – | – | – | T1DM | Neg |
| 13 | M/8y | 12 | 37 | 0.4 | – | – | – | – | Neg |
| 14 | M/10y | 11 | 40 | N/A | – | – | – | – | Neg |
| 15 | M/5y | 8 | 34 | 0.37 | – | – | – | – | Neg |
| 16 | F/5y | 8 | 40 | 0.49 | – | – | – | – | Neg |
| 17 | M/3y | 2 | 38 | N/A | – | – | – | – | Neg |
| 18 | M/4y | 0 | 39 | N/A | – | – | – | – | Neg |
| 19 | M/3y | 2 | 33 | N/A | – | – | – | – | Neg |
| 20 | F/4y | 3 | 37 | 0.53[a] | – | – | – | – | Neg |
| 21 | M/1y | 0 | 34 | N/A | – | – | – | – | Neg |
| 22 | M/2y | 8 | 37 | N/A | – | – | – | – | Neg |
| 23 | F/7y | 2 | 39 | 0.35 | – | – | – | – | N/A |
| 24 | F/5y | 4 | 37 | N/A | – | – | – | – | N/A |
| 25 | M/10y | 7 | 39 | 0.37 | – | – | – | – | N/A |

[a] CSF: blood glucose ratio >25th percentile, FS: febrile seizure, DD:developmental delay, C: cognition delay, M: motor delay, S: speech delay, N/A: not available, Neg: negative results.
[b] Both patients outgrew the developmental delay after early intervention and attended regular elementary school now.
[c] Both had negative results from sanger sequencing and MLPA analysis.

Table 2
Comparisons of the adverse outcomes between children with hypoglycorrhachia and those without hypoglycorrhachia in the young infantile period.

| | Hypoglycorrhachia group n = 25 | Non-hypoglycorrhachia group n = 50 | *P* |
|---|---|---|---|
| Male sex, n (%) | 15 (60.0%) | 30 (60.0%) | 1.000 |
| Age at CSF exam (wk) | 4.2 ± 3.3 | 5.5 ± 4.1 | 0.199 |
| CSF glucose levels (mg/dL) | 37.0 ± 3.0 | 53.0 ± 9.9 | <0.001 |
| CSF to blood glucose ratio | 0.47 ± 0.07 | 0.50 ± 0.08 | 0.198 |
| Adverse outcomes, n (%) | 4 (16.0%) | 2 (4.0%) | 0.091 |
| Mixed type developmental delay, n (%) | 3 (12.0%) | 0 (0.0%) | 0.034 |
| Autism, n (%) | 1 (4.0%) | 0 (0.0%) | 0.333 |
| T1DM, n (%) | 1 (4.0%) | 0 (0.0%) | 0.333 |

Data are means ± standard deviation or number (%); T1DM: type 1 diabetes mellitus.

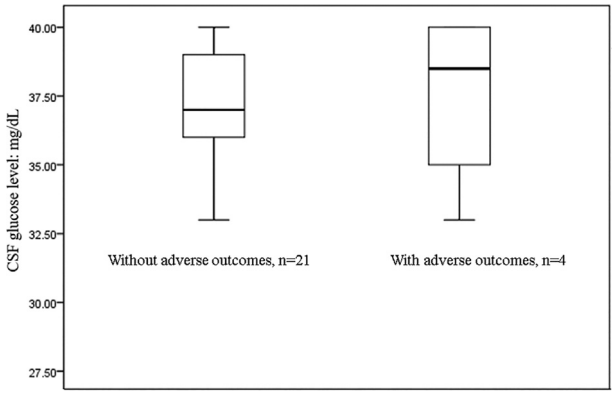


Fig. 2. The comparison of the CSF glucose levels between the infants with and those without adverse outcomes in the hypoglycorrhachia group. No difference in the CSF glucose levels was found between the infants with adverse outcomes (median 38.5 mg/dL; IQR 6 mg/dL; ranged 33–40 mg/dL) and those without adverse outcomes (median 37 mg/dL, IQR 3.5 mg/dL; ranged 27–40 mg/dL) in the hypoglycorrhachia group ($P = 0.625$).

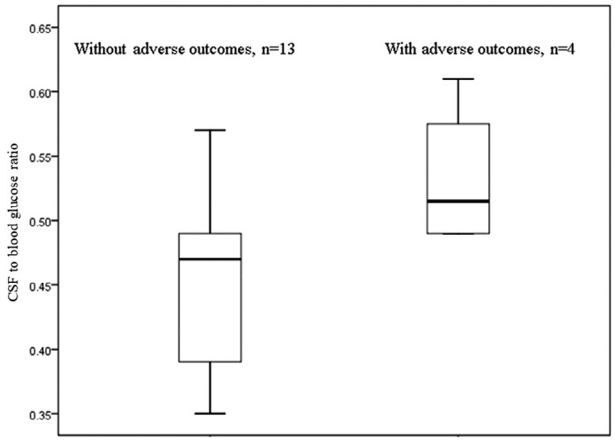


Fig. 3. The comparison of the CSF to blood glucose ratio between the infants with and those without adverse outcomes in the hypoglycorrhachia group. Infants with adverse outcomes (median 0.52; IQR 0.10; ranged 0.49–0.61) had higher CSF to blood glucose ratio compared to those without adverse outcomes (median 0.47; IQR 0.12; ranged 0.35–0.57) in the hypoglycorrhachia group ($P = 0.041$).

have neurological symptoms during this critical period, except some patients who exhibit less impaired glucose

Table 3
Genotype Frequencies of *SLC2A1* Gene Polymorphism in Young Infants with Hypoglycorrhachia

| SNP/Genotype | Hypoglycorrhachia n = 22 | Taiwan reference n = 1517 | *P* |
|---|---|---|---|
| **rs1385129** | | | 0.628 |
| GG | 14 (63.6%) | 783 (51.7%) | |
| GA | 7 (31.8%) | 616 (40.7%) | |
| AA | 1 (4.6%) | 116 (7.7%) | |
| **rs11537641** | | | 0.677 |
| GG | 17 (77.3%) | 1215 (80.1%) | |
| GA | 5 (22.7%) | 286 (18.9%) | |
| AA | 0 (0.0%) | 16 (1.1%) | |
| **rs2229682** | | | 0.603 |
| CC | 18 (81.8%) | 1287 (85.0%) | |
| CT | 4 (18.2%) | 218 (14.4%) | |
| TT | 0 (0.0%) | 9 (0.6%) | |

SNP: Single nucleotide polymorphism.

transporter 1 functions and show later-onset motor symptoms, such as dystonia, ataxia, or paroxysmal dyskinesia [27]. The types of developmental delay observed in our hypoglycorrhachia patients were motor and speech delay, motor and cognition delay, and global delay with autism, diagnosed between 1 and 3 years of age. Their motor disabilities were relatively mild and none had the typical motor symptoms, such as paroxysmal behavioral disturbance, ataxia, and exercise intolerance as described in the genetically confirmed GLUT1DS patients. The hypoglycorrhachia infants had a high rate of adverse outcomes after years of follow-up despite none of them having GLUT1DS, suggesting hypoglycorrhachia in infancy may have adverse effects on early brain development.

The detection rate of *SLC2A1* pathogenic variants for GLUT1DS varies with different clinical presentations. The detection rate reaches almost 100% in hypoglycorrhachia patients presented with classic neurological presentations, such as intractable infantile seizures, acquired microcephaly, and developmental delay. However, the genetic detection rate is low among those with minor symptoms without hypoglycorrhachia. For example, the detection rate of *SLC2A1* variants was

1% in patients with idiopathic generalized epilepsies and 10% in patients with early-onset absence epilepsy [11,28]. Very few studies have investigated the *SLC2A1* variants in young infants with hypoglycorrhachia who routinely have CSF examinations as a part of a fever workup. The CSF data derived from these infants may be more representative of the rate of hypoglycorrhachia than those from patients with abnormal neurological presentations in this age group.

We observed no *SLC2A1* pathogenic variants among the febrile infants with hypoglycorrhachia that that was comparable to GLUT1DS, even though those patients had a high rate of the history of mixed-type of developmental delays. These results suggest that long-term follow-up of the infants who exhibit isolated hypoglycorrhachia is necessary because these patients may have a high rate of adverse outcomes. A genetic analysis of the *SLC2A1* gene in these patients might not be necessary if they have normal development or do not develop the classic symptoms of GLUT1DS.

In patients with GLUT1DS, the severity of clinical symptoms usually correlates inversely with the CSF glucose levels and the CSF to blood glucose ratios [7]. In the hypoglycorrhachia group, we didn't find significant difference in CSF glucose levels between the patients with and those without adverse outcomes. However, we found that patients with adverse outcomes seemed to have higher CSF to blood glucose ratio than those without adverse outcomes. Although our study had a relatively large cohort of febrile infants with CSF data, the number of infants who had hypoglycorrhachia was relatively small. Subgroup analysis between even smaller numbers of patients might lead to some degree of uncertainty. A multicenter prospective study recruiting a larger number of febrile infants with hypoglycorrhachia for a longer time of follow up will be necessary to validate our findings.

There were some other limitations in this study. 32% (8/25) of the patients in the hypoglycorrhachia group did not have available record of blood glucose data at the time of CSF examination, which implied that hypoglycemia might be one of the causes of hypoglycorrhachia in some of the infants with hypoglycorrhachia. The fasting time before blood and CSF examinations usually cannot be standardized in the febrile young infants. We only performed MLPA in infants with mixed-type developmental disorder instead of in all the patients in the hypoglycorrhachia group. However, patients who had duplication or deletion in *SLC2A1* gene were all reported to have severe clinical presentations of GLUT1DS [7]. Our results might be post-hoc findings because of the small sample size. Therefore, a prospective study with a larger sample size using Sanger sequencing, MLPA and an erythrocyte 3-OMG uptake test is needed to address the long-term neurodevelopmental outcome and the prevalence rate of *SLC2A1* pathogenic variants in neurologically normal young infants who have hypoglycorrhachia [29].

In summary, one in 25 young infants who exhibit a normal CSF cell count and sterile CSF had hypoglycorrhachia that was comparable to GLUT1DS. One in 6 children with infantile hypoglycorrhachia had adverse outcomes. In contrast, none of the infants with hypoglycorrhachia showed pathogenic variants in the *SLC2A1* gene. Hypoglycorrhachia diagnosed in young infants who are neurologically asymptomatic may be a potential biomarker for neurodevelopmental delay instead of for GLUT1DS.

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## Ethics approval and consent to participate

This study was approved by the Institutional Review Board of National Cheng Kung University Hospital. 25 participants that received genetic testing of *SLC2A1* in this study provided written informed consents following a full explanation of the procedure undertaken; consents for children were obtained through the parents, caregivers or guardians. As for the 50 participants that completed questionnaire via telephone, oral informed consents were obtained through the parents, caregivers or guardians following a full explanation.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2019.07.004.

## References

[1] Mergenthaler P, Lindauer U, Dienel GA, Meisel A. Sugar for the brain: the role of glucose in physiological and pathological brain function. Trends Neurosci 2013;36:587–97.
[2] Pascual JM, Wang D, Hinton V, Engelstad K, Saxena CM, Van Heertum RL, et al. Brain glucose supply and the syndrome of infantile neuroglycopenia. Arch Neurol 2007;64:507–13.
[3] Viola GM. Extreme hypoglycorrhachia: not always bacterial meningitis. Nat Rev Neurol 2010;6:637–41.
[4] Silver TS, Todd JK. Hypoglycorrhachia in pediatric patients. Pediatrics 1976;58:67–71.

[5] Chow E, Troy SB. The differential diagnosis of hypoglycorrhachia in adult patients. Am J Med Sci 2014;348:186–90.
[6] Wang D, Pascual JM, Yang H, Engelstad K, Jhung S, Sun RP, et al. Glut-1 deficiency syndrome: clinical, genetic, and therapeutic aspects. Ann Neurol 2005;57:111–8.
[7] Leen WG, Klepper J, Verbeek MM, Leferink M, Hofste T, van Engelen BG, et al. Glucose transporter-1 deficiency syndrome: the expanding clinical and genetic spectrum of a treatable disorder. Brain 2010;133:655–70.
[8] Pascual JM, Wang D, Lecumberri B, Yang H, Mao X, Yang R, et al. GLUT1 deficiency and other glucose transporter diseases. Eur J Endocrinol 2004;150:627–33.
[9] De DV, Leary L, Wang D. Glucose transporter 1 deficiency syndrome and other glycolytic defects. J Child Neurol 2002;17:3S15-23.
[10] De Vivo DC, Trifiletti RR, Jacobson RI, Ronen GM, Behmand RA, Harik SI. Defective glucose transport across the blood-brain barrier as a cause of persistent hypoglycorrhachia, seizures, and developmental delay. N Engl J Med 1991;325:703–9.
[11] Larsen J, Johannesen KM, Ek J, Tang S, Marini C, Blichfeldt S, et al. The role of SLC2A1 mutations in myoclonic astatic epilepsy and absence epilepsy, and the estimated frequency of GLUT1 deficiency syndrome. Epilepsia 2015;56:e203–8.
[12] Mullen SA, Suls A, De Jonghe P, Berkovic SF, Scheffer IE. Absence epilepsies with widely variable onset are a key feature of familial GLUT1 deficiency. Neurology 2010;75:432–40.
[13] Overweg-Plandsoen W, Groener J, Wang D, Onkenhout W, Brouwer OF, Bakker HD, et al. GLUT-1 deficiency without epilepsy – an exceptional case. J Inherit Metab Dis 2003;26:559–63.
[14] Joshi C, Greenberg CR, De Vivo D, Wang D, Chan-Lui W, Booth FA. GLUT1 deficiency without epilepsy: yet another case. J Child Neurol 2008;23:832–4.
[15] Weber YG, Storch A, Wuttke TV, Brockmann K, Kempfle J, Maljevic S, et al. GLUT1 mutations are a cause of paroxysmal exertion-induced dyskinesias and induce hemolytic anemia by a cation leak. J Clin Invest 2008;118:2157–68.
[16] Nickels K, Wirrell E. GLUT1-ous maximus epileptics. The expanding phenotype of GLUT-1 variants and epilepsy. Neurology 2010;75:390–1.
[17] Leen WG, Wevers RA, Kamsteeg E-J, Scheffer H, Verbeek MM, Willemsen MA. Cerebrospinal fluid analysis in the workup of GLUT1 deficiency syndrome: a systematic review. JAMA Neurol 2013;70:1440–4.
[18] Willemsen MA, Verrips A, Verbeek MM, Voit T, Klepper J. Hypoglycorrhachia: a simple clue, simply missed. Ann Neurol 2001;49:685–6.
[19] Fujii T, Ito Y, Takahashi S, Shimono K, Natsume J, Yanagihara K, et al. Outcome of ketogenic diets in GLUT1 deficiency syndrome in Japan: a nationwide survey. Brain Dev 2016;38:628–37.
[20] Klepper J, Scheffer H, Leiendecker B, Gertsen E, Binder S, Leferink M, et al. Seizure control and acceptance of the ketogenic diet in GLUT1 deficiency syndrome: a 2-to 5-year follow-up of 15 children enrolled prospectively. Neuropediatrics 2005;36:302–8.
[21] Baraff LJ. Management of infants and young children with fever without source. Pediatr Ann 2008;37:673.
[22] Lieu TA, Baskin MN, Schwartz JS, Fleisher GR. Clinical and cost-effectiveness of outpatient strategies for management of febrile infants. Pediatrics 1992;89:1135–44.
[23] Leen WG, Willemsen MA, Wevers RA, Verbeek MM. Cerebrospinal fluid glucose and lactate: age-specific reference values and implications for clinical practice. PLoS One 2012;7 e42745.
[24] Thomson J, Sucharew H, Cruz AT, Nigrovic LE, Freedman SB, Garro AC, et al. Cerebrospinal fluid reference values for young infants undergoing lumbar puncture. Pediatrics 2018;141 e20173405.
[25] Taiwan Biobank.c2012 [cited 2018 Feb 01]. Available from: https://taiwanview.twbiobank.org.tw/index.
[26] Chugani HT, Phelps ME, Mazziotta JC. Positron emission tomography study of human brain functional development. Ann Neurol 1987;22:487–97.
[27] Rotstein M, Engelstad K, Yang H, Wang D, Levy B, Chung WK, et al. Glut1 deficiency: inheritance pattern determined by haploinsufficiency. Ann Neurol 2010;68:955–8.
[28] Arsov T, Mullen SA, Rogers S, Phillips AM, Lawrence KM, Damiano JA, et al. Glucose transporter 1 deficiency in the idiopathic generalized epilepsies. Ann Neurol 2012;72:807–15.
[29] Yang H, Wang D, Engelstad K, Bagay L, Wei Y, Rotstein M, et al. Glut1 deficiency syndrome and erythrocyte glucose uptake assay. Ann Neurol 2011;70:996–1005.