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Vitamin K prophylaxis in neonates: comparing two different oral regimens

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OBJECTIVE: This prospective study compared PIVKA-II and PT-INR levels in infants who received two vitamin K (VK) prophylactic regimens.

METHODS: A single institution administered 119 healthy newborns 2 mg of VK syrup. Infants were assigned to a 3-time regimen ($n = 56$) with VK at birth, five days (5D), and 1-month-old (1 M), or a 13-time regimen ($n = 63$) with VK at birth, 5D, and then weekly for 11 weeks.

RESULTS: The 13-time regimen significantly lowered PIVKA-II and reduced PT-INR at 1 M in both breastfed (PIVKA-II: 18–16 mAU/mL, $p = 0.02$; PT-INR: 1.37–1.13, $p < 0.01$) and formula-fed infants (PIVKA-II: 18–15 mAU/mL, $p = 0.01$; PT-INR: 1.54–1.24, $p < 0.01$), compared to baseline measurements taken at 5D. The 3-time regimen did not significantly alter PIVKA-II levels and only improved PT-INR (2.00–1.50, $p < 0.01$) in formula-fed infants.

CONCLUSION: The 13-time VK regimen significantly enhanced coagulation profiles more effectively than the 3-time regimen.

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INTRODUCTION

Hemorrhagic disease of the newborn (HDN) is a life-threatening bleeding disorder that occurs within a few weeks of life and is characterized by bleeding from various tissues and organs, including subcutaneous tissue, gastrointestinal tract, and intracranial spaces. HDN involves multifactorial etiologies, including inherited clotting factor deficiencies, birth trauma, asphyxia, sepsis, and vitamin K (VK) deficiency [1].

VK is an essential nutrient that plays a crucial role in coagulation. Newborn babies are susceptible to VK deficiency because of limited fetal stores and poor placental transfer from the mother [2]. VK deficiency bleeding (VKDB) in infancy and childhood leads to VK-dependent coagulopathy (factors II, VII, IX, and X deficiencies) but is treatable by VK supplementation [3].

Since 1961, the American Academy of Pediatrics has recommended VK administration to all newborns [4]. While a single intramuscular injection (IM) at birth has been the global standard, thigh muscle contracture following repeated IM, other than VK medications, in the 1970s [5] contributed to a hesitancy towards IM since the 1980s [6], fostering a parental preference for oral administration in our country [7–9].

In 1988, the Ministry of Health, Labor and Welfare Research Group of Japan recommended oral VK supplementation three times: at birth, upon hospital discharge at 5 days old (5D), and 1 month (1 M) [10]. Our revision for a 13-time regimen of weekly oral administration until 3 months of age was influenced by comprehensive practices against late-onset VKDB in infants with underlying diseases of the hepatobiliary system [11]. Inspired by the Dutch regimen of 1 mg oral at birth followed by 25 µg/day up to 3 months and the Danish regimen of 2 mg oral at birth and then 1 mg weekly for 3 months, we adapted these approaches to align with domestic healthcare practices [12, 13].

The diagnosis of VK deficiency involves detecting elevated proteins induced by VK absence or antagonist-II (PIVKA-II) levels or normalizing coagulation parameters such as activated partial thromboplastin time or prothrombin time-international normalization ratio (PT-INR) after VK administration. PIVKA-II, an undercarboxylated precursor of VK-dependent coagulation factors produced by the liver, becomes detectable in the bloodstream in VK-deficient states [14]. This early detection allows for timely intervention before the manifestation of bleeding complications [3, 15].

Reflecting on these developments, our hospital changed its prophylactic regimen from the conventional 3-time to a comprehensive 13-time regimen in February 2019. Taking advantage of this timing, we investigated VK-associated coagulability using PT-INR and PIVKA-II values in healthy infants who received two distinct regimens, offering insights into the efficacy of extended oral VK supplementation in preventing VKDB.

MATERIALS AND METHODS
Subjects enrollment

This prospective observational study recruited all newborns delivered at Fukuda Hospital between January 1, 2015, and December 31, 2022. The

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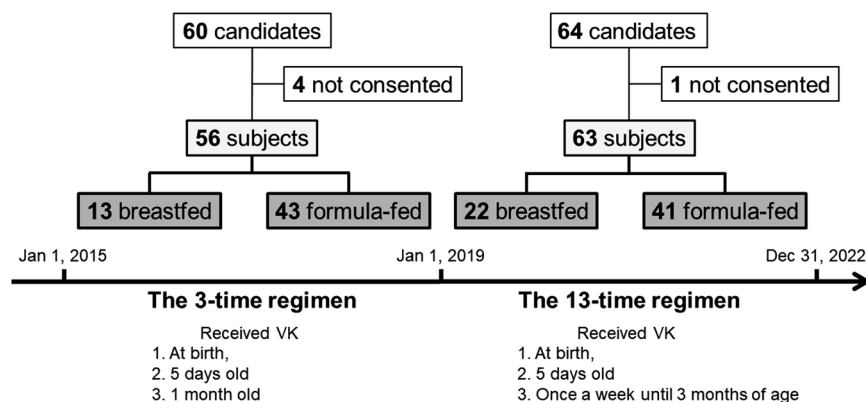
Fig. 1 **Overview of the comparative cohort.** Our hospital altered the vitamin K (VK) deficiency bleeding prophylaxes from the 3-time to the 13-time regimen at the beginning of 2019. Before this alteration, we planned a cohort to compare the coagulability of the two methods. The candidate newborns were presumed to be healthy infants who did not have a definite hemorrhagic disease of the newborn, a history of maternal medication that affected VK metabolism, gestational age <36 weeks, birth weight <2 000 grams, or hepatointestinal disabilities. We obtained parental consent for the study protocol from the 56 and 60 subjects in 2015–2019 and 2019–2022, respectively. Exclusively breastfed newborns were defined as the breastfed group, and those who received formula milk were defined as the formula-fed group.
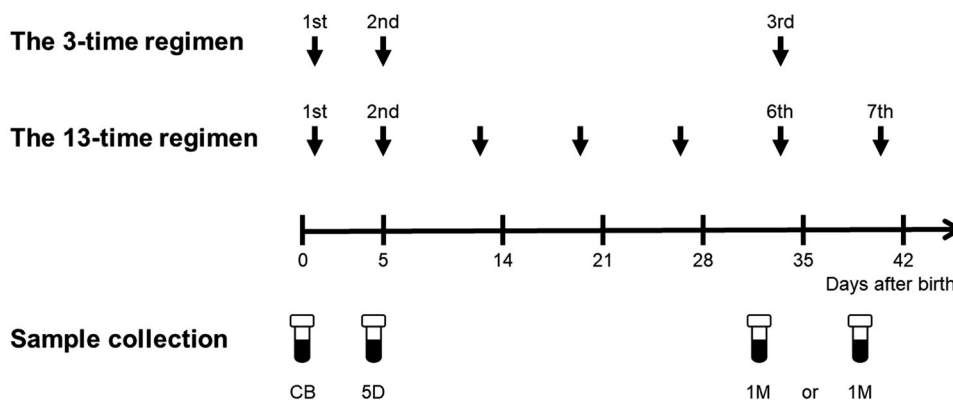
Fig. 2 **Chronological order of blood samplings and vitamin K (VK) administrations.** The first (cord blood [CB]) and second (5 days old [5D]) samples were collected before the first and second VK administrations, respectively. The third (1 month old [1 M]) sample was obtained before the third administration using the 3-time regimen and before the sixth or seventh administration using the 13-time regimen.

candidates did not include the following pathological newborns with definite HDN: a history of maternal medication affecting VK metabolism, gestational age <36 weeks, birth weight <2 000 grams, hepatointestinal disabilities, or suspected of having disseminated intravascular coagulation.

Prophylactic regimens
The enrolled subjects were divided into two groups according to the timing of the alteration of the prophylactic regimens at our hospital (Fig. 1). We use VK2 syrup (Kaytwo syrup, Eisai Co Ltd, Tokyo, Japan) as the oral preparation of menaquinone-4. The 3-time regimen group, born between January 1, 2015, and December 31, 2018, received a 2 mg VK2 syrup at birth, 5D (the day of hospital discharge), and 1 M. The 13-time regimen group, born between January 1, 2019, and December 31, 2022, received the same 2 mg VK2 syrup at birth, 5D, and once a week until 3 months of age (Fig. 2). The caregivers monitored for adverse effects following oral VK administration for gastrointestinal symptoms (vomiting, diarrhea) and allergic reactions (rash, erythema) within 24 h post-administration and any refusal to feed. Cases suspected of adverse effects underwent a detailed medical evaluation and were documented in the medical record.

Nutrition
We collected data on the infants' nutritional status through parent interviews during the hospital stay and at the follow-up visit one month after birth. Newborns exclusively receiving breast milk were categorized as 'breastfed.' All other infants, including those on mixed breast milk and formula diets, were classified as 'formula-fed' (Fig. 1), aligning with previous categorizations in VK deficiency [13].

Sampling
To assess VK-associated coagulability, we measured the plasma levels of PIVKA-II, PT-INR, and D-dimer at birth, 5D, and 1 M (Fig. 2). The first sample was obtained from the cord blood (CB) at birth. The second sampling was established on discharge from the maternity ward, usually five days after delivery. Blood samples were collected before the first and second VK administrations. At 1 month old, the third sampling was set before the third VK administration in the 3-time regimen group or before the sixth or seventh administration in the 13-time regimen group.

Coagulation tests
Whole blood in a 3.2% sodium citrate tube was centrifuged at 3000 rpm for 5 min, and the supernatant plasma was stored in a cryopreservation tube at $-30\,°C$. PIVKA-II was measured using Lumipulse presto PIVKA-II-N® (Sekisui Medical Inc., Tokyo, Japan). This assay's limit of detection and limit of quantitation are 1 mAU/mL, allowing for an accurate assessment of subtle VK status in newborns. At the same time, PT-INR was determined using Thromboel S® (Siemens Healthcare Diagnostics Inc., Tarrytown, USA), which is capable of precise measurement up to the hundredth decimal place. This level of precision ensures accurate PT-INR value assessment, reflecting subtle variances in coagulation status. Lias Auto D-dimer Neo® (Siemens Healthcare Diagnostics Inc., Tarrytown, USA) was used for

Table 1. Characteristics of the study subjects.

Variables	The 3-time regimen $n = 56$		The 13-time regimen $n = 63$		*p* value
Maternal age (years)	32	29–35	32	27–38	0.85
*Primipara	21	37.5	23	36.5	1.00
*Cesarean section	29	52.0	30	48.0	0.72
Gestational age (weeks, days)	38w4d	38w0d–39w5d	38w6d	38w3d–40w1d	0.01
Apgar score at 5 min. (point)	9	9–9	9	9–9	0.24
Umbilical artery pH	7.33	7.31–7.36	7.33	7.30–7.35	0.33
*Male	29	52	25	40	0.20
Birth height (meters)	0.495	0.481–0.500	0.500	0.490–0.505	0.26
Birth weight (grams)	2 895	2 651–3 176	2 958	2 762–3 178	0.39
*Exclusively breastfed	13	23.0	22	35.0	0.23

The 3-time regimen method administrated oral preparation of menaquinone-4 (Kaytwo syrup, Eisai Co Ltd, Tokyo, Japan) for newborns at birth, 5D, and 1 M. The 13-time regimen method was used at birth, 5D, and once a week until 3 months. Those variables represent the median and interquartile ranges or *the number and percentage. The Wilcoxon rank-sum test was used to compare continuous variables.

D-dimer detection. We excluded PT-INR values from samples with elevated D-dimer levels (>2.5 times the facility's standard or ≥2.5 µg/dL) owing to suspicious post-sampling coagulation. These assays were performed at Kyusyu University Hospital but were not standardized to set a reference range or cut-off level for PIVKA-II and PT-INR in healthy neonates or VKDB.

Outcome
Our primary endpoint was to assess the impact of two prophylactic VK regimens on newborn coagulability, as indicated by changes in PIVKA-II and PT-INR levels. Secondary included the incidence of VKDB and any adverse effects of oral VK administration.

Statistical analyses
The chi-square or Fisher's exact test was used for dichotomous variables to evaluate the distribution difference, whereas Wilcoxon's rank sum or signed-rank tests were used to compare continuous variables. A multiple regression model yielded *t*- and *p*-values to identify independent predictors for the values of PIVKA-II or PT-INR at 1 M. Both models included the values of the same item in the CB and the prophylactic methods as covariates but not other potential influencing factors, such as birth weight and gestational age. Statistical significance was set at a *p* value of <0.05. Statistical analyses were performed using the JMP Pro11 software program (version 11.2.0 for Windows; JMP Inc. SAS Institute, Tokyo, Japan).

RESULTS
Characteristics
Of the 124 candidates, we obtained informed consent from the parents of 119 subjects: 56 in the 3-time regimen group and 63 in the 13-time regimen group (Fig. 1). Table 1 compares the characteristics of the 3- and 13-time groups. Differences in gestational age were statistically significant ($p = 0.01$). Breastfeeding accounted for a larger population in the 13-time regimen group (35.0%) than in the 3-time group (23.0%). None of the infants showed symptoms of bleeding during the study periods. No adverse events related to the VK administration were observed.
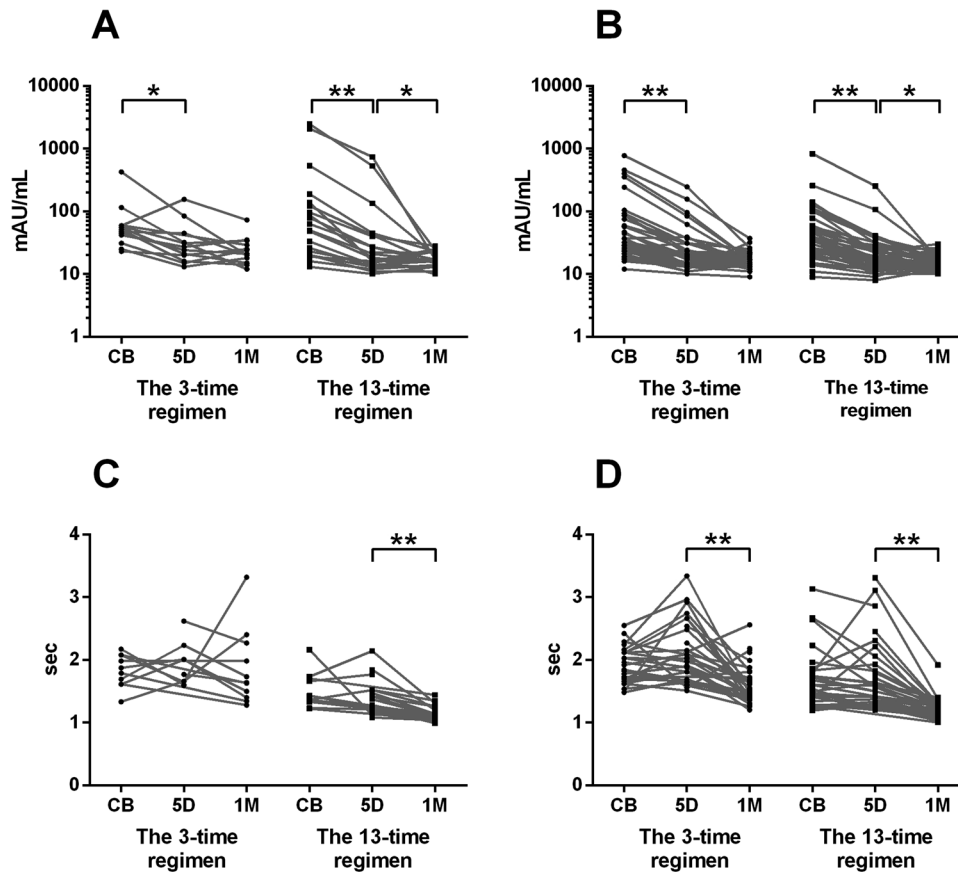
VK-associated coagulability
Of all the blood collections, 58 samples were excluded from measuring PT-INR based on elevated D-dimer levels (28 samples from CB, 22 at 5D, and 8 at 1 M). Table S1 presents PIVKA-II and PT-INR measurement values across two prophylactic regimens and nutritional statuses at birth, 5D, and 1 M. In breastfed infants (Fig. 3A), the 3-time regimen reduced PIVKA-II from 51 to 26 mAU/mL ($p = 0.03$), while the 13-time regimen decreased levels from 57 to 18 mAU/mL ($p < 0.01$) by 5D. Formula-fed infants (Fig. 3B) also saw reductions from 30 to 18 mAU/mL ($p < 0.01$) and 35 to 18 mAU/mL ($p < 0.01$) for the 3-time and 13-time regimens,

respectively. By 1 M, only the 13-time regimen further decreased PIVKA-II in breastfed infants to 16 mAU/mL ($p = 0.02$) and in formula-fed to 15 mAU/mL ($p = 0.01$), with no significant changes observed with the 3-time regimen. From birth to 5D, there were no significant changes in PT-INR for either regimen in breastfed (Fig. 3C) or formula-fed infants (Fig. 3D). However, beyond 5D, the 13-time regimen significantly improved PT-INR in breastfed infants, reducing from 1.37 to 1.13 ($p < 0.01$), and formula-fed infants from 1.54 to 1.24 ($p < 0.01$). The 3-time regimen also improved PT-INR in formula-fed infants from 2.00 to 1.50 ($p < 0.01$), but no significant change was observed in breastfed infants. The multivariate regression analysis indicated that prophylactic VK regimens significantly impacted the PIVKA-II and PT-INR levels at 1 M (Table S1). However, the formula-fed infants did not experience significant changes in PIVKA-II levels (t = −1.30, $p = 0.20$).

DISCUSSION
The novel regimen of weekly oral dosing until 3 months of age (13-time method) improved PIVKA-II and PT-INR values in breastfed infants and PIVKA-II values in formula-fed infants more than the conventional method (3-time regimen). Adverse events related to oral VK administration did not occur during the study period for either method. The present results support the novel 13-time method as the standard regimen in Japan to prevent VKDB in infancy and childhood. The substantial reduction in PIVKA-II levels in infants following the 13-time regimen underscores the regimen's potential to improve neonatal coagulation profiles, especially in exclusively breastfed infants.

By establishing a reference range for PIVKA-II levels in healthy infants who received prophylactic VK, our study not only adds to the clinical understanding of effective VKDB prevention but also calls attention to the need for further investigation into the optimal management of VK prophylaxis across diverse neonatal populations. A PIVKA-II threshold based on the 90th percentile of values from a healthy infant cohort could reduce the risk of VKDB misdiagnosis, but it would require a large study population and long observational periods. The absence of VKDB cases in our study cohort conducted a literature review to understand the PIVKA-II levels associated with VKDB. Electronic bibliographic databases, including PubMed and Google Scholar up to December 20, 2022, searched on the terms "newborn OR infant* OR neonate*" AND "vitamin K prophylaxis" AND "PIVKA-II," revealed that the median PIVKA-II value in VKDB infants ranged from 5000 to 86,000 mAU/mL [16–19]. The substantial differences between those levels in the obtained patients and our healthy newborns emphasize the effectiveness of both regimens. Notably, within our

Fig. 3 Kinetics of vitamin K (VK)-associated coagulability. The 3-time regimen was administrated oral VK at birth, 5D, and 1 M. The 13-time regimen was administered at birth, 5D, and once a week for 3 months. Levels of vitamin K absence or antagonist (PIVKA-II) during the neonatal period in the breast (**A**) and formula-fed (**B**) infants were significantly decreased within 5D but differed by the prophylactic methods and the nutritional status between 5D and 1 M. The prothrombin time-international normalization ratio (PT-INR) did not decrease in the breastfed (**C**) or the formula-fed infants (**D**) within 5D, regardless of the prophylactic methods. The 3-time regimen did not shorten the PT-INR after 5D under formula feeding. CB cord blood, D days old, M month old; *$p < 0.05$ and **$p < 0.01$ on the Wilcoxon signed-rank sum test.

cohort of healthy neonates, PIVKA-II levels did not surpass the >1 µg/mL (10 000 mAU/mL) threshold commonly indicative of VKDB [20]. This observation further substantiates the efficacy of the 13-time regimen in maintaining safe levels of PIVKA-II, suggesting that this regimen could be effectively utilized to prevent VKDB in infancy without the risk of reaching the high PIVKA-II levels associated with VKDB.

A single IM injection is the global method for the prophylactic VKDB regimen. However, some regions select alternative oral administration [4, 7, 8]. There is no significant difference between the two methods in terms of coagulation status [7, 9]. The incidence of late-onset VKDB was 0.44 per 100 000 infants in the 3-time regimen, with no patients receiving daily or weekly oral dosing [21]. A repeated oral regimen may be impractical because of parental compliance. An epidemiological study conducted in developed countries confirmed the inferiority of the 3-time oral regimen to the 1-time IM injection, but the efficacy of daily oral dosing was similar to that of IM injection [22]. Variations in oral regimens across regions cannot completely prevent late-onset VKDB. In addition, these weekly regimens may help improve VK-associated coagulopathy in infants with underlying hepatobiliary diseases [21]. Further nationwide surveillance is needed to validate whether or not the 13-time regimen prevents secondary VKDB.

Several studies have used an enzyme-linked immunosorbent assay (ELISA) to measure PIVKA-II levels with a limited detection sensitivity of 0.1 to 0.2 AU/mL [23]. Consequently, the distribution of PIVKA-II levels below the detection sensitivity of an ELISA method remains to be investigated. Our study measured PIVKA-II

levels using a chemiluminescent enzyme immunoassay (CLEIA) with a ≥ 1 mAU/mL sensitivity [24]. The high sensitivity of this CLEIA method allowed us to compare the coagulability of the two prophylactic regimens in ostensibly healthy newborns. A recent study measured PIVKA-II levels using an electrochemilumines-cence immunoassay (ECLIA) in preterm low-birth-weight infants receiving prophylactic IM injection of VK [25]. The measured PIVKA-II values using this method ranged from 3 to 700 mAU/mL, which is comparable to our results. A CLEIA and ECLIA can thus be used to evaluate VK deficiency even in pathological newborns.

Several limitations associated with the present study warrant mention. The single-center setting and cohort design of this study limited the generalizability of the findings. The small sample size led to limited statistical power and produced an inevitable statistical difference in gestational age between the groups receiving the two regimens. Due to the technical difficulties associated with neonatal blood sampling, some samples were suspected of excessive coagulation and were excluded from the study. Proton sulfate testing should be utilized to confirm the absence of coagulation in samples before analysis. However, due to constraints in our testing environment, we resorted to using elevated D-dimer levels as a surrogate marker to screen for post-sampling coagulation. The observation that only eight samples were excluded at the one-month mark due to elevated D-dimer levels prompts important considerations regarding the natural variability of D-dimer in neonates. This pattern likely indicates the maturation and stabilization of the coagulation system in neonates over the first month of life [26]. Future research should

explore improved blood sampling techniques, coagulation prevention measures, and methods for assessing coagulation in neonatal samples. The lack of a comparison with other prophylactic regimens, such as a single IM injection or daily oral administration, made it difficult to determine the superiority of the 13-time regimen over the others. A medical limitation in our country is that we cannot evaluate the long-term outcomes of healthy subjects beyond one month old. Furthermore, the improvements in PT-INR and PIVKA-II values during the neonatal period did not reduce actual bleeding events in infancy and childhood.

The present study evaluated coagulability during the first month of life in healthy subjects receiving the 3- and 13-time prophylactic regimens and added biological evidence concerning weekly oral dosing. Further large-sampled, long-time cohorts may highlight the effectiveness and safety of these regimens for preventing VKDB in late infancy and infants with underlying hepatointestinal disabilities.

DATA AVAILABILITY
The datasets generated and analyzed during the current study are not publicly available due to institutional ethical policy.

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AUTHOR CONTRIBUTIONS
Takahashi D, Egami N, and Ochiai M conceptualized and designed the study, designed the data collection instruments, collected the data, carried out initial analyses, drafted the initial manuscript, and critically reviewed and revised the manuscript. Hotta T measured and analyzed the collected samples and critically reviewed and revised the manuscript. Suga S conducted the initial analyses and critically reviewed and revised the manuscript. Suga S, Ishimura M, Kawaguchi C, Uchiumi T, Nishikubo T, Nogami K, and Goto K conceptualized and designed the study and critically reviewed and revised the manuscript. Ohga S supervised the study design, drafted the initial manuscript, and critically reviewed and revised the manuscript. All authors had access to the data, approved the final manuscript as submitted, and agreed to be responsible for all aspects of this work.

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COMPETING INTERESTS
The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
The study protocol was reviewed and approved by the Fukuda Hospital institutional review board (27-1-2). Written informed consent was obtained from each parent of candidate newborns.

ADDITIONAL INFORMATION
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