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MATERNAL-FETAL MEDICINE

Intrauterine exposure to chorioamnionitis and neuroanatomical alterations at term-equivalent age in preterm infants

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Abstract

Purpose Infants born to mothers with chorioamnionitis (CAM) are at increased risk of developing adverse neurodevelopmental disorders in later life. However, clinical magnetic resonance imaging (MRI) studies examining brain injuries and neuroanatomical alterations attributed to CAM have yielded inconsistent results. We aimed to determine whether exposure to histological CAM in utero leads to brain injuries and alterations in the neuroanatomy of preterm infants using 3.0- Tesla MRI at term-equivalent age.

Methods A total of 58 preterm infants born before 34 weeks of gestation at Nagoya University Hospital between 2010 and 2018 were eligible for this study (CAM group, n = 21; non-CAM group, n = 37). Brain injuries and abnormalities were assessed using the Kidokoro Global Brain Abnormality Scoring system. Gray matter, white matter, and subcortical gray matter (thalamus, caudate nucleus, putamen, pallidum, hippocampus, amygdala, and nucleus accumbens) volumes were evaluated using segmentation tools (SPM12 and Infant FreeSurfer).

Results The Kidokoro scores for each category and severity in the CAM group were comparable to those observed in the non-CAM group. White matter volume was significantly smaller in the CAM group after adjusting for covariates (postmenstrual age at MRI, infant sex, and gestational age) ($p = 0.007$), whereas gray matter volume was not significantly different. Multiple linear regression analyses revealed significantly smaller volumes in the bilateral pallidums (right, $p = 0.045$; left, $p = 0.038$) and nucleus accumbens (right, $p = 0.030$; left, $p = 0.004$) after adjusting for covariates.

Conclusions Preterm infants born to mothers with histological CAM showed smaller volumes in white matter, pallidum, and nucleus accumbens at term-equivalent age.

Keywords Chorioamnionitis · Magnetic resonance imaging · Neurodevelopment · Nucleus accumbens · Pallidum · Preterm infants

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What does this study add to the clinical work

Preterm infants born to mothers with histological chorioamnionitis (CAM) showed smaller white matter, pallidum, and nucleus accumbens volumes than those born to mothers without CAM at term-equivalent age. However, the brain injury and abnormality scores and levels of the brain injury marker, S100B, were comparable.

Introduction

Chorioamnionitis (CAM) is a common pregnancy complication, accounting for approximately 30–40% of preterm births [1]. Growing evidence indicates that maternal infections, particularly CAM, are associated with various neonatal morbidities, including sepsis, respiratory disorders, and neurodevelopmental impairment [2, 3]. Furthermore, these adverse complications can be severe, particularly in extremely preterm infants. Several meta-analyses and population-based studies have highlighted significant associations between histological CAM and increased risks of severe brain injury and neurodevelopmental disorders, including intraventricular hemorrhage, cerebral palsy, autism spectrum disorder (ASD), and attention-deficit/hyperactivity disorder (ADHD) [4–7]. Children with these neurodevelopmental impairments may require lifelong healthcare and social support. Therefore, elucidation of the mechanisms underlying such disorders caused by intrauterine exposure to CAM is a pressing issue in perinatal medicine.

The neurodevelopmental impairment attributed to CAM has a complex, multifactorial etiology that includes the following: (1) activation of a systemic inflammatory cascade (e.g., TNF-α, IL-6, IL-8, and IL-1β); (2) injury of oligodendrocytes and neurons by inducing apoptosis and disrupting their differentiation; (3) dysfunction of glial cells, including microglia and astrocytes, with subsequent production of free radicals [8]; and (4) impaired integrity of the blood–brain barrier and disturbances in cerebral hemodynamics [9, 10]. However, most of this evidence is based on preclinical studies in rodents and nonhuman primates and not in human subjects through the use of brain magnetic resonance imaging (MRI) or pathological brain tissue specimens. In particular, there is insufficient evidence regarding morphological and volumetric brain assessments of neonates born to mothers with CAM.

To date, clinical MRI studies examining brain injury and neuroanatomical alterations attributed to CAM have yielded inconsistent results. Several reports have indicated that CAM is not associated with an increased risk of non-cystic white matter injury, recognized as the dominant form of brain injury in preterm infants, or abnormal brain development by evaluating MRI at term-equivalent age [4, 11, 12]. In contrast, Hatfield et al. demonstrated that preterm offspring born to mothers with CAM showed widespread, long-term regional alterations in brain development at six to ten years old [13]. Furthermore, neurodevelopmental disorders such as ASD and ADHD are closely related to structural and functional changes in the subcortical gray matter including the basal ganglia; however, no studies have focused on volumetric alterations in the subcortical gray matter. Further studies are needed to investigate the association between CAM and neuroanatomical changes.

Although morphological brain analyses using MRI have been widely performed for various adult diseases, such as Alzheimer's disease, Parkinson's disease, and schizophrenia [14–16], those for pediatric diseases, especially neonates, are lagging significantly owing to technical and safety issues. For example, FreeSurfer, a useful software platform that achieves automated segmentation of cortical and subcortical brain areas, cannot be applied to children aged less than five years. However, Infant FreeSurfer, released in 2020, enables automated segmentation and surface extraction using brain MRI data from a population of zero to two year olds [17].

Statistical Parametric Mapping software version 12 (SPM12) was designed for various analyses of neuroimaging data, and can segment images into gray matter, white matter, and cerebrospinal fluid. In addition, various scoring systems (e.g., the Kidokoro Global Brain Abnormality Scoring system) for the assessment of brain injury and abnormalities using conventional MRI have been developed and validated to identify infants at a high risk of subsequent neurodevelopmental impairment [18–20]. S100B, an astrocyte related protein, is a well-known early marker of brain injury [21]. S100B levels in umbilical cord blood are useful for predicting brain injury associated with hypoxic-ischemic encephalopathy, preterm birth, and intraventricular hemorrhage [21–24].

This study aimed to investigate the association among histological CAM, neonatal neuroanatomical alterations, and brain injury using neuroimaging techniques and umbilical cord analyses. In this study, we evaluated brain injury and abnormalities using the Kidokoro score among preterm infants born before 34 weeks of gestation using MRI at term-equivalent age. We also evaluated brain volumes at the regional level of the subcortical gray matter using Infant FreeSurfer and at the global level using SPM12. In addition, we evaluated brain injury at birth using S100B levels in the umbilical cord blood. This study may provide insights into

the pathophysiology of the subsequent increased risk of neurodevelopmental and neuropsychiatric disorders in infants exposed to CAM.

Material and methods

Study population

The study population of this retrospective cohort study consisted of preterm infants born at Nagoya University Hospital between January 2010 and December 2018. The inclusion criteria were preterm infants born between $22^{0/7}$ and $33^{6/7}$ gestational weeks who were admitted to the neonatal intensive care unit (Fig. 1). The exclusion criteria were as follows: multiple pregnancies, major congenital and/or chromosomal abnormalities, no MRI (e.g., in-hospital death, transfer to other hospitals before MRI, respiratory and/or circulatory problems not allowing MRI to be performed safely at term-equivalent age, and refusal of participation), low-quality MRI [e.g., 1.5 Tesla MRI or unsuitable for segmentation tools (Infant FreeSurfer and SPM12) due to motion or ghost artifacts], severe brain injury (intraventricular hemorrhage grade III/IV, cystic periventricular leukomalacia, encephalitis, or persistent ventricular dilatation), pregnancy complications (hypertensive disorders of pregnancy, fetal growth restriction, and placental abruption), an infant exposed to chemotherapy during pregnancy, and no pathological examination of the placenta (Fig. 1). In this study, we excluded infants with severe brain injury because accurate segmentation could not be performed due to abnormal structural changes in the brain. The participants and clinical data in this study partially overlapped with those in our previous study [25]. Brain volume and brain injury were assessed in 21 infants born to mothers with histological CAM (CAM group) and in 37 infants born to mothers without histological CAM (non-CAM group). Among the 58 infants, neurodevelopmental assessment at three years of age was performed in 36 (CAM group, n = 11; non-CAM group, n = 25) after excluding 22 infants who were transferred to another hospital after discharge or lost to follow-up.

Clinical data acquisition and ethical approval

Clinical data on maternal and neonatal characteristics were obtained from electronic medical records. Maternal characteristics included age, parity, gestational age, body mass index before pregnancy, mode of delivery, diabetes mellitus, gestational diabetes mellitus, pregnancy via assisted reproductive technology, antenatal corticosteroid treatment, antenatal magnesium treatment, and the stage of histological CAM. Neonatal characteristics included sex, birth weight, height, head circumference, and short-term neonatal outcomes such as respiratory distress syndrome, chronic lung disease, intraventricular hemorrhage (grade I/II), patent ductus arteriosus banding, inotrope use, postnatal steroid use, necrotizing enterocolitis, infection, treated retinopathy of prematurity, and duration of hospitalization. This study was approved by the Institutional Ethics Board of Nagoya University (approval numbers: 2015–0068 and 2018–0026), and written informed consent was obtained from all parents.

Definition

Histological CAM is based on the pathological findings of placental histology, including neutrophil infiltration of the placental membranes [Blanc classification: subchorionic (stage I), chorionic (stage II), amniotic membrane (stage III)], and umbilical cord (funisitis) [26]. Fetal inflammatory response syndrome (FIRS) is defined as elevated interleukin-6 (IL-6) values in umbilical cord blood (IL-6 > 11.0 pg/mL) [27]. Hypertensive disorders of pregnancy were defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg during pregnancy [28]. Fetal growth restriction was defined as birth weight and height < 10^{th} percentile according to curves from the Japanese gender-specific neonatal anthropometric chart in 2000

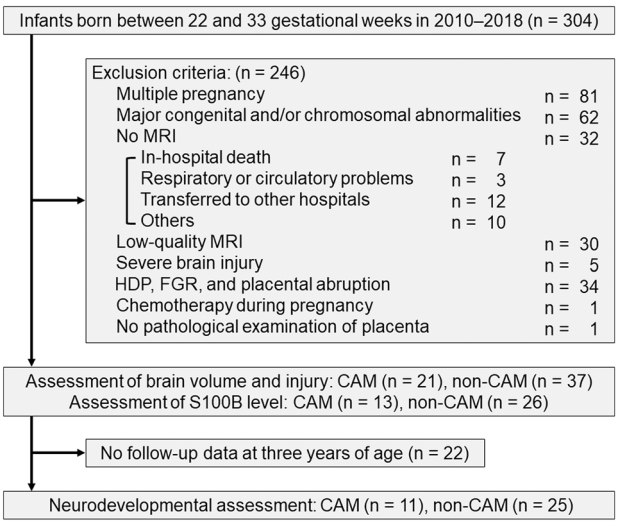

Fig. 1 Flow diagram of the study population. The clinical data of 304 preterm infants born at 22–33 gestational weeks between 2010 and 2018 are available. Brain volume and injuries were assessed in 58 infants [CAM (n = 21), non-CAM (n = 37)] after excluding 246 infants. Among the 58 infants, 36 [CAM (n = 11), non-CAM (n = 25)] underwent neurodevelopmental assessment at three years of age. *MRI* magnetic resonance imaging, *HDP* hypertensive disorders of pregnancy, *FGR* fetal growth restriction, *CAM* chorioamnionitis

[29]. Antenatal corticosteroid treatment was defined as two doses of betamethasone (12 mg) administered before delivery. The definitions of short-term neonatal outcomes have been described previously [25].

MRI acquisition

MRI data were collected using a 3.0 Tesla scanner system (MAGETOM Verio; Siemens, Erlangen, Germany) with a 32-channel head coil at Nagoya University Hospital. To perform automated segmentation and evaluate the Kidokoro score, we obtained high-resolution three-dimensional magnetization-prepared rapid acquisition of gradient echo (3D MPRAGE) T1-weighted sequence (repetition time [TR], 1570 ms; echo time [TE], 2.2 ms; voxel size, $1 \times 1 \times 1$ mm^3) and a turbo spin-echo T2-weighted sequence (TR 3200 ms; TE, 499 ms; voxel size, $1 \times 1 \times 1$ mm^3; echo train length, 123.0), described previously [25].

Infant FreeSurfer

Automated segmentation was performed to obtain brain regional volumes from the 3D MPRAGE T1-weighted sequence using the Neuroimaging Informatics Technology Initiative format in the recon-all pipeline on Infant FreeSurfer [17]. A representative image of segmentation using Infant FreeSurfer is shown in Fig. 2A–D. We visually checked the quality of the automated regional segmentation using FreeView's graphic interface (https://surfer.nmr.mgh.harvard.edu/). We found that cortical segmentation was imprecise, likely due to specific neonatal or other reasons related to Infant FreeSurfer [30]; thus, we only used data on regional volumes of subcortical gray matter in this study. However, we found that SPM12 was more accurate than Infant FreeSurfer for cortical segmentation; therefore, we used SMP12 to evaluate the gray and white matter volumes. The skull-stripped total brain volume, and regional brain volumes of the subcortical gray matter (thalamus, caudate nucleus, putamen, pallidum, hippocampus, amygdala, and nucleus accumbens) were measured based on the "aseg" annotation atlas [31]. The regional volumes of the subcortical gray matter were compared between the CAM and non-CAM groups in two ways: absolute volume and relative volume (percentage of the total brain volume). In addition, multiple linear regression analysis was performed to evaluate the absolute and relative volumes after adjusting for two types of covariates (#1: postmenstrual age at MRI and infant sex, and #2: postmenstrual age at MRI, infant sex, and gestational age).

SPM12

Image processing of the segmentation by SPM12 was described in detail previously [25]. Briefly, global volumetric analysis was conducted based on the SPM12 implemented in MATLAB (version22, MathWorks, INC., MA, USA). SPM12 software was used for the automated segmentation of gray matter, white matter, and cerebrospinal fluid. The quality of the automated segmentations was visually evaluated, and MRI with low-quality segmentation despite motion correction was excluded.

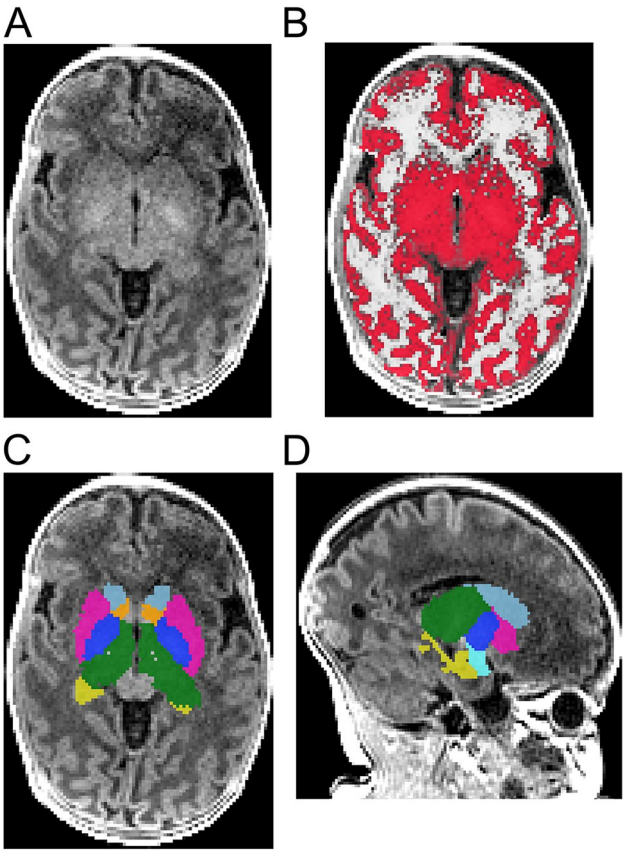

Fig. 2 Representative pictures of automated segmentation by Infant FreeSurfer. Representative images of segmentation using Infant FreeSurfer **A** axial T1-weighted, **B** axial image with automated segmentation, **C** axial image with automated segmentation of subcortical gray matter, **D** sagittal image with automated segmentation of subcortical gray matter)

Assessment of brain injury and abnormalities

Brain injury and abnormalities at term-equivalent age were assessed using the Kidokoro Global Brain Abnormality score, which is a widely accepted scoring system for detecting brain abnormalities and predicting subsequent neurodevelopmental outcomes [18]. The scoring system consisted of four separate categories using T1- and T2-weighted sequences: (1) cerebral white matter abnormality (scores 0–17), (2) cortical gray matter abnormality (scores 0–9), (3) deep gray matter abnormality (scores

0–7), and (4) cerebellum abnormality (scores 0–7). The Kidokoro score is the sum of the scores in each category and is classified as normal (scores 0–3), mild (scores 4–7), moderate (scores 8–11), or severe (scores 12–40). The assessment of the neonatal MRI was reviewed by a single neonatal neurologist who was blinded to the clinical data.

Assessment of brain damage and FIRS at birth by umbilical cord analysis

Umbilical cord blood samples were collected during the delivery. Cord S100B and IL-6 concentrations were measured using a commercial enzyme-linked immunosorbent assay kit (YK151; Yanaihara Institute Inc., Japan) and a high-sensitivity multiple immunoassay using electrochemiluminescence (K151A0H, Meso Scale Discovery, Tokyo, Japan), respectively, as previously described [32].

Assessment of neurodevelopment

Neurodevelopment was assessed by experienced clinical psychologists at three years of age using the Kyoto-Scale Developmental Quotient (DQ). The Kyoto-Scale DQ is comparable to the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley III) [33]. The composite DQ (mean = 100) was derived from three separate categories (postural and motor, cognitive and adaptive, and language and social areas). Generally, a DQ ≥ 85 is defined as normal development, a DQ between 70 and 84 as borderline development, and a DQ < 70 as developmental delay. Preterm infants born before 34 gestational weeks were followed up until at least three years of age. Children who were subsequently diagnosed with ASD or ADHD based on the DSM-5 criteria were included for additional analysis.

Statistical analysis

All statistical analyses were performed using SPSS version 28 (SPSS Inc., Chicago, IL, USA). Demographic and clinical characteristics were compared using the Mann–Whitney U test or unpaired *t*-test for continuous variables and the Chi-squared or Fisher's exact test for categorical variables. The gray and white matter volumes between the CAM and non-CAM groups were compared after adjusting for two types of covariates (#1: postmenstrual age at MRI and infant sex; #2: postmenstrual age at MRI, infant sex, and gestational age). To evaluate the relationship between the stage of CAM and regional volumes, we performed an analysis of variance (ANOVA) or the Kruskal–Wallis test, as appropriate. Pearson's or Spearman's correlation coefficients were used to evaluate the correlation between the S100B levels and regional volumes. Statistical significance for all analyses was set at $p < 0.05$.

Results

During the study period, 304 infants were born between $22^{0/7}$ and $33^{6/7}$ weeks of gestation. After excluding 246 infants, 58 infants in the CAM group (n = 21; stage I: n = 5; stage II: n = 8; and stage III: n = 8) and non-CAM group (n = 37) were included in the study and assessed for brain volumes and injury (Fig. 1). The baseline characteristics of the two groups are presented in Table 1. No significant differences were observed between the two groups in terms of maternal characteristics, gestational age at birth, sex, birth weight, or neonatal complications, aside from neonatal infections. The physical assessment and timing of MRI are shown in Supplementary Table 1.

The Kidokoro scores for each category and severity in the CAM group were comparable to those in the non-CAM group (Table 2). In addition, no significant difference was found in the DQ at three years of age between the two groups (CAM group, n = 11; non-CAM group, n = 25).

The segmented regional absolute and relative volumes evaluated using the Infant FreeSurfer in the CAM and non-CAM groups were compared using univariate and multivariate analyses. Univariate analysis revealed significantly smaller absolute volumes of the bilateral pallidums (right, $p < 0.01$; left, $p = 0.04$) and nucleus accumbens (right, $p < 0.01$; left, $p < 0.01$) in the CAM group than those in the non-CAM group (Table 3). Multiple linear regression analysis also showed significantly smaller absolute volumes in the bilateral pallidums and nucleus accumbens after adjusting for the two types of covariates. In contrast, significantly smaller relative volumes were observed in the left nucleus accumbens ($p < 0.01$) in multivariate analyses (covariates #1 and #2) (Table 3). Supplementary Table 2 shows the segmented regional absolute and relative volumes of subcortical gray matter (14 regions) in the CAM and non-CAM groups. We then evaluated the effect of gestational age on each regional brain volume using multiple linear regression analysis. The volumes of the left pallidum and right hippocampus were significantly associated with the gestational age (Supplementary Table 3).

We then evaluated the relationship between CAM stage and the absolute and relative volumes of the bilateral pallidums and nucleus accumbens (Supplementary Fig. 1). We found significant differences among the four groups (non-CAM and stage I–III CAM) in the absolute volume of the

left pallidum and bilateral nucleus accumbens; however, we could not find any evidence of a consistent trend of these volumes increasing or decreasing from non-CAM to stages I–III CAM.

We evaluated the relationship between funisitis/FIRS and the volumes of the bilateral pallidums and nucleus accumbens (Supplementary Fig. 2 and 3, respectively). We found that funisitis was associated with smaller absolute volumes of the left pallidum and accumbens, and smaller relative volumes of the left pallidum; however, FIRS was not associated with these two regional volumes.

Table 4 presents the global brain volumes evaluated using SPM12 in the CAM and non-CAM groups. Multiple linear regression analysis did not show significant differences in gray matter or total volume; however, white matter volume was significantly smaller in the CAM group after adjusting for covariates (#1 and #2).

We performed additional analysis to investigate the association between these regional and global volumetric alterations and the subsequent risk of ASD or ADHD. Among the 36 infants who were followed up until at least three years of age, seven (two from the CAM group and five from the non-CAM group) were subsequently diagnosed with ASD or ADHD. These seven infants showed smaller white matter, right pallidum, and right nucleus accumbens volumes than the other infants, but the difference was not statistically significant (Supplementary Table 4).

We found no significant differences in S100B levels between the two groups [CAM: median (interquartile range), 0.92 (0.75–1.17), non-CAM: 0.88 (0.76–1.02), $p = 0.74$]. Supplementary Fig. 4 shows the relationship between S100B levels and the volumes of the bilateral pallidum and nucleus accumbens. We observed a poor association between S100B levels and these regional volumes.

Table 1 Baseline maternal and neonatal characteristics in the CAM and non-CAM groups

	CAM (n = 21)	Non-CAM (n = 37)	*p*-value
Maternal characteristics			
Maternal age (years)	34.8 ± 7.2	33.2 ± 4.5	0.90
Pre-pregnancy BMI (kg/m^2)	21.5 ± 3.7	21.9 ± 4.6	0.95
Primiparous (%)	8 (38.1%)	14 (37.8%)	0.99
Cesarean section (%)	16 (76.2%)	32 (86.5%)	0.26
GDM/DM (%)	2 (9.5%)	2 (5.4%)	0.46
Pregnancy via ART (%)	4 (19.0%)	6 (16.2%)	0.53
ACS treatment (%)	14 (66.7%)	26 (70.3%)	0.78
Antenatal Mg treatment (%)	7 (33.3%)	7 (18.9%)	0.22
Blanc's classification (%)			
Stage I	5 (23.8%)	NA	
Stage II	8 (38.1%)	NA	
Stage III	8 (38.1%)	NA	
Funisitis (%)	12 (57.1%)	NA	
Neonatal characteristics			
Male (%)	10 (47.6%)	21 (56.8%)	0.50
Gestational age (weeks)	30.8 ± 3.2	30.4 ± 2.3	0.20
Birth weight (g)	1567 ± 496	1,507 ± 391	0.47
Height at birth (cm)	40.3 ± 4.7	40.1 ± 3.7	0.65
Head circumference at birth (cm)	28.3 ± 2.6	28.2 ± 2.1	0.56
RDS (%)	8 (38.1%)	21 (56.8%)	0.17
Chronic lung disease (%)	1 (4.8%)	8 (21.6%)	0.09
IVH (grade I/II) (%)	1 (4.8%)	2 (5.4%)	0.71
PDA banding (%)	0 (0.0%)	1 (2.7%)	0.64
Inotrope use (%)	1 (4.8%)	2 (5.4%)	0.71
Postnatal steroid use (%)	0 (0.0%)	1 (2.7%)	0.64
Necrotizing enterocolitis (%)	0 (0.0%)	0 (0.0%)	1.00
Infection (%)	5 (23.8%)	1 (2.7%)	0.02
Treated ROP (%)	2 (9.5%)	3 (8.1%)	0.60
Duration of hospitalization (days)	61.9 ± 32.6	64.5 ± 24.2	0.38

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

CAM chorioamnionitis, *BMI* body mass index, *GDM* gestational diabetes mellitus, *DM* diabetes mellitus, *ART* assisted reproductive technology, *ACS* antenatal corticosteroid, *Mg* magnesium sulfate, *RDS* respiratory distress syndrome, *IVH* intraventricular hemorrhage, *PDA* patent ductus arteriosus, *ROP* retinopathy of prematurity, *NA* not applicable

Table 2 The Kidokoro global brain abnormality score and neurodevelopmental assessment in the CAM and non-CAM groups

	CAM (n = 21)	Non-CAM (n = 37)	*p*-value
The Kidokoro score			
Cerebral white matter score (0–17)	2 (1–5)	3 (1–5)	0.64
Cortical gray matter score (0–9)	0 (0–1)	0 (0–2)	0.63
Deep gray matter score (0–7)	0 (0–1)	0 (0–2)	0.25
Cerebellum score (0–7)	1 (0–2)	1 (0–2)	0.99
Total score (0–40)	4 (1–7)	4 (2–8)	0.98
The Kidokoro score severity			
Normal (0–3)	7 (33.3%)	17 (45.9%)	0.62
Mild (4–7)	14 (66.7%)	19 (51.4%)	
Moderate (8–11)	0 (0%)	1 (2.7%)	
Severe (12–40)	0 (0%)	0 (0%)	
DQ at three years of age	(n = 11)	(n = 25)	
Postural and motor area	95.1 ± 10.7	92.3 ± 15.5	0.84
Cognitive and adaptive area	80.4 ± 11.5	85.5 ± 15.2	0.57
Language and social area	85.5 ± 15.3	79.4 ± 20.2	0.38
Total	86.5 ± 11.9	84.5 ± 14.6	0.70

Data are presented as median (range), *n* (%), or mean ± standard deviation

CAM chorioamnionitis, *DQ* developmental quotient

Table 3 Segmented brain volumes of the pallidums and nucleus accumbens in the CAM and non-CAM groups

	CAM (n = 21)	Non-CAM (n = 37)	*p*-value	Adjusted #1 *p*-value	Adjusted #2 *p*-value	FDR
Right pallidum (mm^3)	865 ± 75	951 ± 162	< 0.01	0.043	0.045	0.04
Left pallidum (mm^3)	1036 ± 165	1151 ± 216	0.041	0.052	0.038	0.10
Right nucleus accumbens (mm^3)	280 ± 29	312 ± 55	< 0.01	0.030	0.030	0.03
Left nucleus accumbens (mm^3)	251 ± 40	292 ± 55	< 0.01	< 0.01	< 0.01	0.02
Right pallidum (%)	0.30 ± 0.03	0.32 ± 0.05	0.22	0.21	0.21	0.78
Left pallidum (%)	0.36 ± 0.06	0.38 ± 0.07	0.26	0.15	0.10	0.70
Right nucleus accumbens (%)	0.10 ± 0.01	0.10 ± 0.01	0.06	0.08	0.07	0.66
Left nucleus accumbens (%)	0.09 ± 0.01	0.10 ± 0.01	0.02	0.01	0.01	0.26

Data are presented as mean ± standard deviation. Segmented regional volumes were compared between the two groups after adjusting for covariates #1 (postmenstrual age at MRI and infant sex) and #2 (postmenstrual age at MRI, infant sex, and gestational age)

CAM chorioamnionitis, *MRI* magnetic resonance imaging

Discussion

In this study, we evaluated the impact of histological CAM on brain volume and brain injury at term-equivalent age in preterm infants using neuroimaging techniques, including the latest automated segmentation software. The main finding of this study was that infants born to mothers with CAM showed significantly smaller white matter, pallidum, and nucleus accumbens volumes, indicating that exposure to CAM during pregnancy among preterm infants is associated with altered brain development, even during the neonatal period. It is also noteworthy that these volumetric alterations were detected even though no significant differences in brain injury, abnormalities score at term-equivalent age, and S100B at birth were observed between the CAM and non-CAM groups. The findings of our study suggest that CAM may lead to relatively subtle, local neuroanatomical alterations that are not detectable using conventional MRI.

Although many animal studies have investigated neurological changes attributed to maternal immune activation (MIA) in offspring [34, 35], few have evaluated the association between CAM and brain volumetric alterations in human patients. To date, there is no consensus regarding whether CAM affects offspring brain volumes; however, our study is consistent with a previous study that demonstrated that clinical CAM altered cortical thickness in areas associated with cognitive and motor functions at six to ten years of age [13]. In contrast to our study, Granger et al. demonstrated no significant changes in the total brain, white matter, gray matter, and cerebellar volumes using automated segmentation software (MANTiS) [36]. Although the exact reasons for the discrepancy between these studies remain unclear, differences in the segmentation software or study settings, including exclusion criteria and gestational age, may have affected the results. MANTiS can be used to classify relatively global regions (e.g., white matter, cortical gray matter, subcortical gray matter, brainstem, and cerebellum) [37], whereas Infant FreeSurfer can be used to classify smaller regions, including 14 regions of subcortical gray matter, enabling the detection of small differences. Another possible explanation is that the exclusion criteria of this study may have affected the results because we excluded infants with severe brain injury to evaluate regional brain volumes correctly, and mothers with other pregnancy complications (e.g., hypertensive disorders of pregnancy and fetal

Table 4 Global brain volumes in the CAM and non-CAM groups

	CAM (n = 21)	Non-CAM (n = 37)	*p*-value	Adjusted #1 *p*-value	Adjusted #2 *p*-value
Brain volumes					
Gray matter volume (mL)	175.7 ± 22.7	172.5 ± 23.2	0.62	0.49	0.51
White matter volume (mL)	137.7 ± 10.0	145.1 ± 11.0	0.01	< 0.01	< 0.01
Total volume (mL)	313.5 ± 24.3	317.6 ± 23.7	0.53	0.59	0.53

Data are presented as mean ± standard deviation. Brain volumes were compared between the two groups after adjusting for covariates #1 (postmenstrual age at MRI and infant sex) and #2 (postmenstrual age at MRI, infant sex, and gestational age)

CAM chorioamnionitis, *MRI* magnetic resonance imaging

growth restrictions) to minimize the effect of other factors on brain volume.

Conflicting results have been reported regarding brain injury and abnormalities associated with CAM. There are relatively more reports, including our study, that have demonstrated that CAM is not associated with an increased risk of white matter injury or abnormal brain development at term-equivalent age [11, 36, 38–40]. However, a recent prospective study demonstrated that moderate-to-severe acute histological CAM increases the risk of brain abnormalities and delayed maturation [41]. Regarding whether CAM causes significant brain injury or abnormalities, the interpretation of these studies, including ours, requires attention because the clinical setting and definition of CAM (e.g., Blanc classification and Amsterdam classification system) differ largely according to the studies.

Using the Infant FreeSurfer, we observed significantly smaller volumes of the nucleus accumbens and pallidum in infants exposed to CAM. The nucleus accumbens, located in the basal forebrain, is a major component of the ventral striatum and is involved in cognitive, emotional, and motivational processing [42]. The nucleus accumbens is considered a key structure associated with the social reward response in ASD [43]. The pallidum is composed of the dorsal and ventral pallidum; the dorsal pallidum is known as the globus pallidus. The globus pallidus regulates conscious and voluntary movements and communicates with widespread cortical areas associated with various functions, such as motivation, cognition, and action [44]. The ventral pallidum plays a key role in reward and incentive motivations. Unfortunately, we could not demonstrate a significant association between smaller volumes of the nucleus accumbens or pallidum and the subsequent risk of ASD or ADHD, probably due to the small sample size. Therefore, the clinical and physiological implications of volumetric alterations in these areas, and the association between CAM and neurodevelopmental psychiatric disorders remain unclear. However, interestingly, several previous studies have demonstrated a relationship between smaller volumes of the nucleus accumbens or pallidum and ASD/ADHD [45–47]. The ENIGMA ASD working group showed that ASD is associated with smaller subcortical volumes in the pallidum, putamen, amygdala, and nucleus accumbens [45]. Wegiel et al. reported neuronal volume deficits in the nucleus accumbens (34%) and pallidum (20%) based on pathological autopsies of children aged four to eight years old [46]. Shiohama et al. demonstrated a smaller nucleus accumbens and larger cerebral ventricles in infants prior to receiving a diagnosis of ASD compared to sex- and age-matched controls [47]. Disrupted functional connectivity in the nucleus has also been observed in children with ASD [48]. In animal models, Vlasova et al. showed reduced frontal white matter volume in a monkey model of poly (I:C)-induced MIA, which is consistent with the results of our study [35]. These findings support our hypothesis that offspring brain volumetric alterations attributable to maternal CAM exposure increase the risk of ASD and ADHD.

Accumulating evidence has revealed the mechanisms of brain injury (e.g., white and gray matter injury, periventricular leukomalacia, and ventriculomegal) in neonates exposed to CAM, particularly FIRS [10, 49]. The intricate interplay between the maternal, placental, and fetal triad can be affected by various trimester-specific factors such as maternal health status, placental pathology, stage of brain development, brain vulnerability, and genetic predisposition, all of which significantly contribute to the development of postnatal neonatal brain injury [49, 50]. In this study, we assessed the impact of gestational age on volumetric changes and observed a significant correlation between gestational age and the volume of the left pallidum and right hippocampus using multiple linear regression analysis. However, we were unable to fully account for numerous factors that could potentially affect the maternal, placental, and fetal triad.

Although we demonstrated a significant association between funisitis and volumetric changes in the pallidum and nucleus accumbens, similar to the association observed between CAM and these volumetric changes, we did not observe any significant association between FIRS and the volumetric alterations. The reasons underlying this finding are not entirely clear; however, one potential explanation is that the study design may have influenced the results because we excluded infants with severe brain injury and those with respiratory or circulatory issues at term-equivalent age. A prospective study is required to provide a more precise assessment of the relationship between FIRS and volumetric brain alterations.

This study has several strengths. First, this is the first report to use the Infant FreeSurfer to evaluate regional volumetric alterations attributed to CAM exposure. In this study, we used recent neuroimaging techniques, including the Infant FreeSurfer, to capture subtle structural changes that could not be detected using conventional T1-weighted and T2-weighted imaging. Second, this study is novel in that we performed volumetric analyses at term-equivalent age, which is a very early stage of life compared to a previous report [13].

This study had several limitations. First, the automated segmentation of the cortex by Infant FreeSurfer is imprecise for specific neonatal reasons [30]; therefore, we used SPM12 for cortex segmentation. Second, we defined CAM based on the Blanc classification in this study; however, further studies are required using the definition of CAM based on the Amsterdam classification system. Third, the sample size in this study was smaller than that reported in other studies [36, 41]. Finally, we did not find a significant association between

volumetric alterations in the pallidum and nucleus accumbens and the subsequent risk of ASD or ADHD, because few children were diagnosed with ASD or ADHD.

In conclusion, our study provides evidence that preterm infants born to mothers with histological CAM have smaller volumes of white matter, pallidum, and nucleus accumbens than those without CAM at term-equivalent age. Although the clinical implications and underlying mechanisms behind neuroanatomical alterations remain unclear, these volumetric alterations may be associated with the subsequent risk of neurodevelopmental impairments such as ASD and ADHD.

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Author contributions RN, TU, HK, and TK contributed to the concept and design of the study. YI, NN, and KI collected clinical data and performed umbilical cord blood analysis. RN, TU, HK, MK, and AS performed the statistical analyses. RN, TU, HK, MK, AS, NN, YS, MH, JN, HK, and TK were involved in analyzing and interpreting the data. RN and TU drafted the first version of the manuscript. KI, NN, YS, MH, JN, HK, and TK critically reviewed the manuscript, and all authors approved the final version of the manuscript.

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Data availability Data that support the findings of this study are available from the corresponding author (TU) upon reasonable request.

Declarations

Conflict of interest Jun Natsume is affiliated with the endowed department of the Aichi prefectural government (Department of Developmental Disability Medicine).

Ethical approval This study was approved by the Institutional Ethics Board of Nagoya University (approval numbers: 2015–0068 and 2018–0026).

Consent to participate Written informed consent was obtained from all parents.

Consent to publication Not applicable.

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