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Brain cortical maturation assessed by magnetic resonance imaging in unaffected or mildly affected fetuses with cytomegalovirus infection

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KEYWORDS: cortical development; fetal brain; fetal cytomegalovirus infection; fetal magnetic resonance imaging; pregnancy

CONTRIBUTION

What are the novel findings of this work?
This is the first study demonstrating delayed cortical maturation on prenatal magnetic resonance imaging (MRI) in unaffected or mildly affected fetuses with congenital cytomegalovirus (CMV) infection. CMV-infected fetuses had significantly reduced calcarine and parieto-occipital sulci depth and larger Sylvian fissure angles compared with healthy controls. Mildly affected CMV-infected fetuses also demonstrated lower cortical development grading in the parietal and temporal areas compared with controls.

What are the clinical implications of this work?
Fetal CMV infection, even if it is considered of good prognosis, seems to be associated with an altered pattern of fetal brain cortical maturation compared with healthy non-infected fetuses. Our results reinforce the use of MRI as a complementary tool for assessing brain structure at 32 weeks' gestation in CMV-infected fetuses even if they do not exhibit ultrasound abnormalities.

ABSTRACT

Objectives *To assess by magnetic resonance imaging (MRI) the cortical maturation pattern in fetuses with cytomegalovirus (CMV) infection with mild or no abnormalities on ultrasound (US) and MRI, and to establish possible differences compared with healthy controls.*

Methods *This was a retrospective case–control study of consecutive pregnancies with a CMV-infected fetus undergoing prenatal MRI as a complementary diagnostic tool in two centers, and a control group of singleton low-risk pregnancies without fetal structural abnormalities, with normal fetal growth and with healthy newborns. CMV infection was confirmed by extraction of CMV-DNA from fetal and neonatal samples. Only fetuses with mild (mildly affected) or no (unaffected) neuroimaging abnormalities on US and MRI were included. MRI measurements of fetal parieto-occipital sulcus, cingulate sulcus and calcarine sulcus depth, Sylvian fissure depth and Sylvian fissure angles were performed and cortical development grading of specific cortical areas and sulci were assessed by one operator who was blinded to CMV infection status. Data were compared between controls and fetuses with CMV infection, using linear regression and non-parametric trend analysis.*

Results *Twenty-four CMV-infected fetuses (seven unaffected and 17 mildly affected) and 24 healthy controls that underwent fetal MRI between 27 and 36 weeks' gestation were included. Compared with controls, CMV-infected fetuses showed significantly larger median lateral ventricular width (right side, 7.8 (interquartile range (IQR), 5.9–9.9) mm* vs *3.9 (IQR, 2.6–5.3) mm; left side, 7.5 (IQR, 6.0–10.9) mm* vs *4.2 (IQR, 3.2–5.3) mm),*

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significantly decreased parieto-occipital sulcus depth (right side, 12.6 (IQR, 11.3–13.5) mm vs *15.9 (IQR, 13.5–17.3) mm; left side, 12.3 (IQR, 10.6–13.5) mm* vs *16.0 (IQR, 13.3–17.5) mm) and calcarine sulcus depth (right side, 15.4 (IQR, 14.4–16.3) mm* vs *17.5 (IQR, 16.1–18.7) mm; left side, 14.6 (IQR, 14.1–15.6) mm* vs *16.7 (IQR, 15.6–18.9) mm)* ($P < 0.001$ *for all). Compared with controls, CMV-infected fetuses also had significantly smaller upper (right side, 42.8° (IQR, 35.8–45.8°)* vs *48.9° (IQR, 38.4–64.7°); left side, 40.9° (IQR, 34.2–45.8°)* vs *48.2° (IQR, 41.9–60.7°)) and lower (right side, 41.6° (IQR, 34.4–49.2°)* vs *48.9° (IQR, 40.6–60.9°); left side, 42.2° (IQR, 38.8–46.9°)* vs *48.9° (IQR, 39.5–57.5°)) Sylvian fissure angles* ($P < 0.05$ *for all). In addition, the mildly affected CMV-infected fetuses had a significantly lower cortical development grading in the temporal and parietal areas, and the parieto-occipital and calcarine sulci compared with healthy fetuses* ($P < 0.05$). *These differences persisted when adjusting for gestational age, ipsilateral atrium width, fetal gender and when considering small-for-gestational age as a confounding factor.*

Conclusions *Unaffected and mildly affected CMV-infected fetuses showed delayed cortical maturation compared with healthy controls. These results suggest that congenital CMV infection, even in non-severely affected fetuses that are typically considered of good prognosis, could be associated with altered brain cortical structure. Further research is warranted to better elucidate the correlation of these findings with neurodevelopmental outcomes.* © *2022 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Cytomegalovirus (CMV) is the most common congenital infection and remains a major cause of sensorineural hearing loss and neurodevelopmental abnormalities worldwide[1]. Detection during pregnancy is usually achieved when sonographic signs suggestive of infection are observed during routine ultrasound (US) scans[2]. Maternal screening for CMV infection has recently been recommended, since timely administration of valacyclovir could prevent vertical transmission[3]. After confirmation of fetal infection, the use of magnetic resonance imaging (MRI) as a complementary tool to targeted US for examination of the fetal brain increases the positive predictive value for the diagnosis of brain abnormalities in CMV-infected fetuses[4–7]. The use of these two imaging modalities is useful for appropriate counseling, since the current prognostic assessment in fetal CMV infection is mainly based on cerebral findings[4].

The developing brain is vulnerable to inflammation secondary to fetal CMV infection causing cell injury. Neuronal injury early in pregnancy can lead to significant life-long neurocognitive impairment[8,9]. Long-term sequelae (hearing loss, impaired vision and/or neurodevelopmental delay) are expected in 40–60% of symptomatic survivors[10,11] and in 10–20% of asymptomatic newborns[5,10,12,13]. A significant proportion of fetuses with normal third-trimester US become symptomatic at birth or develop delayed congenital CMV (cCMV)-associated symptoms[14,15]; in these cases, MRI is recommended to better visualize the temporal lobes and better depict gross cortical abnormalities. The cerebral cortex develops in three overlapping stages: cell proliferation, neuronal migration and cortical organization[16,17]. Cortical development appears as gyration, but the structural expression of cortical development is the proper and timely development of gyri and sulci[18–20]. Sulcal development is a marker of cortical maturation and is used as an indicator of cortical development[16,20,21]; it may also be related to the cytoarchitectural organization of the brain[22]. Although the central nervous system (CNS) is a major target of cCMV infection[23], there is scant information on cortical maturation in infected fetuses without US/MRI findings or in those with US/MRI features considered of good or uncertain prognosis. Comprehensive characterization of fetal cortical development *in utero* by MRI can provide more information about underlying structural changes related to mild fetal CMV infection[20,22,24].

The aim of this study was to compare cortical maturation in terms of sulci depth and development grading of cortical areas and sulci between CMV-infected fetuses with mild or no imaging findings, and healthy controls.

METHODS

This was a retrospective case–control study of consecutive pregnancies with a CMV-infected fetus undergoing prenatal MRI as a complementary clinical tool to aid in the prognostic assessment of these patients over an 11 3/4-year period (March 2009 to December 2020) in BCNatal, Hospital Clínic and Hospital Sant Joan de Déu, Barcelona, Spain, and over a 3 1/2-year period (February 2016 to September 2019) in Hôpital Louis-Mourier, Paris, France. The study was approved by the Institutional Review Board of the Hospital Clinic (HCB/2017/0564) and Hôpital Louis-Mourier (CEERB-Paris Nord/2020-012).

Only fetuses with mild (mildly affected) or no (unaffected) abnormalities on US and MRI[25,26], with an MRI study available for analysis, were eligible for inclusion. Of the 23 pregnancies included, 61% were diagnosed after maternal CMV screening according to local hospital policy (France) or by the attending physicians. The remaining 39% of cases were diagnosed by evidence of US abnormalities during the routine second- or third-trimester scan. CMV infection was confirmed by extraction of CMV-DNA from fetal and neonatal samples using the QIAsymphony system (Qiagen, Hilden, Germany). Chromosomal abnormalities and toxoplasmosis infection were ruled out at the time of amniotic fluid study. After fetal US/MRI, the women were counseled about the prognosis of the newborn.

Termination of pregnancy (TOP) was discussed according to Spanish/French laws. In cases of TOP, after obtaining informed consent from the parents, routine postmortem examination of the fetus and the placenta was performed. In cases with a live newborn, cCMV was confirmed by a positive polymerase chain reaction (PCR) of a urine or saliva sample taken within the first 48 h after birth. Administration of high-dose valacyclovir (VCV) treatment for the prevention of progression of fetal brain lesions was recorded. VCV was administered in both centers at an oral dose of 8 g/day (2 g every 6 h) from the time of diagnosis of fetal infection until delivery. VCV was offered in cases without US findings related to CMV infection to prevent fetal anomalies and in those with mild symptoms (extracerebral or mild cerebral manifestation) to limit the consequences of CMV infection. In these cases, patients were informed about the limited evidence concerning the utility of VCV in these circumstances and informed consent was obtained before administration of VCV.

The control group consisted of volunteer women with a singleton low-risk pregnancy with normal fetal growth, without fetal structural abnormalities and with healthy newborns, who attended BCNatal Hospital Clínic and Hospital Sant Joan de Déu for routine screening and US scans. They were selected randomly from a larger prospective cohort of low-risk pregnancies recruited specifically for research purposes[27,28]. Controls did not undergo any additional genetic or infection testing aside from routine blood tests during pregnancy. These children are currently under follow-up for further analysis.

US protocol

Fetal examination and follow-up in CMV-infected fetuses consisted of serial US assessment including detailed neurosonography (NSG). After confirmation of fetal infection, a detailed US examination was performed every 2–3 weeks in the Infection Diseases Unit to assess fetal growth, gross anatomy, amniotic fluid and Doppler parameters. Comprehensive NSG was performed every 2–4 weeks using a standardized protocol according to the guidelines of the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG), including axial[29], sagittal and coronal planes[30]. The sagittal and coronal views were obtained by the transvaginal approach in cases with cephalic position and transabdominally in fetuses with non-vertex presentation. Clips including axial, sagittal and coronal planes were recorded. The control group was evaluated using the same NSG protocol, however, NSG was performed only once, at the time of recruitment. All scans were carried out by experienced examiners using high-resolution US equipment (Voluson 730 Expert and E6 or E8; GE Healthcare, Kretz, Zipf, Austria).

MRI acquisition

MRI was performed in both centers using a clinical MRI system (Hospital Clinic: 1.5-T Magnetom Aera syngo MR D13 (Siemens, Erlangen, Germany); Hôpital Louis-Mourier: 1.5-T GE Signa Horizon, Echo speed, LX MRI scanner (Milwaukee, WI, USA)) with a 5-channel cardiac coil. No maternal or fetal sedation was used[31]. The three orthogonal planes of the fetal head were used to measure sulci depth and evaluate cortical development grading by T-2-weighted sequences obtained using single-shot, fast spin-echo (Hospital Clínic: repetition time, 1000 ms; echo time, 137 ms; slice thickness, 3.5 mm; gap, 3.5 mm; field of view, 225 × 300 mm; voxel size, 0.59 × 0.59 × 3.5 mm; matrix, 256 × 192 mm; flip angle, 135°; acquisition time, 32 s; Hôpital Louis-Mourier: repetition time, 1120 ms; echo time, 92.7 ms; slice thickness, 3.5 mm; gap, 3.5 mm; field of view, 300 × 300 mm; voxel size, 0.55 × 0.55 × 3.5 mm; matrix, 320 × 256 mm; flip angle, 90°; acquisition time, 32 s).

All fetal MR images were reviewed by a specialist fetal radiologist to exclude any additional abnormality of the CNS. The gestational-age limit for MRI assessment was 37 weeks.

MRI analysis

Biparietal diameter (BPD), head circumference, posterior lateral ventricular width and all normative biometry of the fetal brain were determined according to Kyriakopoulou *et al.*[32]. MRI measurements were performed by the first author (A.H.-V.), who was blinded to CMV-infection status.

Sulci depth

Measurements of parieto-occipital sulcus, cingulate sulcus and calcarine sulcus depth were performed offline using HOROS™ Project medical image viewer software version 3.3.6 (LGPL-3.0; horosproject.org) for T2- weighted sequences with a 3.5-mm slice thickness. Laterality was assessed by determining the fetal position *in utero*. Anatomic planes for measurements were assessed using the ISUOG guidelines for sonographic examination of the fetal CNS[29,30]. Measurement of sulcal and Sylvian fissure depth (in mm) in both hemispheres was carried out by tracing a perpendicular line from the midline towards the border of the specific sulcus as described previously by Hahner *et al.*[27]. To ensure a 90° angle, lines were traced using the HOROS software perpendicular lines tool. A straight line was traced from the frontal bone to the occipital bone in the axial plane and vertically from the vault to the skull base in the coronal plane. All measurements were normalized by BPD and multiplied by 100.

Insula depth

Insula depth was measured in an axial transthalamic plane by drawing a perpendicular line from the midline behind the cavum septi pellucidi to the upper border of maximum prominence of the insular cortex, as described previously by Hahner *et al.*[28] and Alonso *et al.*[33].

Sylvian fissure angles

The Sylvian fissure angles (SFAs) on both hemispheres were measured in a coronal transthalamic plane (landmarks used: anterior horns of lateral ventricles, cavum septi pellucidi, third ventricle at the point of maximum development and parietal and temporal lobes), adapting previous methodology[18]. From the midline, a perpendicular horizontal line was drawn on the third ventricle to the extracerebral fluid compartment on both sides. Four lines were then drawn, bilaterally along the upper external and internal sides of the Sylvian fissure (SF). The upper and lower SFAs formed by these lines were measured, using the horizontal line as the reference (0°) (Figure 1a).

Cortical development grading

The development of cortical areas and cortical sulci and the Sylvian fissures was assessed according to the grading system of Pistorius *et al.*[34], which involves a scoring methodology ranging from Grade 0 (no maturation) to Grade 5 (maximum maturation) (Figure 1g,h).

Statistical analysis

Data were compared between healthy fetuses and fetuses with CMV infection, separately for those with no abnormalities seen on US/MRI (unaffected) and those with mild CNS and/or extra-CNS abnormalities seen (mildly affected). Quantitative variables were assessed for normality using the Shapiro–Wilk test, and normally distributed variables were compared using the *t*-test and expressed as mean ± SD. Non-normally distributed quantitative variables were compared using the Mann–Whitney *U*-test and expressed as median and interquartile range (IQR: 25^{th}–75^{th} percentiles). Qualitative variables were compared using the χ-square or Fisher's exact test. To correct for fetal head size, sulcal, insular and SF depth and SFA measurements were adjusted by BPD. For quantitative brain variables, linear regression analysis adjusted for ipsilateral ventricular width and gestational age at MRI was performed. The variable small-for-gestational age (SGA) was analyzed as a probable confounding/interaction factor. A subanalysis of variance and covariance was performed using the Kruskal–Wallis equality of population rank test. Non-parametric tendency analysis (Jonckheere–Terpstra test) was performed according to a possible biological trend (healthy *vs* unaffected CMV-infected fetuses *vs* mildly affected CMV-infected fetuses). To assess the reproducibility of SFA measurements using MRI, interobserver reliability analysis was performed by comparing the measurements obtained by A.H.-V. and K.C. (blinded to each other's measurements) in 13 randomly selected healthy fetuses. Intraclass correlation coefficient (ICC) estimates and their 95% CIs were calculated using a two-way random effects model with absolute agreement. A robust estimation was used to calculate 95% CIs and *P*-values. Power estimation for an alpha error of 0.05 was performed using the two-sample means Satterthwaite's *t*-test. Data were analyzed using STATA version 15.0 (StataCorp., College Station, TX, USA); $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Population characteristics

Included in the study were 47 pregnancies (48 fetuses) that underwent MRI between 27 and 36 weeks' gestation. These comprised 23 pregnancies with 24 CMV-infected fetuses (21 singletons, one dichorionic pregnancy with one infected fetus and one monochorionic diamniotic (MCDA) pregnancy with both fetuses infected) and 24 healthy singleton pregnancies as the control group (Figure 2). Infection was confirmed by a positive PCR test of amniotic fluid in 21 fetuses. The other three patients (all at Hôpital Louis-Mourier) declined amniocentesis and infection was confirmed in the urine of the newborns at birth. Of the 23 pregnancies with fetal CMV infection, 13 (56.5%) patients had a confirmed first-trimester primary CMV infection, two presented second-trimester seroconversion, two had a non-primary infection, and in six (26.1%) patients the type of maternal infection was unknown (including the MCDA pregnancy). The median gestational age at diagnosis of fetal CMV infection was 22.5 (IQR, 21.4–30.0) weeks. The median CMV viral load in amniotic fluid at the time of diagnosis of fetal infection and before maternal treatment was 7.0 (IQR, 1.1–13.0) million copies/mL, with no significant differences according to the presence or absence of US findings. High-dose VCV (8 g/24 h) was administered to 12 (52.2%) patients as tertiary prevention of fetal sequelae at a median gestational age of 26.0 (IQR, 23.7–32.6) weeks, with median treatment duration of 13 (IQR, 7–15) weeks. The median interval between the last US and MRI was 0.79 (IQR, 0.14–1.2) days, with no significant differences between the CMV and control groups. The median gestational age at MRI was 32.6 (IQR, 31.6–33.6) weeks, with no significant differences between the two groups (Table 1). In the CMV-infected pregnancies, compared with controls, there was a significantly higher proportion of parous women and women with a ≤ 3-year-old child, higher rate of SGA and a significantly lower median birth weight (Table 1).

Among CMV-infected fetuses, the most frequent cerebral US findings were subependymal cysts (20.8%), followed by hyperechogenic caudate nucleus (16.7%) and lenticulostriate vasculopathy (16.7%) (Table S1). In one case, mild unilateral ventriculomegaly was diagnosed in the third trimester at NSG follow-up and confirmed by MRI. The most frequent extracerebral findings on US were SGA and hyperechogenic bowel, each seen in 20.8% of cases. With respect to MRI cerebral findings, white matter hyperintensity (WMHS) was observed in 37.5% of cases and temporal lobe cyst in 25.0% (Table S1).

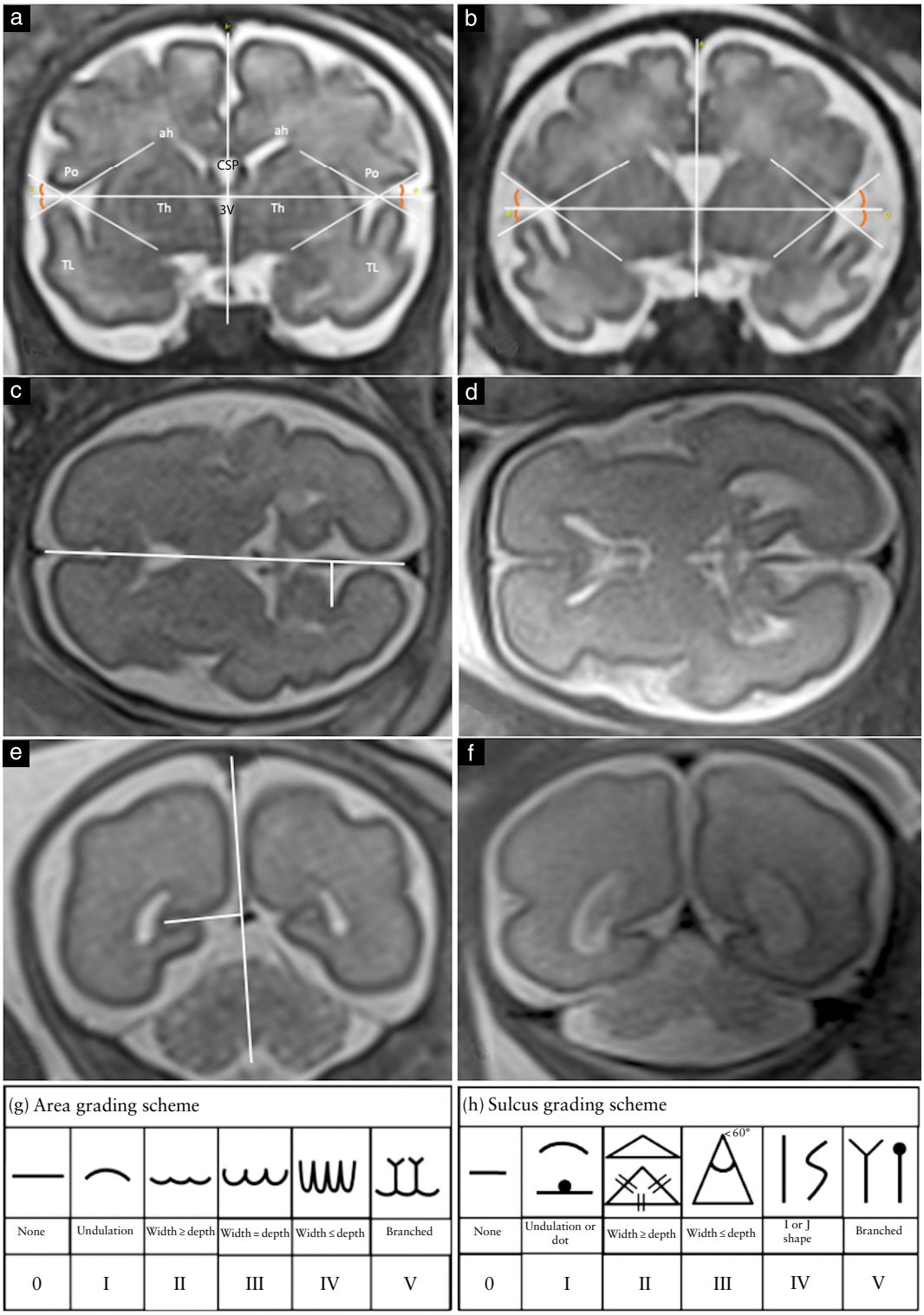

Figure 1 (a,b) Magnetic resonance (MR) images in coronal transthalamic plane showing measurement of upper and lower Sylvian fissure angles (SFA, indicated in orange) in right and left hemispheres in a 33-week healthy fetus (a) and a 33-week cytomegalovirus (CMV)-infected fetus (b). Note wider upper and lower SFAs in the CMV-infected fetus. (c,d) MR images in axial transventricular plane showing measurement of depth and assessment of development grading of parieto-occipital sulcus in a 28-week healthy fetus (c) and a 28-week CMV-infected fetus with unremarkable ultrasound (US)/MR imaging (d). Note larger lateral ventricular width and reduced parieto-occipital sulcus depth in the CMV-infected fetus. Development grading of the parieto-occipital sulcus was Grade III in the healthy control (c) *vs* Grade II in the CMV-infected fetus (d). (e,f) MR images in coronal transcerebellar plane showing measurement of depth and assessment of development grading of calcarine sulcus in a 28-week healthy fetus (same case as in (c)) (e) and a 28-week CMV-infected fetus with unremarkable US/MR imaging (same case as in (d)) (f). Note larger lateral ventricular width and reduced calcarine sulcus depth in the CMV-infected fetus. Development grading of the calcarine sulcus was Grade IV in the healthy control (e) *vs* Grade III in the CMV-infected fetus (f). (g,h) Grading of Pistorius *et al.*[34] for cortical areas (g) and sulci (h). 3V, third ventricle; ah, anterior horns of lateral ventricles; CSP, cavum septi pellucidi; Po, parietal operculum; Th, thalamus; TL, temporal lobe.

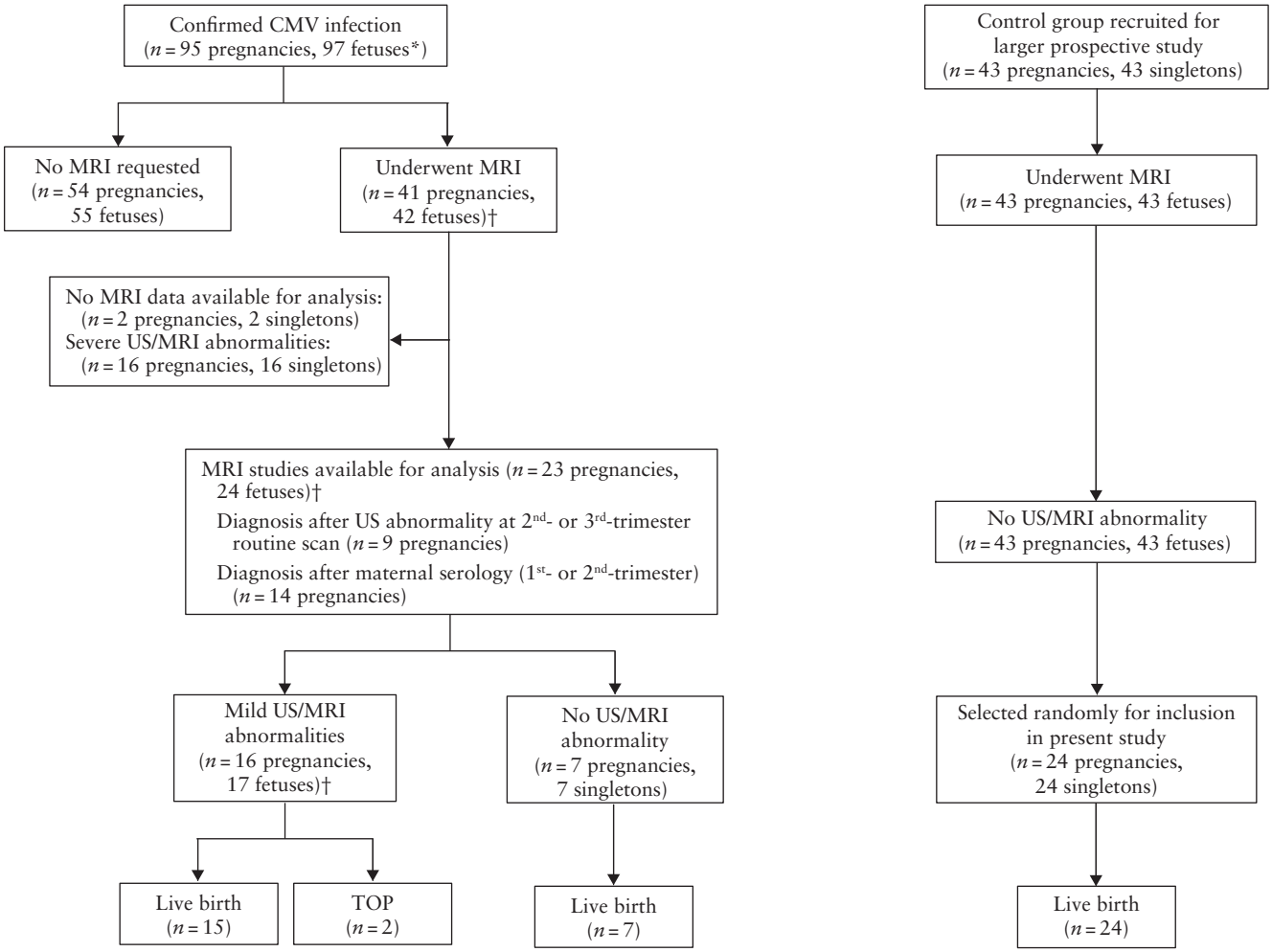

Figure 2 Flowchart showing inclusion and outcome of pregnancies with confirmed fetal cytomegalovirus (CMV) infection and uninfected low-risk pregnancies (control group). *Comprising 92 singletons, one dichorionic (DC) pregnancy with one infected and one uninfected fetus, one DC pregnancy with both fetuses infected and one monochorionic diamniotic (MCDA) pregnancy with both fetuses infected. †Including DC pregnancy with one infected and one uninfected fetus and MCDA pregnancy with both fetuses infected. MRI, magnetic resonance imaging; TOP, termination of pregnancy; US, ultrasound.

Table 1 Baseline characteristics of 24 healthy low-risk pregnancies (controls) and 23 pregnancies with confirmed fetal cytomegalovirus (CMV) infection with no or mild abnormalities on ultrasound and/or magnetic resonance imaging (MRI)

Characteristic	*Controls (*n = *24 pregnancies; 24 fetuses)*	*CMV (*n = *23 pregnancies; 24 fetuses)*	P*
Maternal age (years)	33.1 ± 6.4	32.9 ± 4.4	0.93
Caucasian ethnicity	19 (79.2)	16/23 (69.6)	0.45
Parous	9 (37.5)	19/23 (82.6)	0.003
Low educational level	5 (20.8)	6/23 (26.1)	0.81
≤ 3-year-old child†	6 (25.0)	16/23 (69.6)	0.003
Gestational age at MRI (weeks)	32.8 (29.5–33.8)	32.4 (31.7–33.4)	0.91
EFW at time of MRI (g)	1979 ± 416	1884 ± 621	0.58
Small-for-gestational age	0 (0)	5/24 (20.8)	0.05
Female fetal gender	13 (54.2)	11/24 (45.8)	0.56
Gestational age at birth (weeks)	39.4 (37.8–40.1)	38.8 (37.6–39.4)	0.19
Birth weight (g)	3320 (3006–3610)	2955 (2652–3298)	0.027

Data are presented as mean ± SD, *n* (%), median (interquartile range) or *n*/*N* (%). *P determined using *t*-test, Mann–Whitney *U*-test, χ^2 test or Fisher's exact test. †Young child is risk factor for CMV infection[53]. EFW, estimated fetal weight.

MRI analysis

In the CMV-infected fetuses compared with controls, median lateral ventricular width on MRI was significantly larger (right side, 7.8 (IQR, 5.9–9.9) mm *vs* 3.9 (IQR, 2.6–5.3) mm; left side, 7.5 (IQR, 6.0–10.9) mm *vs* 4.2 (IQR, 3.2–5.3) mm) ($P < 0.001$ for both). There were no significant differences in other brain structures between the two groups (Table S2). When compared according to the degree of involvement, mildly affected CMV-infected fetuses showed wider left and right ventricles compared with the unaffected CMV-infected fetuses (Figure 3), with a significant trend according to severity ($P < 0.001$).

Compared with controls (Figure 1c,e), CMV-infected fetuses showed significantly decreased parieto-occipital sulcus depth (Figure 1d) (right side, 12.6 (IQR, 11.3–13.5) mm *vs* 15.9 (IQR, 13.5–17.3) mm; left side, 12.3 (IQR, 10.6–13.5) mm *vs* 16.0 (IQR, 13.3–17.5) mm)

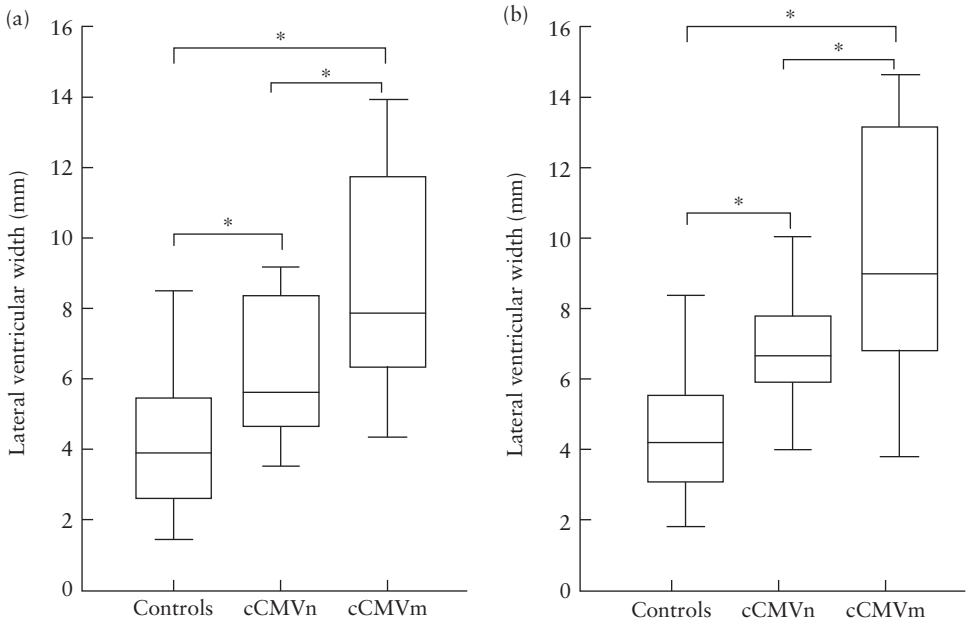

Figure 3 Box-and-whiskers plots showing lateral ventricular width, measured on magnetic resonance imaging (MRI), in right (a) and left (b) brain hemispheres in 24 healthy fetuses (controls) and 24 fetuses with confirmed CMV infection with no (cCMVn) or mild (cCMVm) abnormalities on ultrasound and/or MRI. *P* estimated using Kruskal–Wallis equality of population rank test, adjusted for gestational age at MRI. *$P < 0.05$. Boxes are median and interquartile range and whiskers are range.

Table 2 Cortical development parameters assessed on magnetic resonance imaging (MRI) in 24 healthy fetuses (controls) and 24 fetuses with confirmed cytomegalovirus (CMV) infection with no (unaffected) or mild abnormalities on ultrasound and/or MRI

Characteristic	*Controls* (n = 24)	*CMV*		P*	P†
		Unaffected (n = 7)	*Mildly affected* (n = 17)		
Right hemisphere					
Insula depth (mm)	29.7 (28.3–30.9)	29.4 (28.2–31.4)	29.9 (28.8–31.5)	0.67	0.69
Sylvian fissure depth (mm)	15.9 (14.7–17.1)	15.4 (13.7–16.2)	15.8 (15.1–17.1)	0.46	0.92
Parieto-occipital sulcus depth (mm)	15.9 (13.5–17.3)	12.7 (11.9–13.3)	12.5 (11.3–13.8)	0.002	< 0.001
Cingulate sulcus depth (mm)	4.76 (4.15–6.52)	4.32 (4.04–5.17)	4.77 (4.03–5.61)	0.44	0.61
Calcarine sulcus depth (mm)	17.5 (16.1–18.7)	15.6 (14.7–16.9)	15.4 (13.9–16.0)	0.015	< 0.001
Upper Sylvian fissure angle (°)	42.8 (35.8–45.8)	41.6 (33.5–58.6)	50.1 (40.4–65.5)	0.71	0.012
Lower Sylvian fissure angle (°)	41.6 (34.4–49.2)	42.6 (38.6–49.1)	50.7 (45.8–64.6)	0.79	0.009
Left hemisphere					
Insula depth (mm)	29.5 (28.5–30.7)	29.4 (28.2–31.9)	29.4 (29.0–31.2)	0.90	0.94
Sylvian fissure depth (mm)	16.5 (15.6–17.8)	16.1 (14.7–17.4)	16.1 (15.2–17.6)	0.77	0.95
Parieto-occipital sulcus depth (mm)	16.0 (13.3–17.5)	13.1 (10.1–14.4)	11.6 (10.8–12.4)	0.004	< 0.001
Cingulate sulcus depth (mm)	4.82 (4.02–6.57)	4.59 (3.90–5.40)	4.79 (3.84–5.51)	0.61	0.53
Calcarine sulcus depth (mm)	16.7 (15.6–18.9)	15.4 (14.2–15.9)	14.6 (14.1–15.5)	0.018	< 0.001
Upper Sylvian fissure angle (°)	40.9 (34.2–45.8)	45.3 (40.1–59.5)	51.0 (43.1–61.3)	0.11	< 0.001
Lower Sylvian fissure angle (°)	42.2 (38.8–46.9)	40.7 (34.8–48.9)	54.6 (46.6–61.9)	0.54	< 0.001

Data are presented as median (interquartile range). All variables were normalized by biparietal diameter and multiplied by 100. *P* determined using Kruskal–Wallis equality of populations rank test and adjusted by ipsilateral atrium and gestational age at MRI, and small-for-gestational age as a confounding factor compared with controls. *Controls *vs* unaffected CMV-infected fetuses. †Controls *vs* mildly affected CMV-infected fetuses.

and calcarine sulcus depth (Figure 1f) (right side, 15.4 (IQR, 14.4–16.3) mm *vs* 17.5 (IQR, 16.1–18.7) mm; left side, 14.6 (IQR, 14.1–15.6) mm *vs* 16.7 (IQR, 15.6–18.9) mm) ($P < 0.001$ for all). Differences remained significant on non-parametric tendency analysis when assuming a biological trend ($P < 0.001$). Cortical development measurements in the study groups, separately for unaffected and mildly affected cases, are shown in Table 2.

Compared with controls, CMV-infected fetuses also had significantly smaller upper (right side, 42.8° (IQR, 35.8–45.8°) *vs* 48.9° (IQR, 38.4–64.7°); left side, 40.9° (IQR, 34.2–45.8°) *vs* 48.2° (IQR, 41.9–60.7°)) and lower (right side, 41.6° (IQR, 34.4–49.2°) *vs* 48.9° (IQR, 40.6–60.9°); left side, 42.2° (IQR, 38.8–46.9°) *vs* 48.9° (IQR, 39.5–57.5°)) Sylvian fissure angles ($P < 0.05$ for all). When compared according to the degree of involvement, the upper and lower SFAs were significantly larger bilaterally in mildly affected CMV-infected fetuses compared with healthy controls ($P < 0.05$ for all), but no significant differences were observed between unaffected CMV-infected fetuses and controls (Table 2). There was a significant linear tendency when assuming a biological trend in the upper and lower right SFA and in the upper left SFA ($P < 0.05$ for all).

In addition, significantly lower cortical development grading was observed in the temporal and parietal areas, parieto-occipital sulcus and calcarine sulcus (Figure 1d,f) in mildly affected CMV-infected fetuses *vs* healthy controls ($P < 0.05$ for all) (Figure 4). No significant differences were observed in the cortical development grading of SF, cingulate sulcus, superior temporal sulcus, central sulcus, frontal area and mesial area between CMV-infected fetuses and controls (Figure S1).

There was excellent agreement between the two examiners in the measurement of the upper right SFA (ICC, 0.95 (95% CI, 0.83–0.98)), lower right SFA (ICC, 0.90 (95% CI, 0.50–0.97)), upper left SFA (ICC, 0.95

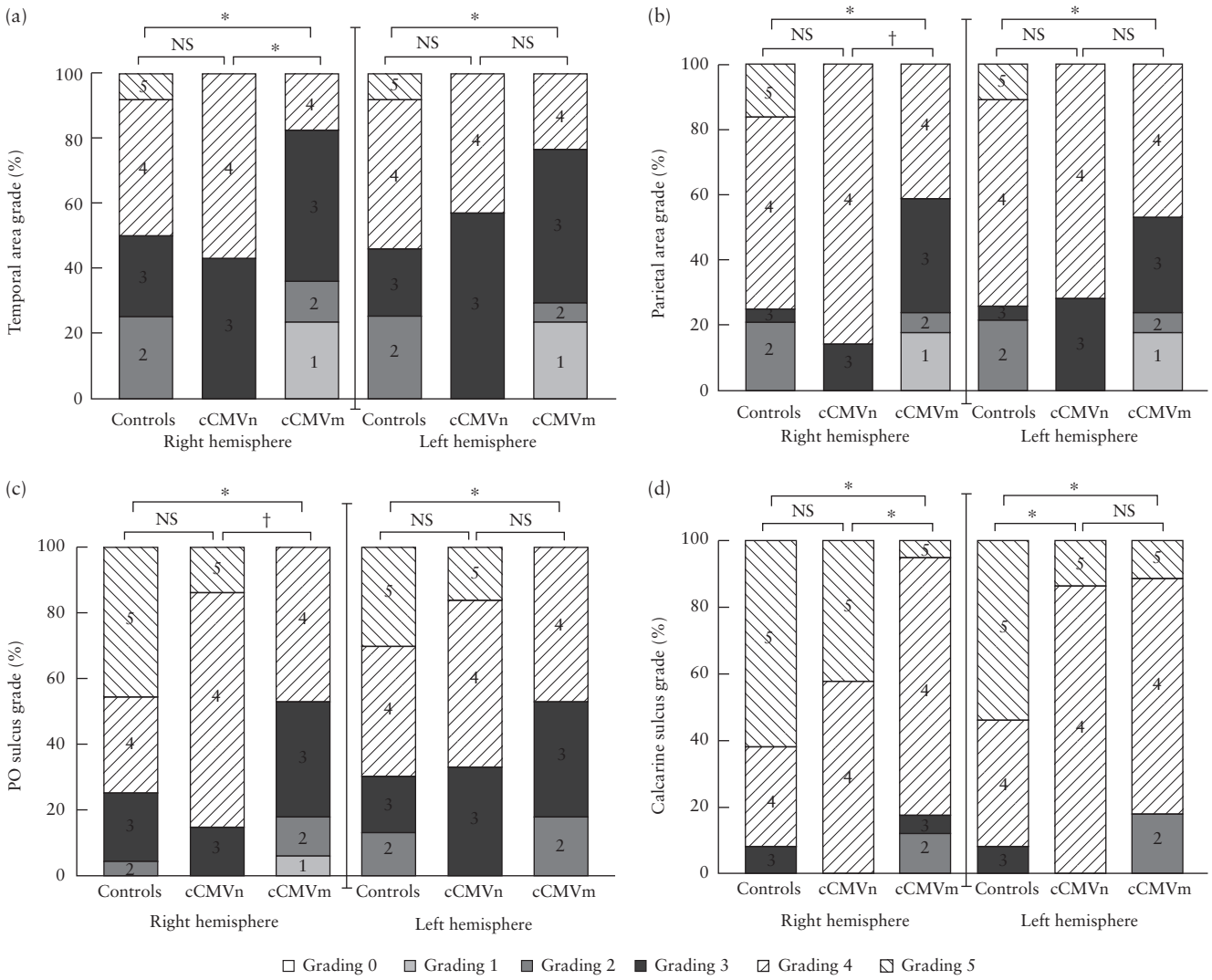

Figure 4 Cortical development grading of temporal area (a), parietal area (b), parieto-occipital (PO) sulcus (c) and calcarine sulcus (d) in right and left hemispheres, based on scoring system of Pistorius *et al.*[34], in 24 healthy fetuses (controls) and 24 fetuses with confirmed cytomegalovirus (CMV) infection with no (cCMVn) or mild (cCMVm) abnormalities on ultrasound and/or magnetic resonance imaging (MRI). Data are expressed as percentage breakdown in each group. *P* estimated by χ^2 or Fisher's exact test. $^*P < 0.05$ and $\dagger P = 0.05$, adjusted for ipsilateral atrium and gestational age at MRI. NS, not significant.

(95% CI, 0.82–0.98)) and lower left SFA (ICC, 0.96 (95% CI, 0.87–0.98)).

DISCUSSION

This study provides evidence that, in congenital CMV infection, fetuses that are unaffected or mildly affected based on imaging findings present a pattern of delayed cortical brain maturation compared with healthy fetuses. Specifically, the parieto-occipital and calcarine sulci depth were significantly reduced in both unaffected and mildly affected fetuses, while significantly larger SFAs and lower cortical grading in the parieto-occipital sulcus, calcarine sulcus and temporal and parietal areas were observed in mildly affected CMV-infected fetuses compared with controls. To our knowledge, this is the first study to describe cortical brain development according to sulci depth and cortical development grading assessed by MRI in CMV-infected fetuses with normal or mild US/MRI findings compared with healthy controls.

Descriptions of structural brain findings in CMV-infected fetuses with mild or no brain involvement are scarce. Hoffmann *et al.*[35] described significantly smaller temporal lobe volume normalized to whole brain volume between 24 and 38 weeks in CMV-infected fetuses without MRI findings or with WMHS compared with controls, with marked changes in those with WMHS. Findings are contradictory regarding the apparent diffusion coefficient (ADC), which allows a quantitative measurement of brain maturation. Katorza *et al.*[36] described higher ADC values in the parietal and temporal lobes of CMV-infected fetuses with WMHS compared with uninfected fetuses with WMHS of unkown etiology, suggesting reduced brain maturation in the former group. However, Kotovich *et al.*[37] found lower ADC values in CMV-fetuses without WMHS and unremarkable fetal MRI results compared with gestational-age matched uninfected controls. These differences could be related to different phases of cellular injury. Of 24 CMV-infected fetuses in our series, 37% had WMHS and almost one-third had unremarkable US/MRI. Likewise, we found significantly lower cortical development grading in the temporal and parietal lobes and higher upper and lower SFAs regardless of the presence or absence of WMHS in mildly affected CMV-infected fetuses.

In our series, 21% of CMV-infected fetuses were SGA, which could be considered a confounding factor; however, our regression analysis showed that being SGA did not confound our results. Moreover, previous data in small fetuses without cCMV showed an increased insular depth and reduced SF depth[38–40], changes which were not observed in our CMV-infected fetuses.

Our data show that, in mildly affected CMV-infected fetuses, the upper SFA was significantly larger compared with controls, probably suggesting delayed cortical maturation of the superior operculization process of the SF[41,42]. Our findings agree with those of Pooh *et al.*[43], who showed that the SFA may be an indicator of the subsequent development of cortical malformation.

CMV infection at different gestational ages may have a distinct pattern of cellular and developmental effects on the fetal brain that may ultimately determine the neurological outcome[12,14,44–49]. More than half our cases were first-trimester primary infections, while two had seroconversion at the beginning of the second trimester, and in a quarter of pregnancies the time of infection was unknown. Although the impact of latent CMV-infection is unclear[9,48], we hypothesized that the larger ventricular width in our CMV-infected fetuses could reflect persisting inflammation due to latent infection, possibly resulting in delayed cortical maturation. Cellular proliferation occurs in the germinal matrix on the ventricular zone of ventricular walls from 8 to 16 weeks, followed by neuronal migration and finally synaptogenesis characterized by the appearance of sulci and gyri[16,22,50], which are critical steps in the sulcation process. We hypothesize that the decreased parieto-occipital and calcarine sulci depth, and lower cortical-development grading in the temporal and parietal areas and parieto-occipital and calcarine sulci in mildly affected CMV-infected fetuses, are related to the cell injury caused by infection during brain embryonic development and strongly related to the ventricular system. Sarnat and Flores-Sarnat[51] showed that the depth of a fissure/sulcus may be influenced by the adjacent ventricular system. The occipital horn is the most recent recess of the lateral ventricle and is therefore most vulnerable between weeks 6 and 9. Although we could not determine the timing of infection in about one-quarter of our sample, these fetuses may have acquired the infection in the first trimester or early second trimester of pregnancy, potentially explaining our findings. Our findings related to significantly higher ventricular width and reduced calcarine and parieto-occipital sulci depth even in CMV-infected fetuses with unremarkable US/MRI reinforce the hypothesis that cell injury in the ventricular system could affect neuronal migration and synaptogenesis, resulting in delayed cortical maturation.

Our results support the use of MRI as a complementary tool for assessing brain structure at 32 weeks' gestation in CMV-infected fetuses even without US abnormalities. In a recent systematic review, fetal MRI detected an additional anomaly in about 6% of sonographically normal CMV-infected fetuses, demonstrating the potential benefit of MRI in the prenatal assessment of congenital CMV infection[52].

The identification of unaffected/mildly affected CMV-infected fetuses at risk of altered neurodevelopment due to subtle prenatal changes in cortical development has potential importance in clinical practice. Evaluation of sulci depth, cortical development grading and SFA is feasible and can provide an overview of brain cortex maturation to detect subtle alterations in specific regions that could explain neurodevelopmental outcomes in infected fetuses. It should be stressed that, owing to the paucity of data on normal sulci depth, we could not provide a cut-off value that would be useful

in clinical practice. Moreover, we acknowledge that our findings may need correlation with neurological outcomes. Similar to our findings in CMV-infected fetuses, in fetuses with isolated non-severe ventriculomegaly, reduced parieto-occipital and calcarine sulci depth and cortical development grading have been reported[27]. These neonates showed a weaker, albeit not significantly so, performance in the motor and range-of-state clusters of the Neonatal Behavioral Assessment Scale compared with healthy controls.

The main strengths of this study include MRI assessment in a well characterized group of CMV-infected fetuses and that manual MRI measurements were performed by a single examiner blinded to the CMV-infection status. We evaluated the SFA, a recently described neurosonographic measurement with excellent interobserver agreement that can serve as a screening tool for malformations of cortical development[18]. We describe the use of the SFA as assessed by MRI to improve our understanding of cortical brain architecture and the pathophysiology of fetal CMV infection.

The main limitations of this study are the retrospective nature of the analysis and the small sample size mainly of unaffected CMV-infected fetuses. Moreover, it was not possible to establish the exact time of infection in almost one-third of the cases, while 52% of the patients were treated with VCV as preventive treatment, but we were unable to determine possible potential effects on our findings. Finally, asymptomatic CMV infection was not excluded among controls, although this probability is very low considering a 0.7% incidence of congenital CMV.

In conclusion, CMV-infected fetuses with unremarkable US/MRI or with mild involvement showed delayed cortical maturation on MRI compared with controls. Postnatal follow-up studies are warranted to understand the consequences of delayed cortical maturation in CMV-infected fetuses without US/MRI findings and in those with mild involvement.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Figure S1 Cortical development grading of frontal area, Sylvian fissure, mesial area, cingulate sulcus, superior temporal sulcus and central sulcus in the right and left hemispheres, based on scoring system of Pistorius *et al.*[34], in 24 healthy fetuses (controls) and 24 fetuses with confirmed cytomegalovirus (CMV) infection. Data are expressed as percentage breakdown in each group. No significant differences were observed between the two groups.

Table S1 Prenatal ultrasound and magnetic resonance imaging (MRI) findings in 17 cytomegalovirus (CMV)-infected fetuses with mild involvement

Table S2 Cerebral structure findings on magnetic resonance imaging in 24 healthy low-risk pregnancies (controls) and 23 pregnancies with confirmed fetal cytomegalovirus (CMV) infection