Third Trimester Brain Growth in Preterm Infants Compared With In Utero Healthy Fetuses

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abstract

BACKGROUND AND OBJECTIVES: Compared with term infants, preterm infants have impaired brain development at term-equivalent age, even in the absence of structural brain injury. However, details regarding the onset and progression of impaired preterm brain development over the third trimester are unknown. Our primary objective was to compare third-trimester brain volumes and brain growth trajectories in ex utero preterm infants without structural brain injury and in healthy in utero fetuses. As a secondary objective, we examined risk factors associated with brain volumes in preterm infants over the third-trimester postconception.

METHODS: Preterm infants born before 32 weeks of gestational age (GA) and weighing <1500 g with no evidence of structural brain injury on conventional MRI and healthy pregnant women were prospectively recruited. Anatomic T2-weighted brain images of preterm infants and healthy fetuses were parcellated into the following regions: cerebrum, cerebellum, brainstem, and intracranial cavity.

RESULTS: We studied 205 participants (75 preterm infants and 130 healthy control fetuses) between 27 and 39 weeks' GA. Third-trimester brain volumes were reduced and brain growth trajectories were slower in the ex utero preterm group compared with the in utero healthy fetuses in the cerebrum, cerebellum, brainstem, and intracranial cavity. Clinical risk factors associated with reduced brain volumes included dexamethasone treatment, the presence of extra-axial blood on brain MRI, confirmed sepsis, and duration of oxygen support.

CONCLUSIONS: These preterm infants exhibited impaired third-trimester global and regional brain growth in the absence of cerebral/cerebellar parenchymal injury detected by using conventional MRI.

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WHAT'S KNOWN ON THIS SUBJECT: Third-trimester brain development is characterized by critical and energy-dependent biological processes needed to support optimal brain growth. Available evidence points to disturbed brain development in ex-preterm infants at term and beyond compared with their term-born peers.

WHAT THIS STUDY ADDS: Third-trimester brain growth is disturbed in preterm infants compared with healthy in utero fetuses in the absence of structural brain injury. Neonatal intensive care likely influences third-trimester brain development in preterm infants.

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Despite marked improvements in neonatal intensive care, life-long neurodevelopmental disabilities remain highly prevalent in survivors of prematurity.[1] Brain injury is a common complication of preterm birth and is diagnosed by using conventional neuroimaging techniques in about one-third of preterm infants.[2] However, the presence of structural brain injury alone does not account for the frequency and scope of neurodevelopmental impairments in surviving preterm infants.[3] Brain development can be altered even without evidence of destructive prematurity-related brain injury.[4] Multiple risk factors have also been implicated with aberrant preterm brain maturation, including infection, medication administration, and respiratory complications.[5] However, the timing, progression, and functional impact of delayed brain maturation in the absence of structural brain injury remain poorly understood.

A growing body of evidence has shown that brain development is impaired in preterm infants by term-equivalent age (TEA) even in the absence of structural brain injury: compared with healthy term newborns, preterm infants at TEA exhibit decreased cerebral and cerebellar volume, altered cortical surface area,[6–9] and microstructural organization,[10] as well as impaired functional connectivity.[11,12] To date, most inferences about third-trimester brain development in preterm infants have been based on comparative quantitative MRI measures versus those of term healthy newborns at a single time point at the end of the third trimester (ie, TEA). However, these cross-sectional studies at the end of the third trimester were not designed to identify the onset and evolution of impaired brain development in the premature infant. Few studies have investigated the third-trimester brain maturation in the absence of brain injury after early exposure to extrauterine life.[13–17] Moreover, another substantial obstacle to progress has been the scarcity of in utero normative fetal MRI studies from which to establish and compare departures from normal third-trimester ex utero brain development.

Recently, we and others have successfully applied advanced quantitative MRI techniques to study in utero brain development in healthy fetuses.[18–22] These studies have provided major insights into the rate and progression of normal second- and third-trimester global, regional, and tissue-specific brain development in utero. However, no study to date has prospectively compared in utero brain volume in healthy fetuses versus that of ex utero preterm infants. The aim of the present study was to compare third-trimester global and regional brain volumes and brain growth trajectories in very preterm infants with no parenchymal brain injury versus healthy in utero fetuses by using 3-dimensional volumetric MRI measures. As a secondary objective, we sought to examine the relationship between clinical risk factors and brain volumes in preterm infants.

METHODS

Participants

We studied preterm infants and in utero healthy control fetuses recruited prospectively from longitudinal observational studies performed at 2 medical centers: Children's Boston Hospital (Boston, MA) and Children's National Health System (Washington, DC). The design of the study was cross-sectional, and it included only a single observation for each participant.

Preterm Cohort

Very preterm infants born before 32 weeks' gestational age (GA) weighing <1500 g were prospectively enrolled. We specifically excluded any preterm infants with known or suspected chromosomal anomalies, congenital malformations, central nervous system infection, and metabolic disorders. Also excluded were preterm infants with any evidence of parenchymal cerebral or cerebellar injury (ie, white matter injury, grade III–IV intraventricular hemorrhage [IVH], cerebellar hemorrhage) according to conventional MRI.

Fetal Cohort

Healthy pregnant women with normal fetal ultrasounds were recruited as normal control subjects in a prospective study comparing brain development in fetuses with congenital heart disease.[23–25] We excluded multiple pregnancies, chromosomal abnormalities, and congenital infection.

The studies were approved by the 2 institutional review boards, and written informed consent was obtained from each participant.

MRI Acquisition

Preterm Cohort

Preterm newborns were scanned under natural sleep by using either a 1.5-T MRI scanner Signa Excite, a 1.5-T Discovery MR450 scanner, or a 3-T Discovery MR750 (all: GE Healthcare, Milwaukee, MI). Preterm infants requiring temperature monitoring underwent scanning by using a Nomag MRI-compatible incubator (LMT Medical Systems GmbH, Luebeck, Germany).

Fetal Cohort

Fetal MRI studies were performed at 1.5 T either on an Achieva scanner (Philips Medical System, Best, Netherlands) or on a Discovery MR450 scanner (GE Healthcare).

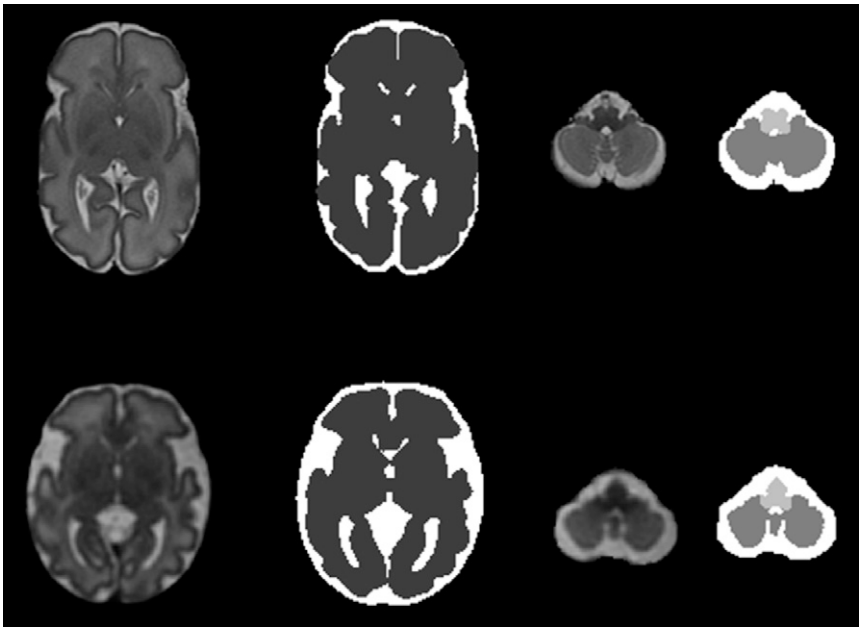

FIGURE 1
Parcellation of preterm (top row) and fetal (lower row) brain. Anatomic T2-weighted images and corresponding parcellation maps in a preterm infant at 31 4/7 weeks GA (top row) and a fetus at 32 2/7 weeks' GA (lower row). Dark gray: cerebrum; intermediate gray: cerebellum; light gray: brainstem; and white: cerebrospinal fluid.

For both cohorts, comparable sagittal, axial, and coronal T2-weighted images were collected by using single-shot fast spin echo sequences (2-mm slice thickness, 0-mm gap). An experienced pediatric neuroradiologist at each site reviewed all the MRI studies and noted the presence or absence of extra-axial blood.

Volumetric Analyses

After visual quality inspection of the anatomic images acquired, images without motion artifacts were selected and corrected for intensity nonhomogeneity.[26] For both cohorts, we reconstructed 3-dimensional images of the brain from the 2-dimensional images acquired[27] and used a parcellation pipeline[18] to delineate the brain regions of interest. The cerebrum, cerebellum, and brainstem were then manually corrected when necessary by using ITK-SNAP software.[28] Figure 1 provides an example of the brain parcellation performed in preterm infants and in utero fetuses. Volumes were computed by multiplying the number of voxels included in each brain region by the volume of each voxel and are expressed in cubic centimeters (milliliters). Total brain volume (TBV) was defined as the sum of cerebral, cerebellar, and brainstem volumes; the intracranial cavity volume (ICV) was the sum of TBV and cerebrospinal fluid.

Prenatal and Postnatal Clinical Data Collection

Prenatal information, mode of delivery, birth weight, GA at birth, Apgar score, and sex were collected for both cohorts. Additional clinical risk factors abstracted in the preterm cohort included length of mechanical ventilation and oxygen support, need for patent ductus arteriosus (PDA) ligation, sepsis (ie, confirmed sepsis by positive blood culture), postnatal dexamethasone treatment, and the presence of extra-axial blood on conventional MRI.

Statistical Analysis

Descriptive statistics were summarized by using median, range, means, and SDs for continuous variables and proportions for categorical factors. Linear regression models were developed by using Stata 13 (Stata Corp, College Station, TX) to evaluate the relationship between global and regional measurements of brain volume according to GA at MRI in healthy fetuses and preterm newborns. During model development, we evaluated the need for cross-product terms to allow for differing growth trajectories in the 2 groups and for higher order effects to introduce curvilinearity if necessary to improve model fit.

Within the preterm cohort, linear regression analysis was first used to evaluate the relationship between the presence/level of each clinical risk factor individually (ie, chorioamnionitis, cesarean delivery, PDA ligation, sepsis, dexamethasone treatment, number of days ventilated /on oxygen support, presence of extra-axial blood) and brain volume controlling for GA at birth, day of life at MRI, and sex. We chose to control for GA at birth rather than birth weight in the models because the 2 factors were highly correlated (r = 0.74) and because GA was judged less likely to interfere with risk factor identification. Results were expressed as regression (slope) coefficients with 95% confidence intervals (CIs). We then performed a multiple regression analysis that evaluated the combined main and interactive effects of each of the clinical risk factors associated with differences in global or regional brain volumes. Only those 2- and 3-way interactions that achieved at least a borderline level of statistical significance ($P < .12$) were retained in final models; the decision on which of the alternative models to use to represent the combined impact of risk factors on brain growth was aided by the use of Akaike information criterion statistics.[29,30] We consider these

TABLE 1 Clinical Characteristics of the Cohort

Characteristic	Preterm Cohort (n = 75)	Fetal Cohort (n = 130)	P
GA at MRI, wk			.007
Mean ± SD	33.95 ± 2.53	32.1 ± 3.27	
Range	27–39	27–39	
Female, *n* (%)	39 (52)	64 (50)	.74
Maternal age, y			.93
Mean ± SD	29.4 ± 6.3	29.5 ± 5.8	
Range	15–41	18–43	
Maternal highest level of education, *n* (%)			.02
Less than seventh grade	1 (1)	0	
Junior high school	2 (3)	0	
Partial junior high school	7 (9)	7 (5)	
High school	14 (19)	30 (23)	
Partial college or specialized training	12 (16)	28 (22)	
Standard college	19 (25)	26 (20)	
Graduate professional training	8 (11)	39 (30)	
Not collected	12 (16)	0	
GA at birth, wk			<.001
Mean ± SD	27.2 ± 2.36	39.3 ± 1.2[a]	
Range	22–32	36–41.3	
Birth weight, g			<.001
Mean ± SD	947 ± 283	3362 ± 432[a]	
Range	400–1490	1953–4310	
Small for GA	10 (13)	7 (5)[a]	.14
Vaginal delivery, *n* (%)	24 (32)	78 (68)[a]	<.001
Apgar score at 5 min, median (range)	8 (0–9)	9 (6–10)[a]	<.001
Ethnicity, *n* (%)			.003
Hispanic	14 (19)	13 (10)	
White	30 (40)	35 (27)	
Asian	0	13 (10)	
Black	30 (40)	61 (47)	
Multiethnic	1 (1)	8 (6)	

[a] Birth data were not available in 10 participants of the fetal cohort.

analyses hypothesis-generating and thus did not correct for multiple comparisons, and we were more lenient in choosing terms to include in the model to avoid missing the identification of potentially important risk factors.

RESULTS

Clinical Characteristics of the Cohort

A total of 205 subjects were studied: 75 very preterm infants (birth GA: 22–32 weeks) with a mean corrected GA at MRI of 34 ± 2.5 weeks (range: 27–39 weeks) and 130 healthy fetuses with a mean GA at MRI of 32 ± 3.3 weeks (range: 27–39 weeks). All MRI scans in the preterm and fetal cohorts were structurally normal (ie, had no parenchymal brain injury/malformations). The clinical characteristics of the cohort are summarized in Table 1.

Comparisons of Brain Volumes by Site

Table 2 summarizes the brain volumes of the preterm and fetal cohorts by site; the ratio of preterm infants to fetuses was similar at each site. Notably, the few significant differences that were present in the preterm and fetal cohorts at a young age (<33 weeks' GA) disappeared after controlling for GA at MRI.

Comparisons Between Fetal and Preterm Brain Volumes

Controlling for GA at MRI and sex, brain volumes were lower in preterm infants compared with in utero fetuses for all the brain regions examined ($P < .05$) (Fig 2). Third-trimester brain growth trajectories by GA at MRI were slower in the ex utero preterm cohort compared with the fetal cohort for the cerebrum ($P = .001$), the cerebellum ($P = .05$), the brainstem ($P = .06$), TBV ($P = .002$), and ICV ($P = .03$).

Preterm Infants: Clinical Risk Factors and Impaired Brain Volume

The clinical risk factors were similar in the 2 preterm cohorts except for length of ventilation (Table 3). The statistically significant relationships between individual clinical risk factors and regional brain volume when controlling for GA at birth, day of life at MRI, and sex are summarized in Table 4. Dexamethasone treatment, the presence of extra-axial blood, sepsis, and length of oxygen support were associated with reduced brain volumes. We then included the aforementioned risk factors in a multiple regression model analysis (Table 5). The 3-way interaction effect among dexamethasone treatment, extra-axial blood, and length of oxygen support explained >75% of the reduction in cerebral/cerebellar volumes, TBV, and ICV. In addition, sepsis was significantly associated with reductions in cerebral volume, TBV, and ICV.

DISCUSSION

To our knowledge, our study reports for the first time decreased third-trimester global and regional brain growth in very preterm infants without evidence of cerebral or cerebellar parenchymal injury compared with healthy in utero control fetuses. Using volumetric MRI, we found differences in brain growth trajectories between preterm infants who experience their "third trimester" of development ex utero and in utero healthy fetuses. Our findings suggest that even in the absence of structural brain injury, the developmental trajectory of the preterm brain is altered over the third trimester. Evidence of clinical

TABLE 2 Brain Volumes of the Preterm and Fetal Cohorts According to Site and Gestational Age

Variable	Preterm Infants (n = 75)						Fetuses (n = 130)					
	Boston (n = 37 [50%])						Boston (n = 55 [42%])					
	Washington DC (n = 38 [50%])						Washington DC (n = 75 [58%])					
	Boston (n = 10)	DC (n = 17)	P	Boston (n = 27)	DC (n = 21)	P	Boston (n = 40)	DC (n = 35)	P	Boston (n = 15)	DC (n = 40)	P
GA at MRI, wk	30.47 ± 1.8 [27–30.9]	31.92 ± 0.8 [30.6–32.9]	.04	35.61 ± 1.5 [33.1–39]	35.4 ± 1.3 [33.3–37.7]	.61	28.96 ± 1.4 [27–32.42]	30.44 ± 1.7 [27–32.86]	.001	34.73 ± 1 [33–36.57]	35.56 ± 1.7 [33–39]	.03
Cerebrum, mL	160.3 ± 27 [123–205]	163.2 ± 20 [113–199]	.76	203.4 ± 40 [124–276]	195.5 ± 45 [113–289]	.83	138.1 ± 23 [100–290]	156.3 ± 35 [88–231]	.01*	248.4 ± 22 [206–280]	237.6 ± 33 [170–315]	.25
Cerebellum, mL	7.3 ± 1.8 [4–9.4]	7.77 ± 1.6 [5–11]	.48	12.61 ± 2.2 [8–16]	11.91 ± 3.2 [7–18]	.38	6.94 ± 1.6 [4–11]	8.13 ± 2.2 [4–12]	.01*	14.17 ± 1.6 [4–18]	13.91 ± 2.7 [8–20]	.66
Brainstem, mL	3.5 ± 0.4 [3–4]	3.2 ± 0.4 [3–4]	.04*	4.3 ± 0.5 [3–6]	4.4 ± 0.8 [3–6]	.7	2.9 ± 0.4 [2–4]	3.1 ± 0.6 [2–5]	.12	4.7 ± 0.5 [4–6]	4.54 ± 0.7 [4–6]	.33
Total brain volume, mL	171.1 ± 29 [131–218]	174.16 ± 22 [122–214]	.76	236.2 ± 34 [168–296]	237.9 ± 47 [169–311]	.88	148 ± 24 [106–205]	167.6 ± 37 [93–247]	.01*	267.3 ± 23 [222–299]	256.1 ± 36 [183–341]	.27
Intracranial cavity volume, mL	225.8 ± 37 [175–283]	241.8 ± 31 [179–294]	.24	315.37 ± 48 [228–413]	324.64 ± 56 [238–441]	.55	262.7 ± 43 [190–372]	284.1 ± 56 [179–398]	.067	418.2 ± 32 [360–460]	394.8 ± 47 [303–495]	.08

* Differences are not significant when controlling for gestational age at MRI.

risk factors associated with reduced brain volumes in preterm infants included individual and synergistic effects of dexamethasone treatment, the presence of extra-axial blood, the length of oxygen support, and confirmed sepsis.

Previous studies suggest that brain volumes in preterm survivors are altered by TEA compared with healthy term newborns and are characterized by decreased parenchymal volumes.[9,31–35] Alterations in brain volumes at TEA in the preterm infants have also been correlated with worse neurocognitive outcomes.[36–38] Moreover, prematurely born children and adults exhibit altered regional volumetric brain growth compared with their term-born peers[39,40] suggesting long-lasting consequences of early-life disturbances in brain growth.[41] However, to date, few studies have investigated third-trimester volumetric brain development in preterm infants and healthy in utero fetuses. Only 1 recent study (by Lefèvre et al[42]) used a retrospective cross-sectional design to compare 27 preterm infants (birth GA: 25–35 weeks) and 14 fetuses; the investigators found that brain volumes were similar, whereas cortical folding trajectories were altered in preterm infants compared with healthy control fetuses. Our data showed reductions in brain volumes across the third trimester of ex utero life in preterm infants compared with the in utero healthy control fetuses. Our larger sample size (N = 205) suggest that we were better-powered to detect subtle but important differences in regional brain growth. The present results extend earlier findings of altered brain growth in preterm infants by TEA[9,32] and demonstrate a progressive fall-off/dysmaturation of the brain after preterm birth over the third trimester.

The third trimester of gestation is a critical period of prolific brain

TABLE 3 Clinical Risk Factors

Variable	All Preterm Infants (n = 75)	Preterm Cohort From Boston (n = 37)	Preterm Cohort From Washington, DC (n = 38)	P
Chorioamnionitis	15 (20)	4 (11)	11 (28)	.05
Cesarean delivery	51 (68)	25 (49)	26 (51)	.94
Dexamethasone	12 (16)	5 (14)	7 (18)	.56
PDA ligation	14 (19)	8 (22)	6 (16)	.52
Sepsis	11 (15)	8 (22)	3 (8)	.09
Extra-axial blood	16 (21)	9 (24)	7 (18)	.53
Length of mechanical ventilation, d	19.87 ± 23.7 [0–91]	28.1 ± 24.9 [1–81]	12 ± 19.5 [0–80]	.002
Length of oxygen support, d	55.9 ± 35.3 [2–144]	60.5 ± 33.2 [3–122]	51.4 ± 37.2 [2–142]	.27

Data are presented as n (%) or mean ± SD [range].

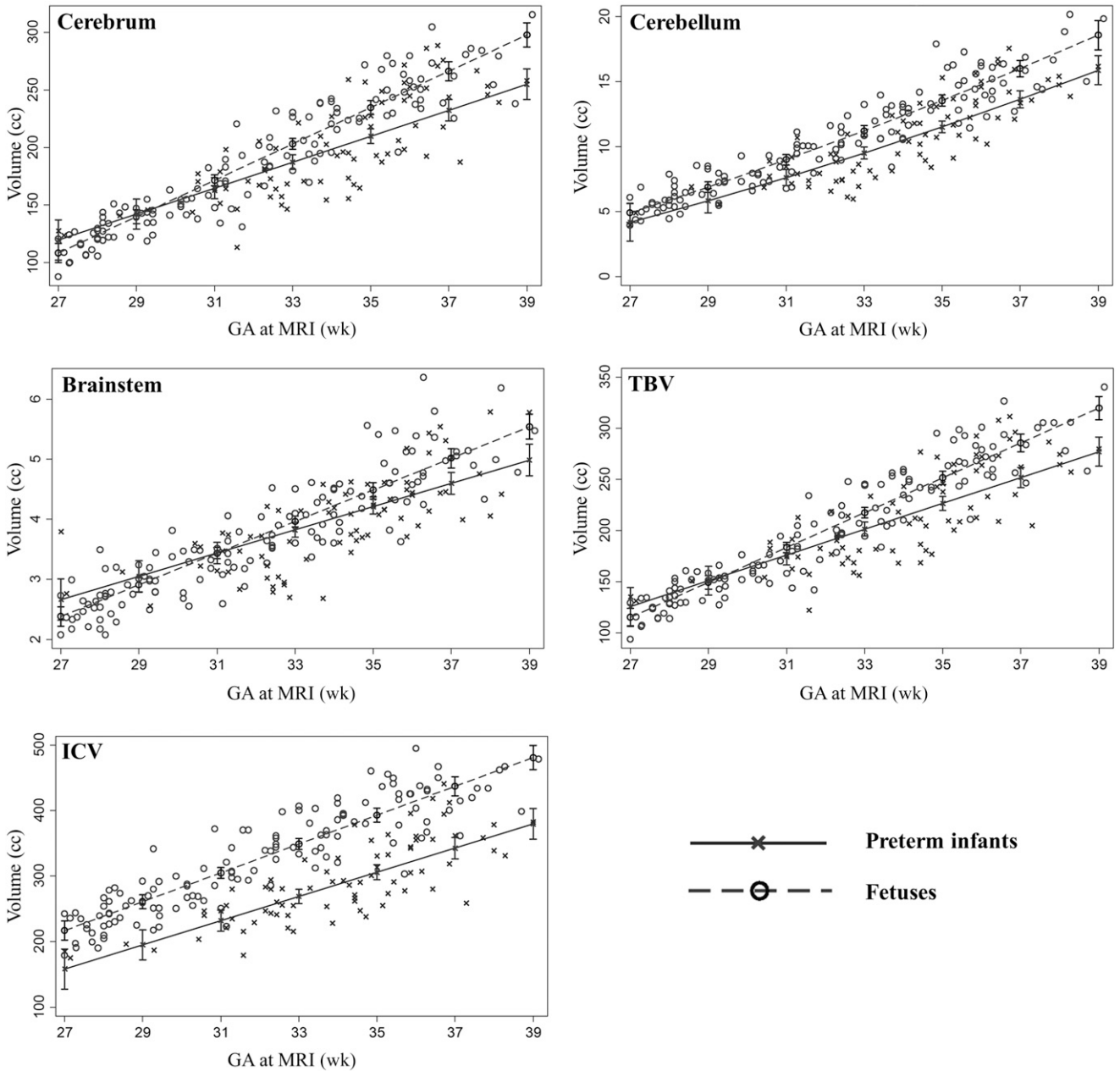

FIGURE 2
Brain volumes plotted against GA at MRI in the preterm and fetal cohorts (controlling for sex).

TABLE 4 Relationship Between Brain Volumes and Individual Clinical Risk Factors in the Preterm Cohort (n = 75)

Variable	Cerebrum	Cerebellum	Brainstem	TBV	ICV
Chorioamnionitis	—	—	—	—	—
Cesarean delivery	−0.07 [−0.1; -9×10^{-3}] (.03)	—	—	−0.07 [−0.1; -6×10^{-3}] (.03)	—
Dexamethasone	−0.128 [−0.22; −0.04] (.004)	−0.167 [−0.3; −0.05] (.008)	−0.66 [−1; −0.3] (<.001)	−0.13 [−0.2;−0.05] (.003)	−38.87 [−60; −18] (<.001)
PDA ligation	—	—	—	—	—
Sepsis	−0.075 [−0.15; -3×10^{-3}] (.04)	—	—	−0.072 [−0.15; −0.001] (.05)	−29.25 [−52;−6.5] (.01)
Extra-axial blood	−0.093 [−0.15; −0.04] (.002)	−0.188 [−0.29; −0.09] (<.001)	—	−0.95 [−0.15; −0.04] (.002)	−27.3 [−44.5; −10.1] (.002)
Length of mechanical ventilation, d	—	—	—	—	—
Length of oxygen support, d	−0.002 [-3×10^{-3}; -3×10^{-4}] (.02)	−0.002 [-4×10^{-3}; -7×10^{5}] (.04)	—	−0.001 [-3×10^{-3}; -4×10^{4}] (.02)	—

Regression coefficient [95% CI] in milliliters and corresponding (P value) of the clinical risk factor of interest controlling for GA at birth, age at MRI (days), and sex. Em dashes indicate the absence of a significant relationship between a given risk factor and brain volume.

TABLE 5 Relationship Between Brain Volumes and Combined Clinical Risk Factors in the Preterm Cohort (n = 75)

Brain Areas	Model[a]	Percentage of Variance Explained
Cerebrum	Dexamethasone | Extra-axial blood | Length of oxygen support | Sepsis	78
Cerebellum	Dexamethasone | Extra-axial blood | Length of oxygen support	85
Brainstem	Dexamethasone	60
Total brain volume	Dexamethasone | Extra-axial blood | Length of oxygen support | Sepsis	79
Intracranial cavity volume	Dexamethasone | Extra-axial blood | Length of oxygen support | Sepsis	77

[a] The estimate of variance explained includes the main effects as well as 2- and 3-way interactions (not shown); all models had a P value < .001.

development. Multiple biological processes are actively taking place, including the beginning of myelination, neuronal organization, spinogenesis, and synaptogenesis.[43] It is well established that there is a fourfold increase in brain size during the third trimester of gestation[18] accompanied by a dramatic increase in brain surface area with the formation of tertiary sulci and gyri.[44] This well-orchestrated and precisely timed development of the neural circuitry may be vulnerable to insults associated with preterm birth. Longitudinal studies have explored the relationship between clinical risk factors and neurodevelopmental outcomes in preterm born infants, including postnatal infections,[45–47] medications,[48,49] and bronchopulmonary dysplasia.[50] At TEA, several clinical risk factors have been associated with decreased brain volume, including white matter brain injury,[31,34,35] respiratory illness,[31,34] small birth weight for GA,[34,35] increasing prematurity,[9] and low-grade IVH.[9,51] Our study extends these observations and shows that among the clinical risk factors examined, postnatal dexamethasone treatment, the presence of extra-axial blood, respiratory illness, and sepsis were inversely associated with brain growth in preterm infants during the third trimester.

We report an association between dexamethasone treatment and impaired cerebral, cerebellar, and brainstem growth during the third trimester. Our findings are in keeping with previously published research suggesting a detrimental effect of dexamethasone treatment on preterm brain volumes at TEA[52,53] and during adolescence.[54] Animal models have shown that dexamethasone administration during the critical developmental period is associated with abnormal apoptosis and alterations in synaptic function.[55,56] Respiratory distress in the preterm infants results in alterations in cerebral hemodynamics that can also lead to impaired brain development.[57] In our study, indicators of respiratory illness such as the length of oxygen support were inversely associated with third-trimester brain volumes in preterm infants, consistent with previous findings reported at TEA.[9,31,34]

Prenatal, intrapartum, and/or postnatal infections result in inflammatory substances that have been linked to both brain injury and altered brain development in the preterm population.[58,59] In this study, we also report an association between confirmed sepsis and stunted cerebral growth. This specific finding contrasts with 2 other studies, which found no relationship between brain volumes

and sepsis in preterm infants at TEA.[9,31] The severity, time, and occurrence of sepsis might play a role in the varied findings. Longitudinal data sets will allow elucidating whether there is a catch-up in brain growth by TEA in preterm infants with confirmed sepsis. A large, longitudinal population-based study has shown a negative independent effect of sepsis on neurodevelopmental impairments at 2 years of age.[46] Conversely, we found no relationship between chorioamnionitis and third-trimester brain volume, which is in agreement with previous studies.[35,60] Chorioamnionitis has been associated with an increased risk for developing brain injury,[61,62] and it has been especially correlated with severe IVH.[63,64] The direct influence of chorioamnionitis on neurodevelopmental outcome is unclear, and it is likely mediated by the increased risk of postnatal morbidities associated with prenatal infection, including brain injury and neonatal sepsis.[61,65–67]

The presence of extra-axial blood in our preterm cohort was linked to decreased brain volumes. Low-grade IVH has previously been related to decreased brain volumes in near-term and preterm infants at TEA.[9,51] Potential mechanisms include free radical injury mediated by extra-axial blood and IVH, which adversely affects the proliferating cells and leads to subsequent downstream events in cerebral and cerebellar development.[68–70] Additional studies are needed to quantify the effects of blood on the developing preterm brain.

Surprisingly, the need for surgical PDA ligation was not associated with impaired brain volumes over the third trimester in the ex utero preterm infants in our study. This finding conflicts with previous literature in which PDA ligation was associated with reduced brain volumes at term age,[8,9] but it is in line with a recently published study. Lemmers et al[71] postulated that the duration of reduced cerebral oxygenation associated with PDA is likely the main factor responsible for brain growth impairment associated with PDA ligation rather than the need for surgery itself. Ongoing research is warranted to address this intriguing question.

Our study provides new insights into the role of prematurity-related clinical risk factors on brain maturation during the third trimester in ex utero preterm infants with no evidence of parenchymal brain injury. Ongoing prospective studies are urgently needed to better ascertain what aspects of neonatal intensive care may be harmful versus protective for the developing preterm brain.[72]

Our study has several limitations. First, the design of the study was cross-sectional; thus, the brain growth trajectories examined are population based (ie, the trajectories are computed from a cohort with a single observation rather than from individuals with longitudinal MRI data). Serial MRI scans would allow for better delineation of third-trimester longitudinal brain growth in normal in utero versus ex utero environments, and study of this topic is currently underway. Second, we combined participants from 2 regionally distinct cohorts, introducing differences in the MRI scanners used and variability in clinical care. However, all participants were scanned with comparable T2-weighted anatomic sequences, and we found no significant difference after controlling for GA at MRI in brain volumes in both fetal and preterm cohorts performed at the 2 sites. Furthermore, the clinical risk factors were similar in the 2 preterm cohorts. The third limitation relates to our sample size that restricted the number of clinical risk factors we could investigate. We decided to include in our multivariate analysis only the clinical risk factors that were correlated with brain volumes on our univariate analysis. Other statistical approaches could lead to different models; consequently, our results are only hypothesis-generating and need to be evaluated and confirmed by other studies. Moreover, our findings are not brain tissue specific. A segmentation pipeline applied to this data set could provide additional information about different tissue types (ie, cortical gray versus white matter) that may be preferentially affected by preterm birth. Finally, although we specifically excluded preterm infants with evidence of structural brain injury on conventional MRI, it is possible that smaller brain lesions below the current resolution of clinical MRI field strength were missed.[73] Specifically, we cannot exclude the presence of white matter microscopic necrosis, which can only be resolved by using a higher MRI field strength (eg, 12 T).[74]

CONCLUSIONS

Our data strongly support the notion that preterm delivery is associated with disturbances in third-trimester global and regional brain growth even in the absence of parenchymal brain injury. Clinical risk factors associated with preterm birth and impaired brain development include the use of steroids, greater respiratory illness, sepsis, and the presence of extra-axial hemorrhage. Ongoing studies are needed to clarify the effects of altered third-trimester regional brain growth on neurodevelopmental outcome.

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ABBREVIATIONS

GA: gestational age
ICV: intracranial cavity volume
IVH: intraventricular hemorrhage
PDA: patent ductus arteriosus
TBV: total brain volume
TEA: term-equivalent age

Dr Limperopoulos co-conceptualized and designed the study, coordinated and supervised the progress of the study at the 2 sites, analyzed the results, and contributed to the writing of the manuscript; and all authors approved the final manuscript as submitted.

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REFERENCES

1. Blencowe H, Lee AC, Cousens S, et al. Preterm birth-associated neurodevelopmental impairment estimates at regional and global levels for 2010. *Pediatr Res*. 2013;74(suppl 1):17–34
2. Kidokoro H, Anderson PJ, Doyle LW, Woodward LJ, Neil JJ, Inder TE. Brain injury and altered brain growth in preterm infants: predictors and prognosis. *Pediatrics*. 2014;134(2). Available at: www.pediatrics.org/cgi/content/full/133/2/e444
3. Miller SP, Ferriero DM. From selective vulnerability to connectivity: insights from newborn brain imaging. *Trends Neurosci*. 2009;32(9):496–505
4. Ortinau C, Neil J. The neuroanatomy of prematurity: normal brain development and the impact of preterm birth. *Clin Anat*. 2015;28(2):168–183
5. Bennet L, Van Den Heuij L, Dean JM, Drury P, Wassink G, Gunn AJ. Neural plasticity and the Kennard principle: does it work for the preterm brain? *Clin Exp Pharmacol Physiol*. 2013;40(11):774–784
6. Ajayi-Obe M, Saeed N, Cowan FM, Rutherford MA, Edwards AD. Reduced development of cerebral cortex in extremely preterm infants. *Lancet*. 2000;356(9236):1162–1163
7. Kapellou O, Counsell SJ, Kennea N, et al. Abnormal cortical development after premature birth shown by altered allometric scaling of brain growth. *PLoS Med*. 2006;3(8):e265
8. Limperopoulos C, Soul JS, Gauvreau K, et al. Late gestation cerebellar growth is rapid and impeded by premature birth. *Pediatrics*. 2005;115(3):688–695
9. Padilla N, Alexandrou G, Blennow M, Lagercrantz H, Ådén U. Brain growth gains and losses in extremely preterm infants at term. *Cereb Cortex*. 2015;25(7):1897–1905
10. Ball G, Srinivasan L, Aljabar P, et al. Development of cortical microstructure in the preterm human brain. *Proc Natl Acad Sci USA*. 2013;110(23):9541–9546
11. Smyser CD, Snyder AZ, Shimony JS, Mitra A, Inder TE, Neil JJ. Resting-state network complexity and magnitude are reduced in prematurely born infants. *Cereb Cortex*. 2016;26(1):322–333
12. Scheinost D, Kwon SH, Shen X, et al. Preterm birth alters neonatal, functional rich club organization. *Brain Struct Funct*. 2016;221(6):3211–3222
13. Dubois J, Benders M, Borradori-Tolsa C, et al. Primary cortical folding in the human newborn: an early marker of later functional development. *Brain*. 2008;131(pt 8):2028–2041
14. Viola A, Confort-Gouny S, Schneider JF, et al. Is brain maturation comparable in fetuses and premature neonates at term equivalent age? *AJNR Am J Neuroradiol*. 2011;32(8):1451–1458
15. Nossin-Manor R, Card D, Raybaud C, Taylor MJ, Sled JG. Cerebral maturation in the early preterm period—a magnetization transfer and diffusion tensor imaging study using voxel-based analysis. *Neuroimage*. 2015;112(C):30–42
16. Schneider J, Kober T, Bickle Graz M, et al. Evolution of T1 relaxation, ADC, and fractional anisotropy during early brain maturation: a serial imaging study on preterm infants. *AJNR Am J Neuroradiol*. 2016;37(1):155–162
17. Makropoulos A, Aljabar P, Wright R, et al. Regional growth and atlasing of the developing human brain. *Neuroimage*. 2016;125(C):456–478
18. Clouchoux C, Guizard N, Evans AC, Plessis du AJ, Limperopoulos C. Normative fetal brain growth by quantitative in vivo magnetic resonance imaging. *Am J Obstet Gynecol*. 2012;206(2):173.e1–8
19. Clouchoux C, Kudelski D, Gholipour A, et al. Quantitative in vivo MRI measurement of cortical development in the fetus. *Brain Struct Funct*. 2012;217(1):127–139
20. Habas PA, Scott JA, Roosta A, et al. Early folding patterns and asymmetries of the normal human brain detected from in utero MRI. *Cereb Cortex*. 2012;22(1):13–25
21. Wu J, Awate SP, Licht DJ, et al. Assessment of MRI-based automated fetal cerebral cortical folding measures in prediction of gestational age in the third trimester. *AJNR Am J Neuroradiol*. 2015;36(7):1369–1374
22. Wright R, Kyriakopoulou V, Ledig C, et al. Automatic quantification of normal cortical folding patterns from fetal brain MRI. *Neuroimage*. 2014;91(C):21–32

23. Limperopoulos C, Tworetzky W, McElhinney DB, et al. Brain volume and metabolism in fetuses with congenital heart disease: evaluation with quantitative magnetic resonance imaging and spectroscopy. *Circulation*. 2010;121(1):26–33

24. Clouchoux C, du Plessis AJ, Bouyssi-Kobar M, et al. Delayed cortical development in fetuses with complex congenital heart disease. *Cereb Cortex*. 2013;23(12):2932–2943

25. Brossard-Racine M, du Plessis AJ, Vezina G, et al. Prevalence and spectrum of in utero structural brain abnormalities in fetuses with complex congenital heart disease. *AJNR Am J Neuroradiol*. 2014;35(8):1593–1599

26. Tustison NJ, Avants BB, Cook PA, et al. N4ITK: improved N3 bias correction. *IEEE Trans Med Imaging*. 2010;29(6):1310–1320

27. Gholipour A, Estroff JA, Barnewolt CE, Connolly SA, Warfield SK. Fetal brain volumetry through MRI volumetric reconstruction and segmentation. *Int J CARS*. 2011;6(3):329–339

28. Yushkevich PA, Piven J, Hazlett HC, et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage*. 2006;31(3):1116–1128

29. Akaike H. Information theory and an extension of the maximum likelihood principle. In: *Selected Papers of Hirotugu Akaike. Springer Series in Statistics*. New York, NY: Springer New York; 1998:199–213

30. Akaike H. Likelihood of a model and information criteria. *J Econom*. 1981;16(1):3–14

31. Inder TE, Warfield SK, Wang H, Hüppi PS, Volpe JJ. Abnormal cerebral structure is present at term in premature infants. *Pediatrics*. 2005;115(2):286–294

32. Peterson BS, Anderson AW, Ehrenkranz R, et al. Regional brain volumes and their later neurodevelopmental correlates in term and preterm infants. *Pediatrics*. 2003;111(5 pt 1):939–948

33. Mewes AU, Hüppi PS, Als H, et al. Regional brain development in serial magnetic resonance imaging of low-risk preterm infants. *Pediatrics*. 2006;118(1):23–33

34. Thompson DK, Warfield SK, Carlin JB, et al. Perinatal risk factors altering regional brain structure in the preterm infant. *Brain*. 2007;130(pt 3):667–677

35. Parikh NA, Lasky RE, Kennedy KA, McDavid G, Tyson JE. Perinatal factors and regional brain volume abnormalities at term in a cohort of extremely low birth weight infants. *PLoS ONE*. 2013;8(5):e62804

36. Soria-Pastor S, Padilla N, Zubiaurre-Elorza L, et al. Decreased regional brain volume and cognitive impairment in preterm children at low risk. *Pediatrics*. 2009;124(6). Available at: www.pediatrics.org/cgi/content/full/124/6/e1161

37. Keunen K, Išgum I, van Kooij BJ, et al. Brain volumes at term-equivalent age in preterm infants: imaging biomarkers for neurodevelopmental outcome through early school age. *J Pediatr*. 2016;172(0):88–95

38. Lind A, Parkkola R, Lehtonen L, et al; PIPARI Study Group. Associations between regional brain volumes at term-equivalent age and development at 2 years of age in preterm children. *Pediatr Radiol*. 2011;41(8):953–961

39. Nosarti C, Nam KW, Walshe M, et al. Preterm birth and structural brain alterations in early adulthood. *Neuroimage Clin*. 2014;6(C):180–191

40. Scheinost D, Kwon SH, Lacadie C, et al. Alterations in Anatomical Covariance in the Prematurely Born. *Cereb Cortex*. 2015;(October):bhv248–bhv10

41. Bjuland KJ, Rimol LM, Løhaugen GCC, Skranes J. Brain volumes and cognitive function in very-low-birth-weight (VLBW) young adults. *Eur J Paediatr Neurol*. 2014;18(5):578–590

42. Lefèvre J, Germanaud D, Dubois J, et al. Are developmental trajectories of cortical folding comparable between cross-sectional datasets of fetuses and preterm newborns? *Cereb Cortex*. 2016;26(7):3023–3035

43. Kostović I, Jovanov-Milosević N. The development of cerebral connections during the first 20-45 weeks' gestation. *Semin Fetal Neonatal Med*. 2006;11(6):415–422

44. Kostovic I, Vasung L. Insights from in vitro fetal magnetic resonance imaging of cerebral development. *Semin Perinatol*. 2009;33(4):220–233

45. Stoll BJ, Hansen NI, Adams-Chapman I, et al; National Institute of Child Health and Human Development Neonatal Research Network. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. *JAMA*. 2004;292(19):2357–2365

46. Schlapbach LJ, Aebischer M, Adams M, et al; Swiss Neonatal Network and Follow-Up Group. Impact of sepsis on neurodevelopmental outcome in a Swiss National Cohort of extremely premature infants. *Pediatrics*. 2011;128(2). Available at: www.pediatrics.org/cgi/content/full/128/2/e348

47. Mitha A, Foix-L'Hélias L, Arnaud C, et al; EPIPAGE Study Group. Neonatal infection and 5-year neurodevelopmental outcome of very preterm infants. *Pediatrics*. 2013;132(2). Available at: www.pediatrics.org/cgi/content/full/132/2/e372

48. Steinhorn R, McPherson C, Anderson PJ, Neil J, Doyle LW, Inder T. Neonatal morphine exposure in very preterm infants-cerebral development and outcomes. *J Pediatr*. 2015;166(5):1200–1207.e4

49. Rozé JC, Denizot S, Carbajal R, et al. Prolonged sedation and/or analgesia and 5-year neurodevelopment outcome in very preterm infants: results from the EPIPAGE cohort. *Arch Pediatr Adolesc Med*. 2008;162(8):728–733

50. Short EJ, Klein NK, Lewis BA, et al. Cognitive and academic consequences of bronchopulmonary dysplasia and very low birth weight: 8-year-old outcomes. *Pediatrics*. 2003;112(5). Available at: www.pediatrics.org/cgi/content/full/112/5/e359

51. Vasileiadis GT, Gelman N, Han VK, et al. Uncomplicated intraventricular hemorrhage is followed by reduced cortical volume at near-term age. *Pediatrics*. 2004;114(3). Available at:

www.pediatrics.org/cgi/content/full/114/3/e367

52. Murphy BP, Inder TE, Huppi PS, et al. Impaired cerebral cortical gray matter growth after treatment with dexamethasone for neonatal chronic lung disease. *Pediatrics*. 2001;107(2):217–221
53. Parikh NA, Lasky RE, Kennedy KA, et al. Postnatal dexamethasone therapy and cerebral tissue volumes in extremely low birth weight infants. *Pediatrics*. 2007;119(2):265–272
54. Cheong JL, Burnett AC, Lee KJ, et al; Victorian Infant Collaborative Study Group. Association between postnatal dexamethasone for treatment of bronchopulmonary dysplasia and brain volumes at adolescence in infants born very preterm. *J Pediatr*. 2014;164(4):737–743.e1
55. Howard E. Reductions in size and total DNA of cerebrum and cerebellum in adult mice after corticosterone treatment in infancy. *Exp Neurol*. 1968;22(2):191–208
56. Kreider ML, Tate CA, Cousins MM, Oliver CA, Seidler FJ, Slotkin TA. Lasting effects of developmental dexamethasone treatment on neural cell number and size, synaptic activity, and cell signaling: critical periods of vulnerability, dose-effect relationships, regional targets, and sex selectivity. *Neuropsychopharmacology*. 2006;31(1):12–35
57. Neubauer V, Junker D, Griesmaier E, Schocke M, Kiechl-Kohlendorfer U. Bronchopulmonary dysplasia is associated with delayed structural brain maturation in preterm infants. *Neonatology*. 2015;107(3):179–184
58. du Plessis AJ, Volpe JJ. Perinatal brain injury in the preterm and term newborn. *Curr Opin Neurol*. 2002;15(2):151–157
59. Hagberg H, Mallard C, Ferriero DM, et al. The role of inflammation in perinatal brain injury. *Nat Rev Neurol*. 2015;11(4):192–208
60. Reiman M, Kujari H, Maunu J, et al; PIPARI Study Group. Does placental inflammation relate to brain lesions and volume in preterm infants? *J Pediatr*. 2008;152(5):642–647, 647.e1–e2
61. Ericson JE, Laughon MM. Chorioamnionitis: implications for the neonate. *Clin Perinatol*. 2015;42(1):155–165, ix
62. Lu HY, Zhang Q, Wang QX, Lu JY. Contribution of histologic chorioamnionitis and fetal inflammatory response syndrome to increased risk of brain injury in infants with preterm premature rupture of membranes. *Pediatr Neurol*. 2016;61:94–98.e1
63. Stark MJ, Hodyl NA, Belegar V KK, Andersen CC. Intrauterine inflammation, cerebral oxygen consumption and susceptibility to early brain injury in very preterm newborns. *Arch Dis Child Fetal Neonatal Ed*. 2016;101(2):F137–F142
64. Soraisham AS, Singhal N, McMillan DD, Sauve RS, Lee SK, Canadian Neonatal Network. A multicenter study on the clinical outcome of chorioamnionitis in preterm infants. *Am J Obstet Gynecol*. 2009;200(4):372.e1–6
65. Alexander JM, Gilstrap LC, Cox SM, McIntire DM, Leveno KJ. Clinical chorioamnionitis and the prognosis for very low birth weight infants. *Obstet Gynecol*. 1998;91(5 pt 1):725–729
66. Andrews WW, Cliver SP, Biasini F, et al. Early preterm birth: association between in utero exposure to acute inflammation and severe neurodevelopmental disability at 6 years of age. *Am J Obstet Gynecol*. 2008;198(4):466.e1–466.e11
67. Bersani I, Thomas W, Speer CP. Chorioamnionitis—the good or the evil for neonatal outcome? *J Matern Fetal Neonatal Med*. 2012;25(suppl 1):12–16
68. Gram M, Sveinsdottir S, Ruscher K, et al. Hemoglobin induces inflammation after preterm intraventricular hemorrhage by methemoglobin formation. *J Neuroinflammation*. 2013;10(1):100
69. Tam EW, Miller SP, Studholme C, et al. Differential effects of intraventricular hemorrhage and white matter injury on preterm cerebellar growth. *J Pediatr*. 2011;158(3):366–371
70. Del Bigio MR. Cell proliferation in human ganglionic eminence and suppression after prematurity-associated haemorrhage. *Brain*. 2011;134(pt 5):1344–1361
71. Lemmers PM, Benders MJ, D'Ascenzo R, et al. Patent ductus arteriosus and brain volume. *Pediatrics*. 2016;137(4):e20153090
72. Pickler RH, McGrath JM, Reyna BA, et al. A model of neurodevelopmental risk and protection for preterm infants. *J Perinat Neonatal Nurs*. 2010;24(4):356–365
73. Back SA. brain injury in the preterm infant: new horizons for pathogenesis and prevention. *Pediatr Neurol*. 2015;53(3):185–192
74. Riddle A, Dean J, Buser JR, et al. Histopathological correlates of magnetic resonance imaging-defined chronic perinatal white matter injury. *Ann Neurol*. 2011;70(3):493–507