Delayed Cortical Development in Fetuses with Complex Congenital Heart Disease


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Neurologic impairment is a major complication of complex congenital heart disease (CHD). A growing body of evidence suggests that neurologic dysfunction may be present in a significant proportion of this high-risk population in the early newborn period prior to surgical interventions. We recently provided the first evidence that brain growth impairment in fetuses with complex CHD has its origins in utero. Here, we extend these observations by characterizing global and regional brain development in fetuses with hypoplastic left heart syndrome (HLHS), one of the most severe forms of CHD. Using advanced magnetic resonance imaging techniques, we compared in vivo brain growth in 18 fetuses with HLHS and 30 control fetuses from 25.4–37.0 weeks of gestation. Our findings demonstrate a progressive third trimester fall-off in cortical gray and white matter volumes (P < 0.001), and subcortical gray matter (P < 0.05) in fetuses with HLHS. Significant delays in cortical gyri formation were also evident in HLHS fetuses (P < 0.001). In the HLHS fetus, local cortical folding delays were detected as early as 25 weeks in the frontal, parietal, calcarine, temporal, and collateral regions and appear to precede volumetric brain growth disturbances, which may be an early marker of elevated risk for third trimester brain growth failure.

Keywords: brain development, congenital heart disease, cortical surface, fetal, magnetic resonance imaging

Introduction

Neurologic disability has long been recognized as a serious complication of congenital heart disease (CHD) (Goldberg et al. 2000; Shillingford et al. 2007; Hirsch et al. 2011). Initially, the primary mechanisms for brain injury were thought to be chronic cyanosis and hypoxia during the delay until cardiac repair became possible. The advent of early neonatal corrective cardiac surgery was met with the expectation that neurologic complications would be minimized or eradicated in CHD survivors (Wernovsky, Mayer et al. 1995; Tweddell et al. 2002). Paradoxically, substantial neurologic morbidity became ascribed to the very support techniques used to enable these complex cardiac procedures (Wernovsky, Wypij et al. 1995; Petko et al. 2011). Consequently, there has been an intense pursuit of optimal neuroprotection during neonatal cardiac repair, with considerable success (Newburger et al. 1984, 2008; Bellinger, Bernstein et al. 2003; Bellinger, Wypij et al. 2003; Jonas et al. 2003; Wypij et al. 2008). However, more recent studies have demonstrated that neurologic abnormality may antedate these early neonatal surgeries, even in the absence of overt chromosomal/dysmorphic conditions or brain injury (Limperopoulos et al. 1999, 2000; Mahle et al. 2002; Licht et al. 2004, 2009; Miller et al. 2007). Conditions at greatest risk are likely to be those most liable to cause chronic cerebral hypoxemia and/or hypoperfusion during fetal development. To better understand the nature of impaired brain development in CHD, we performed the first ever study of in vivo brain growth and metabolism in the fetus with CHD using the quantitative magnetic resonance imaging (MRI) techniques (Limperopoulos et al. 2010). We showed that whole brain volumes were similar in the 2 groups until the late second trimester. However, there was a progressive third trimester decline in global brain growth in CHD fetuses versus controls. Similarly, third trimester metabolic profiles showed a progressive fall-off in N-acetylaspartate/choline (NAA/Cho) ratio among CHD fetuses. In addition, cerebral lactate was detected in a sub-population of CHD fetuses with the lowest cerebral volumes and NAA/Cho ratios, suggesting a potential role for anaerobic metabolism in this impaired brain development (Limperopoulos et al. 2010). A recent case report described abnormal white matter development in 3 fetuses with severe CHD using diffusion-weighted imaging, supporting the finding of abnormal in utero brain development in CHD fetuses (Berman et al. 2011).

The third trimester is a period of intense development of the cerebral cortex, a process that follows a rigid timetable in the different cortical regions (Chi et al. 1997; Garel et al. 2001). This process is highly energy-dependent and, therefore, potentially vulnerable to conditions that restrict cerebral oxygen/substrate supply, such as hypoplastic left heart syndrome (HLHS), in which both cerebral hypoxemia and/or hypoperfusion may occur. Innovative methods have been described that provide quantitative measures of cortical structure (Dubois, Benders, Cachia et al. 2008; Hill et al. 2010). Recently, our group and others have developed novel MRI techniques for studying cortical gyriation in the living fetus (Clouchoux, Kudelski et al. 2012; Habas et al. 2012).

The objective of this study was to compare cerebral cortical development and regional brain tissue growth in HLHS fetuses and controls. We hypothesized that in utero regional cortical maturation in HLHS would be delayed compared with controls. Furthermore, we predicted that gray and white matter volumes would be decreased in fetuses with HLHS versus controls.
Materials and Methods

Subjects
We prospectively recruited pregnant women at Boston Children’s Hospital with a confirmed diagnosis of fetal HLHS, as well as volunteers with normal fetuses. Our current study cohort represents a subgroup of a larger cohort previously reported (Limperopoulos et al. 2010). Briefly, controls included healthy pregnant volunteers, and pregnant women with suspected CHD or a family history of CHD with a normal fetal echocardiogram. Controls underwent a postnatal MRI, which showed no evidence of a brain abnormality in any fetus. Exclusion criteria included multiple gestation pregnancy, congenital infection, chromosomal abnormalities, fetal ultrasound findings of dysmorphic features, dysgenetic brain lesions, or anomalies of other organ systems (Limperopoulos et al. 2010). The study was approved by the Children’s Hospital Boston Committee on Clinical Investigation, and written consent was obtained from all participants.

MRI Acquisition
Each fetal MRI study was performed on a 1.5 T scanner (Achiva, Philips Medical System, The Netherlands) using a 5-channel phased-array coil. Multiplanar single-shot turbo spin-echo imaging were performed (echo time_eff = 120 ms, repetition time = 12 500 ms, 0.625 signal averages, 330 mm field of view, 2 mm slice thickness, no interslice gap, acquisition time 30–60 s, 256 × 204 acquisition matrix). For each subject, we performed multiple axial, coronal, and sagittal acquisitions providing multiple in-plane high-resolution images along the 3 axes. All MRI studies were reviewed by a pediatric neuroradiologist who was blinded to fetal status and clinical history.

MRI Analysis

$T_2$ Volume Correction and Segmentation
All fetal MR images were first corrected for nonuniformity intensities using a nonparametric nonuniform intensity normalization method (Sled et al. 1998). Isotropic high-resolution fetal brain images were then reconstructed using a previously published methodology (Gholipour et al. 2010; Clouchoux, Kudelski et al. 2012). Briefly, this reconstruction pipeline uses multiple images acquired for each fetal brain (at least once in each of the 3 principal planes, namely sagittal, coronal, and axial). This super-resolution technique relies on maximum likelihood and robust motion estimation error minimization. First, relative motion of each two-dimensional (2D) slice is estimated using the slice-to-volume registration method. Then a high-resolution 3D volume is reconstructed from motion-corrected slices using a model-based super-resolution reconstruction technique. Motion estimation and volume reconstruction are performed in an iterative manner. This procedure eliminates interslice motion artifacts and provides images with enhanced contrast and resolution, and coherent anatomic boundaries in 3D, which is necessary for delineating the cortical folding process. For each subject, a single high-resolution isotropic 1-mm volume was reconstructed from the original multiple low-resolution volumes (Fig. 1A). This single reconstructed volume was used to quantify regional brain tissue volumes and gyriﬁcation.

Once we completed this reconstruction step, we segmented the developing white matter (which comprised several lamination layers including unmyelinated white matter, subplate, intermediate layer, and myelinated white matter, depending on the gestational age [GA]; Kostovic and Judas 2002), cortical gray matter, subcortical gray matter, and lateral ventricles (Fig. 1B). We carried out a first delineation of the tissues using a previously validated atlas-based segmentation method (Guizard et al. 2008). This process relies on the registration (linear and nonlinear) of a segmented fetal atlas to match each individual brain. We then manually corrected each segmentation. The accuracy of this manual step has been previously demonstrated (Clouchoux, Kudelski et al. 2012). Secondly, the cerebrum, the lateral ventricles, and the subcortical gray matter were extracted using the methodology described in previous study (Clouchoux, Guizard et al. 2012), and segmentations were manually corrected. Cortical gray matter volume was computed by subtracting the combined white matter and subcortical gray matter volumes from the cerebrum volume.

Cortical Gray Matter Surface Reconstruction and Sulci Delineation
The reconstruction of the inner surface of the cortical gray matter was performed using CLASP (McDonald et al. 2000). The algorithm is based on a spherical mesh deformation method, fitting a triangulated mask composed with 10 240 vertices (20 480 triangles) to the previously delineated white matter volume. As a result, the surface mesh represents each fetal hemisphere (right and left), giving access to important geometrical information such as the sulcal depth, the curvature, and the sulcal fundus. All surfaces were coregistered together and resampled (McDonald et al. 2000) to reduce the interindividual anatomical variability induced by an incorrect vertex sampling (Clouchoux, Kudelski et al. 2012).

Throughout gestation, particularly during the third trimester, both shape and size of the fetal brain change dramatically (Chi et al. 1977; Garel et al. 2001; Batchelor et al. 2002; Hu et al. 2009; Clouchoux, Guizard et al. 2012; Clouchoux, Kudelski et al. 2012). To provide a generic representation of the changes occurring during the studied age window, we divided the cohort into 4 GA groups: 25–27 weeks GA, 27–30 weeks GA, 30–33 weeks GA, 33–37 weeks GA (Clouchoux, Kudelski et al. 2012). We then computed the volume of white matter, cortical gray matter, subcortical gray matter, and the lateral ventricles.
as well as the cortical surface area, cortical depth (Boucher et al. 2009), and the gyriﬁcation index (GI; Zilles et al. 1988; Luders et al. 2006) for each hemisphere. We calculated the volumes of white matter, cortical gray, subcortical gray matter, and ventricular volume by counting the number of voxels of each tissue type and multiplying by the volume of each voxel. The GI was deﬁned as the ratio between the areas of the cortical surface and the convex hull (Zilles et al. 1988; Luders et al. 2006). The cortical surface area was calculated by summing the area of each triangle of the mesh, for each reconstructed cortical surface. The term cortical depth must be distinguished from cortical thickness and is deﬁned using the depth potential function (DPF; Boucher et al. 2009). The DPF is an advanced cortical surface folding quantiﬁcation tool, based on a complex set of mathematical notions. Applied to the reconstructed fetal cortical surface, the DPF enables the quantiﬁcation of both inward (sulci) and outward (gyri) cortical surface curves. We also extracted the sulcal fundi, as it allowed us to precisely identify sulcation, that is, the bottom of each sulcus on the cortical surface. This step relied on a robust feature extraction algorithm, based on both mean and Gaussian curvatures, as described elsewhere (Clouchoux, Kedelski et al. 2012). As a result, the sulcal lines were delineated directly on the cortical gray matter surface, which enabled the quantiﬁcation of the sulcal pattern for each fetal brain (sulcation maps), providing crucial information on the cortical folding process and the sulcal organization throughout gestation.

Fetal Echocardiography
Fetuses with HLHS underwent echocardiography and Doppler studies. The speciﬁc parameters have been previously detailed (Limperepoulos et al. 2010). Briefly, aortic and pulmonary arterial ﬂows were calculated using pulsed-wave Doppler, as the product of valve area, velocity-time integral, and heart rate. Combined ventricular output was calculated as the sum of aortic and pulmonary ﬂow (indexed to fetal weight). The absence of antegrade blood ﬂow in the transverse aortic arch was also evaluated. Middle cerebral and umbilical arterial pulsatility and resistance indices were calculated including the cerebroplacental resistance (CPR) ratio using standard methods (Donofrio et al. 2003).

Statistical Analysis
Means and standard deviations were used to compare the HLHS and control cohorts at baseline, and t-tests were used to evaluate the statistical differences between the 2 groups. Multiple linear regression models were developed in Stata 11 (StataCorp 2009) to compare the changes between groups in gray matter, white matter, subcortical gray matter, and lateral ventricles volumes, as well as the GI and surface area during gestation. The t-statistic was used to evaluate differences between the 2 groups. Partial correlation analyses were carried out to examine the association between anatomical measurements (cerebral volumes, cortical surface area, GI) and the presence or absence of antegrade blood ﬂow in the transverse aortic arch, percentage of combined ventricular output through the aortic valve, and cerebral ﬂow indices. These correlations were all adjusted for gestation age at MRI. Differences between controls and HLHS fetuses for cortical depth and sulcation maps were tested using the linear mixed-effect model, and results were corrected for multiple comparisons using nonnegative least-squares random ﬁeld theory (Worsley and Taylor 2008). This was done using the SurfStat toolbox in Matlab (The Mathworks Inc. 2010). Differences between the 2 groups were determined within each GA group (25–27 weeks GA, 27–30 weeks GA, 30–33 weeks GA, 33–37 weeks GA). Results were considered statistically signiﬁcant at P < 0.05.

Results
Characteristics of the Cohort
We studied 48 fetuses (18 cases with HLHS, 30 controls) of which 28 were male (11 HLHS, 17 controls). The GA of our cohort ranged from 25.4 to 37.0 weeks (mean GA for controls: 30.5 ± 3.1 weeks versus cases: 30.8 ± 3.8, P = 0.3). The median GA at MRI was 30.14 (range: 25.4–37.0). The average number of MRI acquisitions for each subject was 7.6 ± 1.7 (range: 4–14). The mean number of scans was 7.6 for controls, and 7.5 for HLHS fetuses. There was no signiﬁcant statistical difference between the 2 groups (P = 0.49). All control fetuses had a normal conventional MRI study. The only structural abnormalities seen by conventional MRI in fetuses with HLHS was mild ventriculomegaly in 5. The median duration between MRI and echocardiography for HLHS fetuses was 0 (range: 0–16 days). Among the 18 HLHS fetuses, 9 (50%) showed no aortic output and absent antegrade blood ﬂow in the transverse aortic arch. The percentage of combined ventricular output via aorta was 0 for 8 cases (44.4%) and ranged from 0% to 29%. The median cerebroplacental pulsatility index ratio was 1.26 (range: 1.02–1.94), median CPR index ratio was 1.04 (range: 0.84–1.37), and 8 (44.4%) had a CPR ratio below 1.

Comparison of Cerebral Volumes in Fetuses with HLHS versus Controls
We first compared white matter, cortical gray matter, subcortical gray matter, and ventricular volumes in control fetuses and those with HLHS (Fig. 2). Volumes varied linearly over gestation but with different slopes in the 2 groups. Differences in volume over gestation in both white and gray matter volumes were found to be signiﬁcantly smaller in the HLHS group (P < 0.001), with difference becoming progressively greater after 30 weeks of gestation. Interestingly, the tissue-speciﬁc differences between the 2 groups were not statistically signiﬁcant before 30 weeks GA for either white or cortical gray volumes (P = 0.2 and 0.3, respectively). The difference in subcortical gray matter volume between the 2 groups was less pronounced, although statistically signiﬁcant (P = 0.02). Lateral ventricular volume was not different between the 2 groups (P = 0.6).

Comparison of the Cortical Gray Matter GI and Surface Area in Fetuses with HLHS versus Controls
Overall, the GI and surface area of the cortical gray matter changed in a curvilinear fashion with increasing GA in both groups. We compared the folding state between the 2 populations, and the GI was found to be signiﬁcantly lower in the HLHS group compared with controls (P < 0.001; Fig. 3A). Likewise, the cortical surface area (Fig. 3B) was signiﬁcantly smaller in fetuses with HLHS (P < 0.001). For both measures, the difference between the 2 groups became progressively greater with increasing GA.

Relationship Between Blood Flow and Brain Development in Fetuses with HLHS
Among fetuses with HLHS, low CPR (<1.0) was signiﬁcantly associated with decreased cortical gray matter (P = 0.04), white matter (P = 0.03), subcortical gray matter (P = 0.004), and decreased cortical surface area for both the left (P = 0.03) and right (P = 0.02) hemispheres, after controlling for GA. Similarly, the absence of antegrade blood ﬂow in the transverse aortic arch was associated with decreased white matter (P = 0.02), subcortical gray matter (P = 0.02) volumes and a lower surface area for the right hemisphere (P = 0.04).
There was a borderline relationship between the left cortical surface area and the absence of antegrade blood flow ($P = 0.06$).

**Local Sulcation Quantification and Timetable in Fetuses with HLHS versus Controls**

Figure 4 depicts the timetable of the appearance of sulcal lines (see Materials and Methods) in the 2 groups. Three major differences were evident in the timing of sulcation between the 2 groups: 1) The cingulate sulcus was visible in controls from 25 weeks GA, but not until 28 weeks GA in HLHS fetuses; 2) the superior frontal sulcus was visible from 27 weeks in control fetuses, but not until 30 weeks GA in HLHS fetuses; and 3) the anterior ascendant ramus was visible from 32 to 33 weeks GA in controls, but only at 36 weeks GA in HLHS fetuses.

The differences in folding described above were confirmed by sulcation map analysis, and the precise locations of the sulcation delays in the HLHS fetuses are shown in Figure 5. Between 25 and 37 weeks GA, the overall statistically significant differences identified between fetuses with HLHS and controls were in the superior frontal, postcentral, occipital, cingulate (including the isthmus of the cingulate gyri), calcarine, collateral, superior temporal, and sylvian regions. More precisely, the intersection of the postcentral sulcus and the intraparietal sulcus appeared significantly later in the fetus with HLHS (between 27 and 30 weeks GA) compared with the control (between 25 and 27 weeks GA). From the perspective of connectivity between these regions, it is interesting to note...
that the delayed sulcation in the anterior ascendant ramus (between 30 and 33 weeks GA) was associated with a similar delayed sulcation in the posterior region of the sylvian fissure.

### Local Differences in Cortical Depth Between Fetuses with HLHS and Controls

We also examined differences in cortical depth between controls and HLHS fetuses (Fig. 6). Over the entire GA range (i.e. 25–36 weeks), the morphological differences between the 2 groups occurred in the frontal (superior and inferior), temporal, cingulate, postcentral, calcarine, occipital, and parieto-occipital regions.

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### Discussion

We report the application of quantitative MRI techniques to study global and local developmental delays of the fetal cerebral cortex in vivo, in fetuses with complex CHD. Specifically, we show a region-specific delay in third trimester cortical development in a population known to be at risk for

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Figure 4. Sulcation timetable in control and HLHS fetuses. Color-coded bars represent the percentage of subject showing each sulcus, for both control and HLHS fetuses. Asterisks identify sulci where the difference between HLHS and control fetuses was significant ($P < 0.05$).

Between 25 and 27 weeks GA, the posterior region of the superior temporal sulcus, the superior frontal sulcus, and the isthmus of the cingulate region were deeper in controls ($P < 0.05$). Over the same GA period, the medial prefrontal cortex and the anterior temporal lobe were significantly more developed in the control group ($P < 0.05$). Between 27 and 30 weeks GA, the cortical gray matter in a number of cortical areas was significantly more folded in control fetuses ($P < 0.05$). These differences were most pronounced in the superior frontal and cingulate regions, where sulci were significantly deeper and gyri were more developed in control fetuses ($P < 0.05$). Elsewhere, sulci were deeper in certain regions (e.g. the anterior collateral, inferior postcentral, and marginal sulci), and gyri were more developed in others (e.g. medial prefrontal cortex, fusiform gyrus, and the subparietal lobule) in control fetuses compared with HLHS fetuses. Between 30 and 33 weeks GA, the cortical development was characterized by deeper sulcation in the anterior temporal area, and more developed posterior inferior temporal, and superior temporal (rostral part) regions in controls versus HLHS fetuses. After 33 weeks GA, differences in cortical development between the 2 populations were evident in the frontal (superior and inferior), postcentral, cingulate, temporal, and occipital regions (lateral and medial surfaces). Controls fetuses showed greater development in the anterior midtemporal region, the postcentral gyrus (above the intraparietal and postcentral ramification), and the medial surface of the anterior prefrontal cortex ($P < 0.05$).

Gyrification Asymmetries in Fetuses with HLHS versus Controls

Asymmetries in cortical depth are illustrated in Figure 7. Striking was the fact that the major asymmetries present in control fetuses were not observed in the HLHS fetuses. Specifically, in the control fetus, the most prominent sulcal asymmetry occurred around the planum temporale and the superior temporal region. While the superior temporal sulcus and the supramarginal sulcus were deeper in the right hemisphere, the supramarginal gyrus (Wernicke’s area) was more folded in the left hemisphere. In the fetus with HLHS, a slight asymmetry existed in the operculum area, particularly in the posterior region of the sylvian fissure, with the left hemisphere being deeper. Although this asymmetry was quite marked at early stages, it decreased with increasing GA in the fetus with HLHS. Conversely, the asymmetry in the operculum area was evident in control fetuses throughout the study period. Similar patterns of asymmetries were delineated in both control and HLHS fetuses late in gestation including the inferior frontal areas and the occipital region. Of note, the calcarine sulcus was found to be deeper in the right hemisphere in controls, whereas the same fold was deeper in the left hemisphere in the HLHS fetuses, particularly after 30 weeks of gestation.
cerebral hypoxemia and/or hypoperfusion, that is, fetuses with HLHS. Local cortical regions with the most striking delay in development in our HLHS cohort were in the frontal, temporal, cingulate, postcentral, calcarine, occipital, collateral, sylvian, and parieto-occipital areas. Relative to controls, we also show a progressive decline in fetuses with HLHS over the third trimester in volumetric growth of the cortical gray, subcortical gray, and white matter. However, there was no significant difference in lateral ventricular volumes between HLHS and control fetuses, suggesting that the process was subcortical rather than periventricular in origin. These findings extend our previous observations describing impairment in global volumetric brain growth in fetuses with complex CHD (Limperopoulos et al. 2010). Of note, these delays in cortical maturation and regional tissue growth were not associated with obvious encephaloclastic or dysgenetic abnormalities on conventional MRI.

In an earlier autopsy study in infants with HLHS, Glauser et al. (1990) described malformations of the cortical mantle, including underdeveloped secondary folds and convolutional malformations of the temporal lobe. Importantly, local delayed cortical gyriﬁcation appears to precede impairments in regional volumetric growth of the gray and white matter suggesting that delayed gyriﬁcation may be an early marker of enhanced risk for brain growth failure in the third trimester.

Figure 5. Differences in sulcation between control and HLHS fetuses for each gestation age range. Bottom row shows the differences in sulcation between the 2 groups between 25 and 37 weeks of gestation. Highlighted regions show the areas where sulci were delineated in control but not in HLHS fetuses (P < 0.05).
Previous studies have described delayed brain maturation in newborn infants with CHD prior to open-heart surgery (Limperopoulos et al. 1999, 2000; Manzar et al. 2005; Shillingford et al. 2007; Barbu et al. 2009; Licht et al. 2009). Microcephaly has been reported in up to one-third of newborns with CHD (Limperopoulos et al. 2000) and is particularly prevalent in infants with HLHS (Manzar et al. 2005; Shillingford et al. 2007). Recent ultrasound studies have demonstrated that fetuses with HLHS develop a disproportionate restriction of head circumference growth in the latter half of gestation (Hinton et al. 2008). We reported impaired third trimester volumetric growth of the brain parenchyma by MRI in the fetus with CHD (Limperopoulos et al. 2010); this finding was particularly striking in the fetus with HLHS.

The major event in third trimester brain development is accelerated growth and organization of the cerebral cortex, characterized by dendritic outgrowth, synapse formation, and cortical connectivity (Kostovic 1990; Kostovic and Jovanovic-Milosevic 2006). These cortical maturation processes are driven by neuronal activation, which is in turn dependent on energy-demanding enzymes such as Na-K/ATP-ase (Milh et al. 2007). Therefore, it is not surprising that in fetal sheep, cerebral oxygen consumption, and blood flow increase 3-fold over the third trimester of gestation (Gleason et al. 1989;
This surge in third trimester cortical energy utilization corresponds to the gestational period in which our CHD fetuses show a progressive fall-off in the rate of global brain growth (Limperopoulos et al. 2010) as well as the global and regional delays in cortical development.

In this study, we describe both global and regional differences in cortical development between HLHS and control fetuses. Between 25 and 37 weeks of gestation, the major differences in gyri
cification between these groups were detected in a number of regions, including frontal, temporal, cingulate, postcentral, calcine, occipital, collateral, sylvian, and parieto-occipital. In these regions, the folding was less complex in HLHS fetuses, with deeper sulci and more developed gyri in the control fetuses. We also describe focal gyrification delays in the operculum, the supramarginal gyrus (Wernicke’s area), the anterior ascending ramus (which plays an important role in the formation of the Broca’s area), the cingulate region (limbic system), and the anterior temporal lobe. These observations provide the first in vivo evidence of delayed opercular development in the HLHS fetus and corroborate previous neonatal studies describing incomplete opercular closure in infants with HLHS (Glauser et al. 1990; Mahle et al. 2002; Licht et al. 2004; Awate et al. 2009). Given the relatively small size of our cohort (18 HLHS and 30 controls), ongoing studies using a larger sample size are needed to validate these observations.

In our control fetuses, we describe asymmetries in cortical development that corroborate previous observations in post-mortem fetuses (Chi et al. 1977), premature babies (Dubois, Benders, Cachia et al. 2008) and in vivo fetuses (Habas et al. 2012). We also report differences in the cortical asymmetries between control and HLHS fetuses. The opercular asymmetry seen in control fetuses decreases with increasing GA in the HLHS fetus. In addition, the asymmetry observed in the calcine region of the control fetus is reversed in the HLHS fetus. Both the mechanisms and functional significance of these findings remain unclear.

The underlying biological mechanisms of brain growth delays in fetuses affected by a complex CHD are largely unknown (Glauser et al. 1990; Miller et al. 2007; Hinton et al. 2008; Watanabe et al. 2009; Limperopoulos et al. 2010). In this study, we postulated that major circulatory disturbances induced by HLHS would have a negative impact on brain development. Normally, the left heart supplies blood to the myocardium and to the brain (Edelstone and Rudolph 1979). In the case of HLHS, blood flow is affected because of reduced outflow from the left heart. Therefore, both glucose and oxygen supply to the developing brain are potentially disrupted (Mahle et al. 2002; Limperopoulos et al. 2010). A previous study in fetuses with complex CHD showed that abnormalities in blood flow were correlated with small head circumferences, and that fetuses with single ventricular physiology were most affected (Donofrio et al. 2003). A low CPR (<1) was most prevalent among fetuses with HLHS. We previously demonstrated that fetuses with HLHS that had absent antegrade blood flow in the transverse aortic arch had significantly lower total brain volume and metabolism. In the
present study, we show that decreased regional brain white matter and subcortical gray matter volumes, and low cortical surface area correlate with absent antegrade blood flow in the transverse aortic arch.

There are well-described fetal compensatory mechanisms that are in place to preserve cerebral metabolism during the periods of fetal hypoxemia. The so-called “brain-sparing” effect of circulatory centralization (Donofrio et al. 2003) depends upon a preferential decrease in cerebrovascular resistance, measured clinically as the CPR. However, these compensatory responses are at best temporizing, and during severe or sustained hypoxemia, the “brain-sparing” effect is inadequate. In our study, lower CPR was associated with low regional cerebral volumes and reduced cortical surface areas in fetuses with HLHS, compared with controls.

The long-term functional implications of these early prenatal findings remain unclear. A range of neurodevelopmental deficits have been described in CHD populations, including increased attention and self-awareness (Posner and DiGirolamo 1998; Mesulam 2000; Stuss and Knight 2002; Forn et al. 2011), motor impairments (Limperopoulos et al. 2001; Karl et al. 2004; Majnemer et al. 2009; Watanabe et al. 2009; van der Rijksen et al. 2011), speech, and language delays, and feeding problems (Chen et al. 1995; Mahle et al. 2000; Hövels-Güürich et al. 2002, 2006; Bellinger, Bernstein et al. 2003; Bellinger, Wypij et al. 2003; Crewe et al. 2005; Licht et al. 2009; Majnemer et al. 2009). From a structure–function perspective, very few studies have correlated morphometric changes with later neurodevelopmental outcomes in CHD infants (Soul et al. 2009; Bellinger et al. 2011; Ibuki et al. 2012). Subtle hemorrhagic brain injury has been associated with lower psychomotor development in infants with CHD (Soul et al. 2009). Long-term neuropsychological impairment has also been associated with conventional brain MRI abnormalities, in adolescents with corrected d-transposition of the great arteries (Bellinger et al. 2011). More recently, Ibuki et al. (2012) demonstrated a relationship between mental development and frontal lobe volume, as well as psychomotor development and total and regional brain volume in infants with transposition of great arteries or HLHS. We postulate that regional delays in gray and white matter volumes, as well as gyriﬁcation described herein may contribute to the high prevalence of neurodevelopmental disturbances children with CHD. Long-term outcome studies are currently underway to determine the functional significance of delayed in utero cerebral cortical development described in our study.

Very few studies have reported cortical folding differences between healthy and high-risk fetuses. Gyriﬁcation of the developing brain has been studied in ex utero premature infants with and without intrauterine growth restriction (Dubois, Benders, Borradori-Tolsa et al. 2008). The authors showed reduced gyriﬁcation in premature infants with intrauterine growth restriction, compared with control premature newborns. Cortical complexity has also been described in neonates with CHD, prior to open-heart surgery (Awate et al. 2009). The authors demonstrated regional differences in gyriﬁcation between controls and neonates with CHD and described the operculum as being more “opened” in the CHD cohort. More recently, we reported differences in global gyriﬁcation between in utero fetuses and premature infants at equivalent postconceptional ages, suggesting an adverse impact of early extraterine exposure on the brain development (Clouchoux, Kudelski et al. 2012). The functional impact of these observations awaits further study.

This study has a number of strengths, but also several limitations. From a computational perspective, the manual correction of brain tissue segmentation is both tedious and time-consuming, and potentially subjective. We have addressed this concern in a previous report, showing that our manual brain tissue segmentations have high intrarater reliability (Clouchoux, Guizard et al. 2012; Clouchoux, Kudelski et al. 2012). Ongoing work is aiming at improving automatic segmentation algorithms, using a patch-based approach (Coupé et al. 2011). From a validation perspective, the size of the identified cortical regions can potentially be small (<2 mm), and therefore, may be an issue when compared with the original volumes that were acquired at 2 mm thickness. However, we used reconstructed high-resolution isotropic 1-mm voxel size brain volumes, which will reduce problems related to partial volume. Remaining errors in surface and features extraction are dependent on the surface extraction technique itself. Consequently, this should not represent a group bias, just increased noise. Therefore, anything signiﬁcant is likely to be underestimated rather than being detected as a false positive. Moreover, in the current study, we used a previously validated surface and features extraction method (McDonald et al. 2000; Clouchoux, Kudelski et al. 2012) to quantify fetal cortical development (Clouchoux, Kudelski et al. 2012). Nevertheless, ongoing work by our group aims at improving and reﬁning the surface extraction technique, by using a subvoxel approach, overcoming a large voxel resolution bias. Another limitation is the cross-sectional nature of the study, which precludes a reliable assessment of longitudinal brain development in individual cases. Finally, although we excluded HLHS fetuses with genetic or cerebral dysgenetic conditions, undiagnosed or unknown genetic syndromes may have been missed.

In summary, the mechanisms underlying neurologic impairment in survivors of HLHS are undoubtedly complex, multifactorial, and incompletely understood. We report for the ﬁrst time globally and locally delayed in vivo cortical development in HLHS fetuses. Our data suggest that local folding delays precede third trimester regional impairment in gray and white matter volumetric growth. The insights provided by these cortical surface-based analyses may provide early and sensitive markers of brain growth failure in this and other high-risk populations. Although the correlation between these differences in structural brain development and long-term functional outcome warrants further study, these data offer early evidence of in utero brain growth disturbances likely to portend signiﬁcant abnormalities in postnatal brain structure and child function. Quantitative evaluation of gyral development in HLHS fetuses has the potential to identify the onset of impaired brain development, and thereby to guide prenatal and postnatal management, counseling of families, the initiation of timely early intervention services, and the development of future neuroprotective therapies.

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