Title
Fetal Brain Measurements May Predict Neonatal Brain Injury in Patients with Critical Congenital Heart Disease

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Fetal Brain Measurements May Predict Neonatal Brain Injury in Patients with Critical Congenital Heart Disease

by

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THESIS

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Dedication

I would like to dedicate this thesis to the memory of my cousin, Christina Jenny Li (1987-2010). She was born with congenital heart disease and touched everyone around her through her journalism, creative photography, and quirky laugh. I miss her dearly and she is a part of many things that I do in my life. I also want to thank my mother, brother, and relatives for their unconditional love and support. Because of you, I feel I can do and achieve anything in life.

I am grateful for the incredible collaborative opportunity here at UCSF—combining my interests of pediatric cardiology, neurology, and radiology into a research project. I want to first thank my advisor, Dr. Shabnam Peyvandi, for agreeing to meet me over coffee and allocating much of her time to support this research over the last year. Her upbeat attitude and encouragement on my life goals have made it an absolute pleasure to work on this project.

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I thank the families that participated in the study and the UCSF staff for their assistance in the management of the clinical data—all of whom have ultimately made this project possible.

Fetal Brain Measurements May Predict Neonatal Brain Injury in Patients with Critical Congenital Heart Disease

JOANNE LAU

Abstract

Purpose: This research study sought to explore whether fetal total brain volume (TBV) determined by using conventional fetal MR imaging is associated with postnatal preoperative brain injury in neonates with critical congenital heart disease (CHD). A secondary aim was to investigate the trajectory of brain growth from the fetal to neonatal time period in this same patient population.

Methods: Twenty subjects had complex CHD diagnosed in utero and required postnatal neonatal surgery at University of California, San Francisco (UCSF) from 2010-2016. Fetal and neonatal MRI scans from 12 of the 20 eligible subjects were processed and analyzed in this study. These scans and clinical data are a part of an ongoing study to investigate the effects of CHD on brain injury and neurodevelopmental outcome at UCSF.

Results: This study revealed a strong linear relationship between fetal TBV and gestational age (p=0.0012). Subjects with preoperative brain injury tended to have a smaller mean fetal TBV (187.8 cm^3, 95%CI: 156.9-218.7) as compared to those without preoperative brain injury (225.2 cm^3, 95%CI: 189.8-260.6; p=0.165). There was also a trend towards decreasing TBV with a higher brain injury severity (BIS) score (BIS 0, TBV 225.1 ± 38.3 cm^3; BIS 1, TBV 210.9 cm^3; BIS 2, TBV 199.2 cm^3; BIS 3, TBV 176.4 ± 26.6 cm^3; p=0.06). The rate of increase in TBV from the fetal to neonatal time period was similar in those with and without preoperative brain injury (p=0.33).

Conclusions: These preliminary findings suggest a potential relationship between in-utero brain growth and the risk of neonatal preoperative brain injury in patients with critical CHD. A larger sample size will be analyzed to further assess this relationship.

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1 Background

Congenital heart disease (CHD) is the leading cause of birth defect-related infant illness and death [1]. CHD affects approximately 40,000 births per year in the United States [2], where 25% have critical CHD [3]. Survival of infants with CHD depends on the severity, timing of diagnosis, and treatment. During the last two decades, prenatal detection of CHD has increased. Although prenatal diagnosis of CHD has not resulted in improved survival, it has been shown to improve perioperative clinical outcomes [3,4], and potentially lead to improved neonatal brain growth [5] and neurodevelopmental outcomes [6].

1.1 Neuroimaging in Neonates with CHD

Advances in neuroimaging technology have enabled researchers to understand brain development and injury in patients diagnosed with CHD. Magnetic resonance imaging (MRI) can identify qualitative, quantitative, and metabolic abnormalities in this cohort. Longitudinal studies performing pre- and post-operative brain MRIs in neonates with critical CHD have identified brain injury and impaired brain growth in the preoperative time period [7-13]. In fact, approximately 30% of neonates with hypoplastic left heart syndrome (HLHS) and transposition of the great arteries (TGA) have evidence of brain injury even before going to the operating room, suggesting patient-specific and/or physiologic mechanisms that begin in-utero as a cause for brain injury [14]. Interestingly, the patterns of brain injury seen in full-term neonates with critical CHD resemble that of premature infants, namely white matter injury or periventricular leukomalacia [11]. In addition, stroke is seen in this cohort of patients.

Brain growth or development is abnormal in neonates with CHD. Studies have demonstrated abnormal metabolic (lower N-acetyl-aspartate(NAA)-to-choline ratio) and microstructural brain development (higher apparent diffusion coefficient and lower fractional anisotropy) as compared

to a normative cohort [11]. Furthermore, global reductions in brain volumes were observed in neonates prior to surgery compared to controls [15]. These findings reinforce growing evidence of pre-surgical brain abnormalities, suggesting that the origin lies in fetal development.

1.2 Neuroimaging in Fetuses with CHD

Recent studies have demonstrated delayed brain development in fetuses with CHD [16,17]. Brain abnormalities were present in 23% of CHD fetuses compared with 1.5% in controls [18]. In parallel to neonatal findings, CHD fetuses exhibit delays in cortical folding along with a decrease in normal cortical and subcortical gray matter development [17]. Lower NAA-to-choline ratios were also evident in third-trimester fetuses with CHD compared to controls. Using manual segmentation, MRI analysis helped show a progressive decline in age-adjusted total brain volume (TBV) and intracranial cavity volume (ICV) in fetuses with CHD in comparison with control [17].

Brain development in the third trimester is notable for an increase in neuronal connections and activity leading to an increase in metabolism. Consequently, blood flow to the fetal brain increases and is estimated to be a quarter of the combined ventricular output [19]. Depending on the sub-type of CHD, decreased oxygen delivery as a result of poor fetal brain perfusion and/or poor oxygen content may be compromised, suggesting a potential etiology for delayed brain development (Figure 1) [20-23]. In the normal fetus, cerebral blood flow is supplied by highly oxygenated blood from the ductus venosus preferentially streaming across the foramen ovale to the left atrium and ventricle. In TGA, the aorta and pulmonary artery are transposed and thus the higher oxygenated blood reaches the pulmonary vasculature as opposed to the aorta, potentially leading to lower pulmonary vascular resistance and an increase in pulmonary blood flow proportional to systemic blood flow. In HLHS, inadequate left heart structures lead to the reversal

of blood flow in the foramen ovale with mixing of oxygenated and deoxygenated blood in the right ventricle and in cases of aortic atresia, retrograde flow in the ascending aorta.

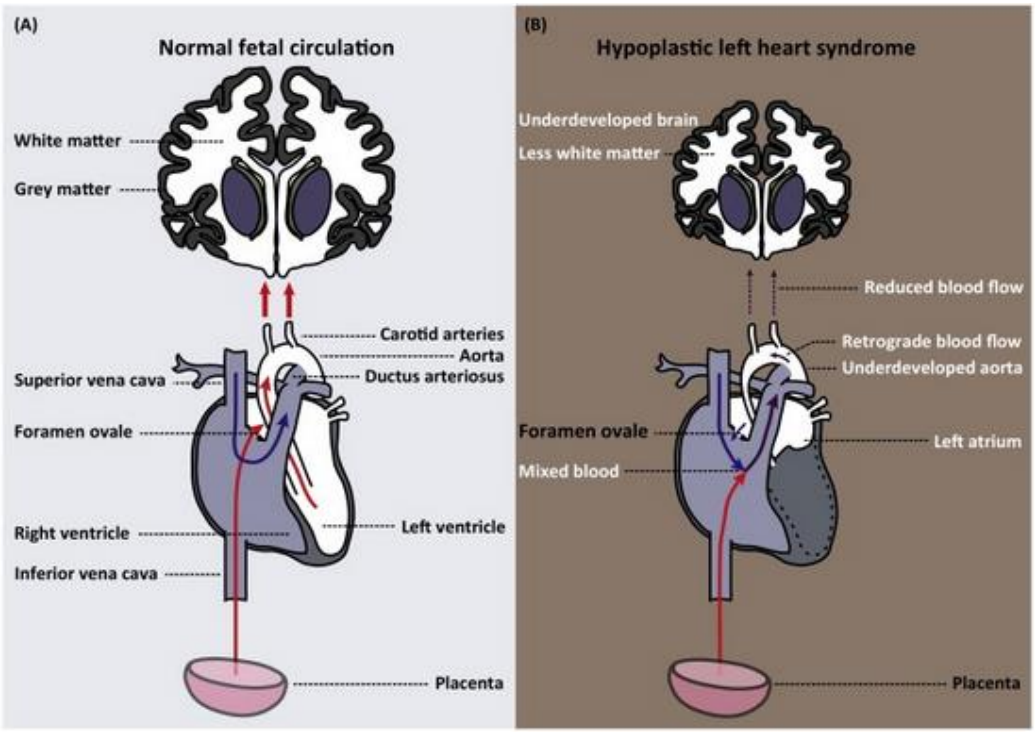

Figure 1: Diagram of Heart and Brain for Normal vs. HLHS Fetus

Adapted from Morton et. al, 2015 with permission [20].

Given these physiologic abnormalities beginning in utero as well as the evidence for cerebral abnormalities originating in the fetus, few studies have compared fetal brain MR imaging with neonatal imaging findings in this patient population. To our knowledge, no studies have quantitatively investigated whether the impairment in fetal brain growth related to CHD is associated with postnatal preoperative brain injury. Our primary aim was to assess whether total brain volume (TBV) in the third-trimester fetus was predictive of postnatal preoperative brain injury in subjects with critical CHD. Our secondary aim was to assess for difference in the trajectory of brain growth from the third-trimester fetus to the neonate in those with preoperative brain injury compared to those without injury.

2 Hypothesis

We hypothesize that neonates with preoperative brain injury have smaller total fetal brain volumes in the third trimester as compared to those that do not have brain injury. In addition, we hypothesize that neonates with preoperative brain injury have a slower rate of brain growth (TBV) from the fetal to neonatal time period as compared to those without injury.

3 Methods

3.1 Patient Selection

This retrospective research study involved 20 patients with critical CHD that were recruited and consented at the University of California, San Francisco (UCSF) Benioff Children's Hospital from 2010-2016. This dataset is a part of an ongoing study performing fetal and neonatal pre-operative MRIs and neonatal post-operative brain MRIs. The Institutional Review Board at UCSF approved this study.

Inclusion criteria consisted of subjects with complex CHD, which included HLHS, TGA, and other critical cardiac lesions. Exclusion criteria included any genetic syndrome or abnormality, an extracardiac anomaly, or prematurity. Of the 20 patients, 8 were excluded in the final data analysis due to the following reasons: 2 with inability to reconstruct MR image; 4 with missing preoperative brain injury scores; and 2 outliers in gestational age window at time of prenatal MRI scan. Of the 12 eligible patients, 8 were diagnosed with single ventricle physiology (SVP) which includes HLHS; 3 were diagnosed with TGA; and 1 was diagnosed with double outlet right ventricle (DORV) with pulmonary atresia.

3.2. Image Acquisition

Fetal MR imaging was performed on a 3T Discovery 750 system using a 32-channel cardiac array (GE Healthcare; Waukesha, WI, USA). The acquisition protocol included T2-weighted

single shot fast spin echo acquired in axial, sagittal, and coronal planes using the following sequence parameters: repetition time: 2500 ms; echo time: 62.0 ms; FOV 256x256 mm; 256 x 256 acquisition matrix; 3 or 4 mm-thickness two-dimensional slices; and in-plane resolution of 1x1 mm^2. Nine to twelve images per axial section were acquired. No contrast or sedation was used for any of the fetal MRIs.

Neonatal MR imaging was performed on a 1.5 Tesla system (GE Healthcare Signa Echo-speed; Waukesha, WI, USA) using GE EXCITE 1.5T software. The acquisition protocol included: 4-mm-thickness T1-weighted sagittal and axial spin echo, 4-mm-thickness dual-echo T2-weighted spin echo, 1.5-mm-thickness coronal or sagittal volumetric three-dimensional gradient echo with radiofrequency spoiling images which could then be reformatted into orthogonal planes. Diffusion tensor sequence (repetition time: 7000 ms; echo time: 99.5 ms; 3-mm section thickness; no gap; 18x36 cm FOV; 128x256 acquisition matrix) was also acquired in the axial plane through the whole brain with an in-plane resolution of 1.4x1.4mm^2, including a T2-weighted reference image (b=0 s/mm^2) and 6-15 diffusion-weighted images (b=700 s/mm^2) in non-collinear gradient directions. Pharmacologic sedation was used as needed for the neonatal MRIs.

3.3 Brain Reconstruction

Fetal brain images were reconstructed based on multiple stacks of the T2-weighted, 2D image slices that were acquired in the axial, sagittal, and coronal planes. A post-processing pipeline [24] performed slice-to-volume registration in order to correct for relatively large motion and to reconstruct volumetric MRI from 2D slices; ultimately creating a super-resolution 3D image volume (Figure 2). Since processing this pipeline was computationally intensive—which potentially impeded its potential clinical use, we accelerated the algorithm based on a multi-GPU/CPU parallel processing technique [25].

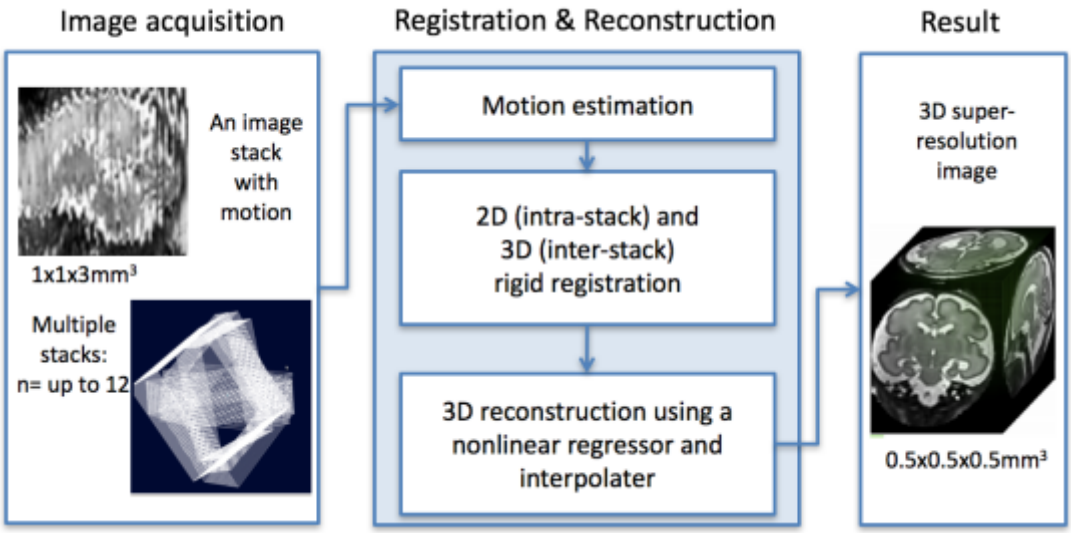

Figure 2: Flow Chart of 3D Brain Reconstruction Processes

3.4 Brain Volume Measurements

After alignment, segmentation of the 3D reconstructed fetal brain was made. The masks for fetal total brain volume (TBV) were initiated automatically using FSL-BET (fMRIB Software Library, Oxford, UK) and subsequently manually refined using ItkSnap software (ITK-SNAP open source software, www.itksnap.org). The ventricle masks were then created on top of the TBV masks using Display software (DISPLAY, Montreal Neurological Institute, Canada). An example of fetal brain segmentation is shown in Figure 3. Volumetric data for the total brain and ventricles were extracted using Display software. Total cranial volume was calculated by adding the total brain and ventricle volumes.

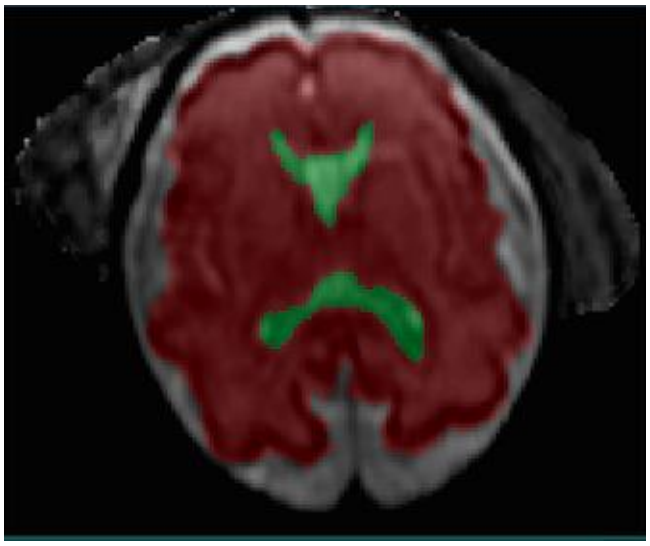

Figure 3: Example of Brain Segmentation Slice (TBV = red and ventricles = green)

The masks for neonatal TBV were segmented using a patch-based brain extraction algorithm (BEaST) [26]. This method is based on non-local segmentation embedded in a multi-resolution framework. A library for the training was constructed by manually segmenting 30 brains that were chosen from the UCSF database of preterm newborn and cardiac cohorts. The neonatal TBV were then visually assessed for accuracy during the automation of TBV masks. None of the 12 subjects required manual intervention in the neonatal MRIs during inspection.

The study member was blinded to the gestational age and clinical data when determining the fetal and neonatal brain volumes. Several masks for the fetal brain volume were also reviewed by an experienced post-doctoral fellow for quality assurance. To address intra-rater variability during the manual refinement process of the fetal total brain masks, 3 randomly selected subjects were repeated in order to observe whether fetal volumetric data could be reproduced. Neonatal brain masks were not repeated since the process was primarily automated due to improved image quality and reduced motion artifact.

3.5 Clinical Data Collection

Neonatal brain MR images were reviewed by a UCSF pediatric neuroradiologist to determine preoperative brain injury and the brain injury severity (BIS) score. Preoperative brain injury was characterized by stroke, white matter injury (WMI), intraventricular hemorrhage (IVH), or global hypoxic-ischemic injury. The BIS score was categorized as follows: 0, normal (no injury); 1, minimal injury (minimal WMI and IVH grade I or II); 2, stroke (all stroke); and 3, moderate to severe injury (moderate and severe WMI, IVH grade III, or global hypoxic-ischemic injury) [27]. Presence of preoperative brain injury and BIS score was retrospectively collected from the UCSF database and REDCap (Research Electronic Data Capture) for this study. Maternal and neonatal demographic information as well as gestational age (GA) at the time of MRI (both fetal and neonatal) were also retrieved.

3.6 Statistical Analysis

A logistic regression was used to examine differences in total fetal brain volume between neonates that have preoperative neonatal brain injury (Group 1) versus non-injury (Group 2). Analysis was conducted on both GA-adjusted and unadjusted data given the small sample size. A non-parametric test for trends was conducted to assess the association between total brain volume and BIS score. A one-sided t-test for continuous parametric variables was also used to compare differences in fetal TBV between neonates that were severely injured (BIS score of 3) versus non-injured (BIS score of 0). To assess changes in TBV over time, each subject contributed 2 outcomes to the analysis from two scans—fetal and neonatal TBV. A generalized estimated equation with a robust variance estimator adjusting for GA at time of MRI was used to account for within-patient correlation when examining brain growth between Group 1 and 2.

The Dice similarity index was used as a statistical validation metric to determine the repeatability of the semi-automated segmentations of fetal TBV by analyzing the spatial overlap accuracy of the two masks. Pearson's correlation coefficient was also used to examine the repeatability of the fetal TBV measurement. All analyses were conducted using Stata Software (IBM, Armonk, New York) with one figure generated by Matlab (Mathworks, MA, USA).

4 Results

4.1 Fetal TBV Changes with Gestational Age

There were a total of 12 subjects with fetal and neonatal MRI images available for analysis. There was a strong linear relationship between fetal TBV and gestational age as shown on Figure 4 (p=0.0012).

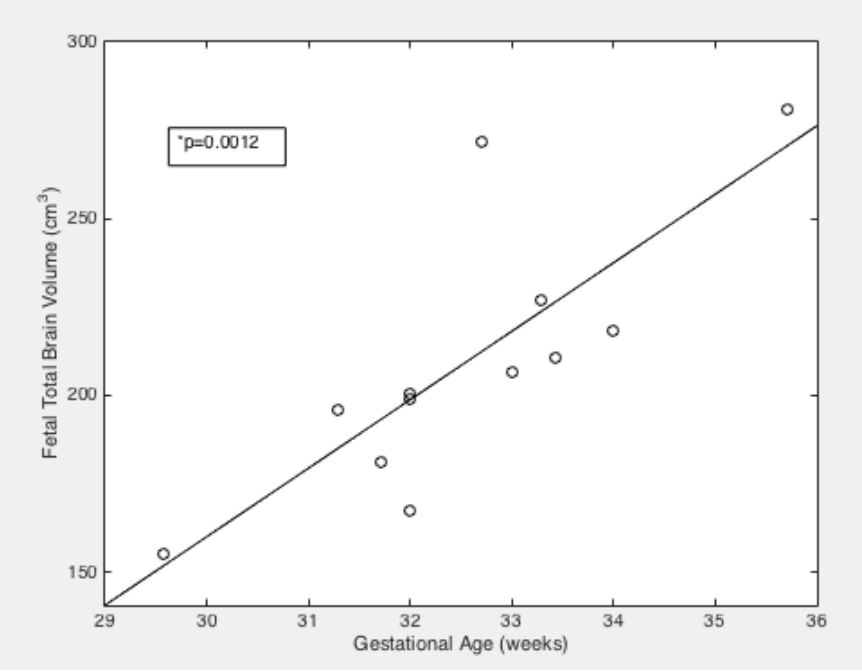

Figure 4: Fetal TBV Corresponds with Gestational Age

4.2 Fetal TBV and Presence of Brain Injury

The median gestational age at the time of fetal MRI for all subjects was 32.3 weeks (IQR 31.3-33.4). Of the 12 subjects, 5 displayed preoperative brain injury and 7 did not. No significant differences were detected in the median gestational age when the fetal brain MRI was performed between those with preoperative brain injury (32 weeks, IQR 32-33) and those without (32.7 weeks, IQR 31.7-34; p=0.56).

There was a trend towards a lower mean fetal TBV in those with preoperative brain injury (187.8 cm^3, 95%CI: 156.9-218.7) as compared to those without (225.2 cm^3, 95%CI: 189.8-260.6; p=0.08; Figure 5). This trend was less significant after adjusting for GA at time of fetal MRI (p=0.165).

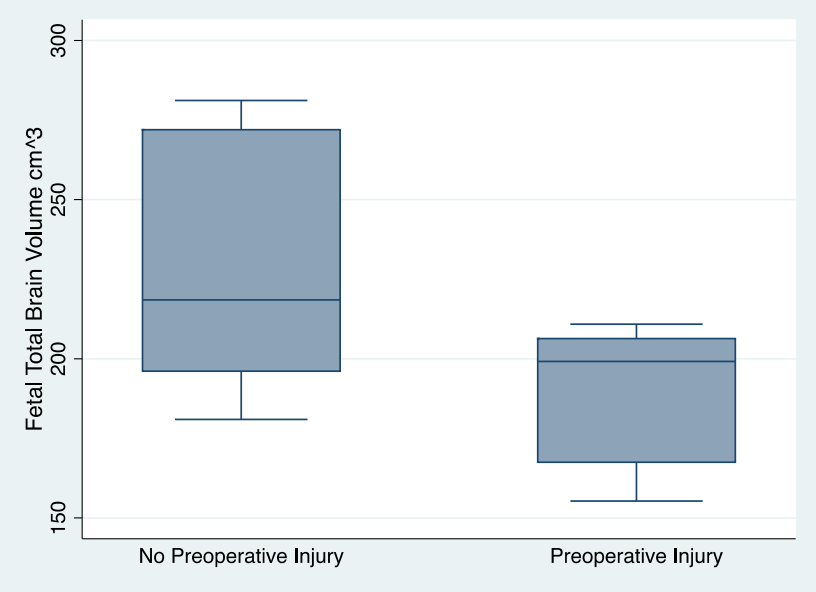

Figure 5: Fetal TBV with and without Presence of Preoperative Brain Injury

4.3 Fetal TBV and BIS Score

Of the 5 that exhibited preoperative brain injury, 3 subjects had a BIS score of 3 while the other 2 neonates displayed a BIS score of either 1 or 2. The BIS score and the corresponding mean TBV and standard deviation are as follows: BIS 0, TBV 225.1 ± 38.3 cm^3; BIS 1, TBV 210.9 cm^3; BIS

2, TBV 199.2 cm^3; BIS 3, TBV 176.4 ± 26.6 cm^3. The test for trends was suggestive of an association between a decreasing TBV and higher BIS score, although this was not significant (p=0.06). This was similarly shown when comparing fetal TBV in those with a BIS score of 0 and 3 (p=0.08). Figure 6 shows the observed relationship between mean TBV and BIS.

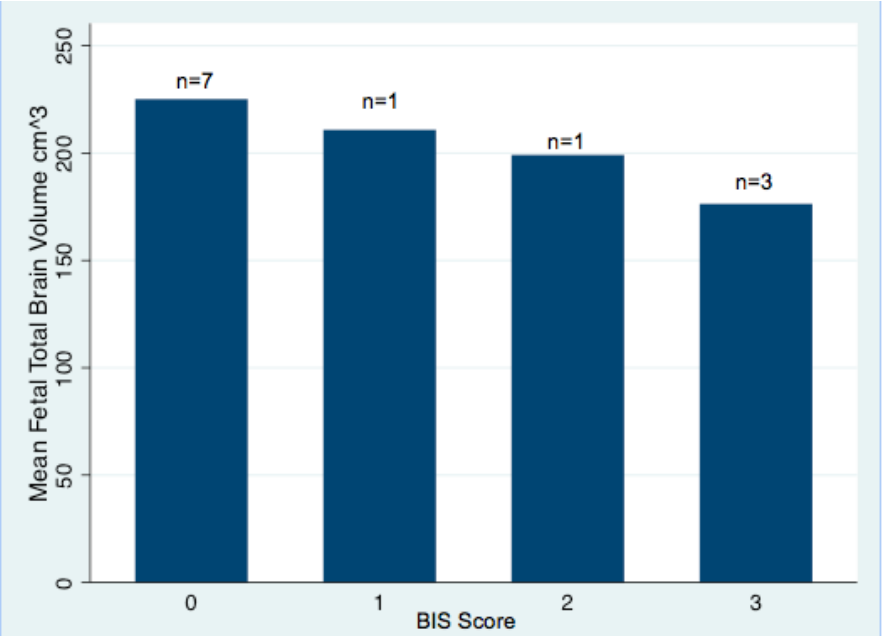

Figure 6: Mean Fetal TBV in Each BIS Score

4.4 Repeatability of Fetal TBV Measurements

The Dice similarity index (DSI) showed a mean spatial overlap of 94.07 ± 0.95% between the initial and repeated segmentation that determined fetal TBV. The measured DSI indicated an excellent agreement between the two separated segmentations. Also, Pearson's correlation coefficient revealed a strong correlation between the two measured fetal total brain volumes (R=0.999). Therefore, the repeatability of fetal TBV segmentation was validated.

4.5 Trajectory of Fetal to Neonatal TBV

The median gestational age at the time of the postnatal preoperative MRI for all subjects was 38.9 weeks (IQR 38.6-39.9). Consistent with the correlation between fetal TBV and GA, a significant linear relationship was established after including neonatal TBV and corresponding gestational age ($p<0.001$). Figure 7 displays the rate of brain growth in those with and without preoperative brain injury. The rate of increase in TBV was similar in both groups before ($p=0.18$) and after adjustment ($p=0.33$) for GA at MRI.

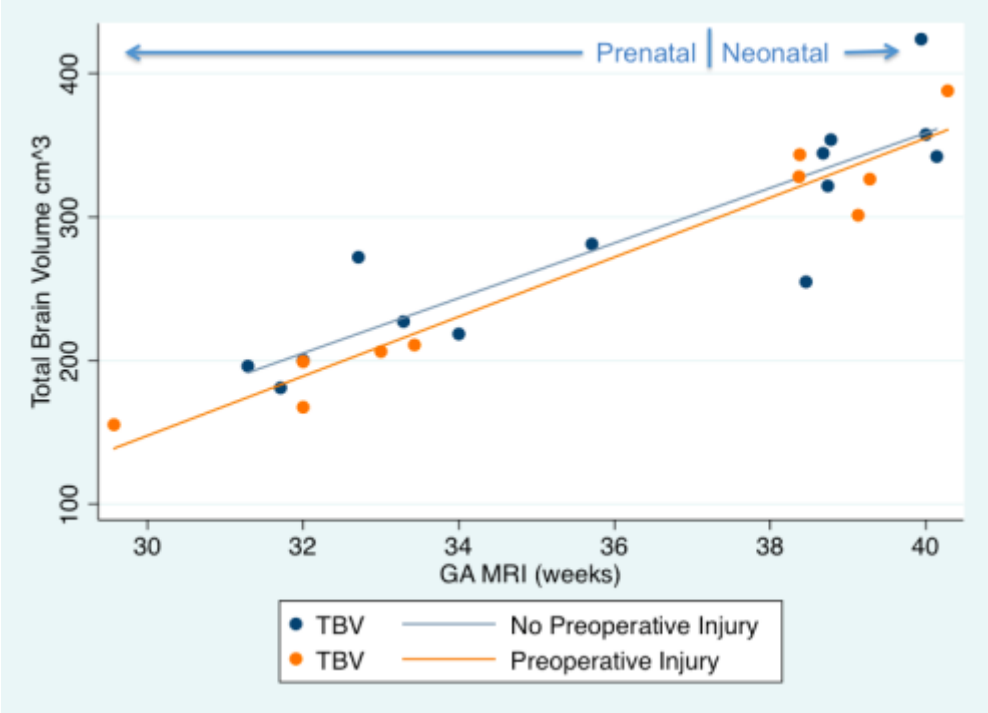

Figure 7: Fetal and Neonatal TBV Trajectory with and without Preoperative Brain Injury

5 Discussion

Thus far, neonatal brain studies and an increasing interest in fetal brain analysis have been critical in helping analyze deviations from the developmental trajectory of subjects with CHD from normal neurodevelopment [16]. This is important because more than half of children born with

critical CHD display some form of disability or neurological deficit—including motor, cognitive, behavioral, social, and attention abnormalities [3,28]. Although conventional fetal MR imaging has demonstrated good specificity in ruling out brain abnormalities, a lack of sensitivity exists in predicting postnatal brain injury. Brossard-Racine et. al. 2016 demonstrated this sensitivity and specificity to be 27% and 89%, respectively [29]. The study also found that CHD cases with abnormal conventional fetal MRI findings had a 90% increased likelihood of having abnormal neonatal brain MRI findings [29]. Although Brossard-Racine established an association between brain abnormalities detected in fetal MR imaging studies and a higher risk for postnatal preoperative brain injury, they were limited to qualitative measures. We therefore examined quantitative fetal TBV in predicting neonatal preoperative brain injury. Our study suggests a potential relationship between fetal brain growth as measured by TBV and the risk of postnatal preoperative brain injury in patients with critical CHD. However, the main findings were not statistically significant, likely a result of small sample size. Despite the lack of statistical significance, these findings suggest a potential relationship and warrant further study.

5.1 Implications

The strong linear relationship between fetal TBV and gestational age seen in this study aligns with the notion that brain volume increases with time due to fetal growth and development. This is consistent with findings from prior studies that investigated both healthy fetuses as well as those diagnosed with CHD in utero [16,30].

Although our subjects with preoperative brain injury were not significant in having a smaller fetal brain volume than those without injury, the general volumetric measurements of each group were observably distinct in their range. There was also a trend towards a lower fetal TBV with a higher BIS score. These findings suggest that fetal TBV may be predictive of preoperative brain

injury in patients with critical CHD. This is clinically relevant for several reasons. Prenatal counseling can be enhanced through added awareness of potential neurological deficits or delays that can be observed after birth in addition to details of the CHD. This can help families with decision-making and ensure early intervention services for their child.

Additionally, the exact timing of brain injury for patients with critical CHD and implications for long-term developmental outcomes remain unknown. The timing of surgery has been shown to affect brain injury. A longer time to surgery was associated with new postoperative white matter injury in neonates with HLHS [31], suggesting that earlier surgery may prevent ongoing injury to the brain. After birth and before surgical correction, the neonate with CHD has persistent fetal circulation. Thus, there are continued aberrations in cerebral oxygen delivery and consumption after birth that may contribute to brain growth and injury. By identifying neonates at highest risk of developing preoperative injury, neuroprotective mechanisms may be utilized to prevent the onset of injury. For example, current ongoing studies are investigating the utility of earlier cardiac operation after birth to minimize brain injury and promote brain growth.

The trajectory of brain growth from fetal to neonatal life has not been examined in the context of CHD. Separate fetal [16] and neonatal [15] studies have shown lower TBVs as compared to controls; however, the rate of brain growth and its relationship to neurologic and developmental outcomes remains unclear. Our study begins to describe brain growth in this cohort and demonstrates similar rates of growth in those with preoperative brain injury and those without.

Although the sample size was small, potential mechanisms to explain these findings includes compensatory mechanisms that enable individuals predisposed to brain abnormalities to achieve comparable neonatal brain volume to their non-injured counterpart within the CHD cohort. This aligns with prior evidence that brain sparing occurs in some CHD fetuses [32]. The brain sparing

effect, a fetal adaptive mechanism, describes the notion that some CHD fetuses with decreased cerebral oxygen supply may have auto-regulation of blood flow that helps enhance cerebral perfusion. The relationship between "brain-sparing" and brain growth warrants further study.

Brain growth trajectory may also vary depending on the type of cardiac lesion. A postnatal, post-operative study observed that those with TGA have a greater increase in TBV as compared to those with HLHS [33]. This suggests that the normalization of cerebral blood flow and correction of hypoxia seen in TGA patients after surgical intervention results in improved brain growth in comparison to palliative treatment provided in HLHS cases, which never achieve normal circulation. Due to the small sample size, our cohort contained a combination of critical CHD subtypes. Future investigations with a larger cohort may be able to assess how differences in cardiac physiologies may affect brain growth from the fetus to neonate.

5.2 Limitations

Several challenges existed over the course of this study. First, the current state of technology in fetal MR imaging is limited. Fetal and maternal motion during fetal MRI scans can cause motion artifacts in anatomical imaging, which may affect the accuracy of the total brain volume masks. Additionally, image resolution and limited acquisition protocols available for fetal imaging increase the variability of the image quality. Collectively, these may subtly vary the volumetric data that was generated. The volumetric replication of the three subjects was added to this study to address this possibility.

Second, the sample size of this study was small, resulting in a small number of patients that exhibited presence of neonatal preoperative brain injury, and therefore limiting statistical power. The small sample size may have resulted in less significant findings. After performing a power

analysis to predict the smallest sample size required to achieve significance, an additional 24 subjects is needed to expand the data set and reinforce this association.

Although this study showed a lack of significant difference in gestational age at fetal MR imaging between subjects with and without brain abnormalities, the timing of the fetal and neonatal MRI may have limited the study's ability to accurately detect delayed brain development. Brain injuries and abnormalities may have been missed as a result of the timing of the MRI scans.

Lastly, there was no control group that was used in predicting neonatal brain injury and in comparing the trajectory of fetal to neonatal TBV. It is possible that this phenomenon may be observed in other populations and may not be unique to the CHD cohort. These limitations will be addressed in the future since data collection is ongoing.

5.3 Future Directions

Additional subjects will be enrolled over time along with neurological follow-up information. Ventricular volume can later be analyzed with a larger sample size. Particularly in cases with ventriculomegaly, the association of ventricular volume and risk of post-natal brain injury may be of interest. Future investigations could also examine whether certain regional volumes in the neonatal brain may be related to severity of brain injury and neurodevelopmental outcome. This may help clarify the hypothesized mechanisms of neurodevelopmental risks in CHD patients.

6 Conclusion

This is the first study to explore whether total fetal brain volume determined by using conventional fetal MR imaging is associated with postnatal preoperative brain injury in neonates with critical CHD. By identifying fetal risk factors for the development of neonatal brain injury in patients with critical CHD, neuroprotective mechanisms can be applied to at-risk patients to further improve ultimate neurodevelopmental outcomes.

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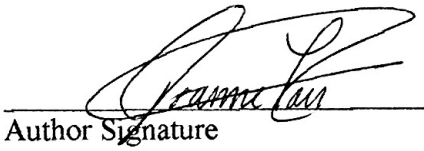

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