学 位 論 文

Evaluation of factors affecting brain volume in newborn infants using Multi-Atlas Labeling on 3D MRI

新生児の脳体積に影響を与える因子の検討 ～頭部３D MRI画像を用いて～

小児科学

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Contents

Introduction

Small brain volume is known to associate with developmental and psychological disorders. However, there are various factors affecting the brain volume including acquired factors such as aging, growing environment, and illnesses. Therefore, findings from the studies in older children and adults are inconsistent. In this study, to minimize effects of the acquired factors, early postnatal brain volume was evaluated from clinical and genetic perspectives by using 3D-MRI images.

This doctoral dissertation is based on two papers.[1,2]

Chapter 1

Clinical factors that affect the relationship between head circumference and brain volume in very-low-birth-weight infants

Abstract

Background and Purpose: Measuring head circumference (HC) in infants is an easy screening procedure with which to detect abnormalities in brain growth. It has been demonstrated that HC can predict total brain volume (TBV) in very-low-birth-weight (VLBW) infants. However, the correlation between HC and TBV was weaker than that observed in healthy term-born toddlers, suggesting that there are factors that influence the relationship between HC and TBV. The aim of this study was to identify the clinical risk factors that caused a deviation from the regression line obtained between HC and TBV.

Methods: The study population was based on 37 VLBW infants, who underwent a clinical MRI examination at a term-equivalent age, during 2013 - 2015, at Toyama University Hospital. The HC and the TBV were both adjusted for sex, multiple births, and postmenstrual age. The relationship between TBV/HC and clinical characteristics was evaluated.

Results: There was a positive correlation between HC and TBV ($r = 0.58$, $p = 0.000168$). Two clinical factors, the lower birth body weight (BBW) ($r = 0.38$, $p = 0.02$) and dolichocephaly ($r = 0.46$, $p = 0.006$), were identified as factors that negatively affected the TBV/HC ratio. After excluding infants with low BBW or with dolichocephaly, the correlation between HC and TBV was higher ($r = 0.63$).

Conclusions: Although HC has predictive value for TBV in VLBW infants, care should be taken in infants with low BBW (BBW less than 600 g) or dolichocephaly (MRI-based Cranial Index less than 0.68), which were related to overestimation of TBV.

Introduction

Growth measurements are an important part of the clinical routine in pediatrics. Measuring head circumference (HC) in infants is a quick, simple, and noninvasive screening procedure to detect abnormalities in brain or skull growth, such as macro- or micro-cephalus, hydrocephalus, or craniosynostosis.[3] Early diagnosis of hydrocephalus enables earlier surgical intervention, which improves the neurological outcome.[4] Detection of macro- or microcephalus also has a significant impact on predicting a child's neurological, cognitive, or behavioral outcomes.[3,5] Therefore, knowledge about how well the HC predicts the total brain volume (TBV), and the factors that affect the accuracy of the prediction, is of central significance in pediatric clinical practice. It has been demonstrated that the correlation between HC and TBV is higher in a younger population. For example, a study based on postmortem fetus and neonatal brains indicated that the correlation coefficient between HC and TBV was $R > 0.979$,[6] and the correlation calculated based on magnetic resonance imaging (MRI) volumetry was $R = 0.928$ in healthy normal-developing toddlers, which was higher than that of children ($R = 0.67$) or adults ($R = 0.69$).[7] The HC correlated well with neurodevelopmental outcome and intelligence in healthy infants,[8] which indicated the usefulness of the HC as a surrogate of TBV.

Preterm birth and very-low-birth-weight (VLBW) are among the major risk factors for the development of cognitive and motor impairment,[9] such as cerebral palsy (5 – 15%) or fine motor, cognitive, learning, and behavioral disorders that persist into young adulthood and affect the individual's adaptive functioning (40%).[10] It has been demonstrated that the HC can also predict TBV in preterm-born and VLBW infants($R = 0.68$).[11] However, the correlation was weaker than that observed in healthy term-born toddlers, suggesting that there are factors associated with preterm birth or VLBW that confound the relationship between HC and TBV.

In this study, I investigated the relationship between HC and TBV in VLBW infants, the majority of which were born very preterm. I hypothesized that known risk factors for poor neurodevelopment, such as lower birth body weight (BBW), smaller gestational age, bronchopulmonary dysplasia, sepsis, and necrotizing enterocolitis (NEC),[12-15] were related to a TBV smaller than that expected from the HC, and resulted in poor correlation between HC and TBV. To test this hypothesis,

the relationship between these risk factors and the TBV/HC ratio were investigated.

Methods

Patients

The study population was based on 40 VLBW infants (BBW of < 1500 g), who underwent clinical MRI examination at a term-equivalent age, during 2013 – 2015, at Toyama University Hospital. Among them were infants with (1) intraventricular hemorrhage (IVH) grades 3-4,[16] or (2) congenital anomalies, chromosomal anomalies, or genetic disorders. Of the 40 preterm-born infants, two infants with IVH, and an infant with a congenital abnormality (21 trisomy), were excluded. After the exclusion, a total of 37 infants were enrolled in this study. The Toyama University Hospital Institutional Review Board approved the study, and written, informed parental consent was obtained from all participants.

Non-image data collection

Demographic information, growth measurements, and clinical factors were obtained from the Toyama University Hospital electronic medical record. Demographic information included the gestational age at birth in weeks, postmenstrual age (PMA) in weeks at scan, sex, and race (all Asian Japanese). Growth measurements included birth body weight (BBW), height at birth, and head circumference at birth. The z-scores were calculated based on the Japanese registry reference data[17] and are demonstrated in Table 1. Clinical factors include: Apgar score at 1 and 5 minutes; antenatal or postnatal corticosteroid use; duration of intubation; duration of oxygenation; presence of bronchopulmonary dysplasia; sepsis; necrotizing enterocolitis; treated patent ductus arteriosus (PDA); treated retinopathy of prematurity; and days of stable enteral nutrition (100 ml/kg/day).[18] The Cranial Index (CI) was calculated and included in the analysis because dolichocephaly, which is defined by a CI < 76%, is often related to prematurity at birth.[19]

Table 1. Participant characteristics

	Characteristics	**N = 37**
Maternal characteristics	Pre-eclampsia	3 (8.1%)
	Antenatal corticosteroids	21 (56.8%)
	Cesarean delivery	32 (86.5%)
Infant characteristics, neonatal period	Gestational age at birth, wk, mean (SD)	29.0 (2.7)
	Birthweight, g, mean (SD)	1116 (314)
	Birthweight z-score, mean (SD)*	-0.8 (1.2)
	Head circumference at birth, cm, mean (SD)	25.8 (2.6)
	Head circumference at birth z-score, mean (SD)*	-0.2 (0.8)
	Small for gestational age	6 (16.2%)
	Apgar at 1 min, mean (IQR)	4.7 (1-8)
	Apgar at 5 min, mean (IQR)	7.0 (2-9)
	Male	20 (54.1%)
	Multiple births	8 (21.6%)
	Received respiratory support	31 (83.8%)
	Neonatal infection	9 (24.3%)
	Necrotizing enterocolitis	0 (0%)
	Bronchopulmonary dysplasia	16 (43.2%)
	Postnatal corticosteroids	8 (21.6%)
	Surgery in the neonatal period	8 (21.6%)
Infant characteristics at scan	Gestational age, wk, mean (SD)	38.6 (2.8)
	Weight, g, mean (SD)	2591 (347)
	Weight z-score, mean (SD)*	-1.2 (1.2)
	Head circumference, cm, mean (SD)	33 (1.2)
	Head circumference z-score, mean (SD)*	-0.1 (0.9)

Data are reported as number of mothers or infants (percentage) unless otherwise specified.

IQR = inter quartile range; SD = standard deviation; N = number of mothers or infants; g = gram; wk = weeks

*The z-scores were calculated according to the Japanese registry reference data (17).

Small for gestational age indicates birth weight of less than −2 SDs for age and gender.

Interpretation of the Apgar score: 7 and above: normal; 4–6: fairly low; and 3 and below: critically low

The CI is usually defined by the ratio of the fronto-occipital diameter (FOD) and the biparietal diameter (BPD).[20] In this study, I used a minor diameter to major diameter ratio (MRI-based CI or mCI), measured by MRI, with the anterior- and

posterior-commissure lines aligned in the Talairach space (Fig. 1) because the FOD and the BPD were not available for all infants.

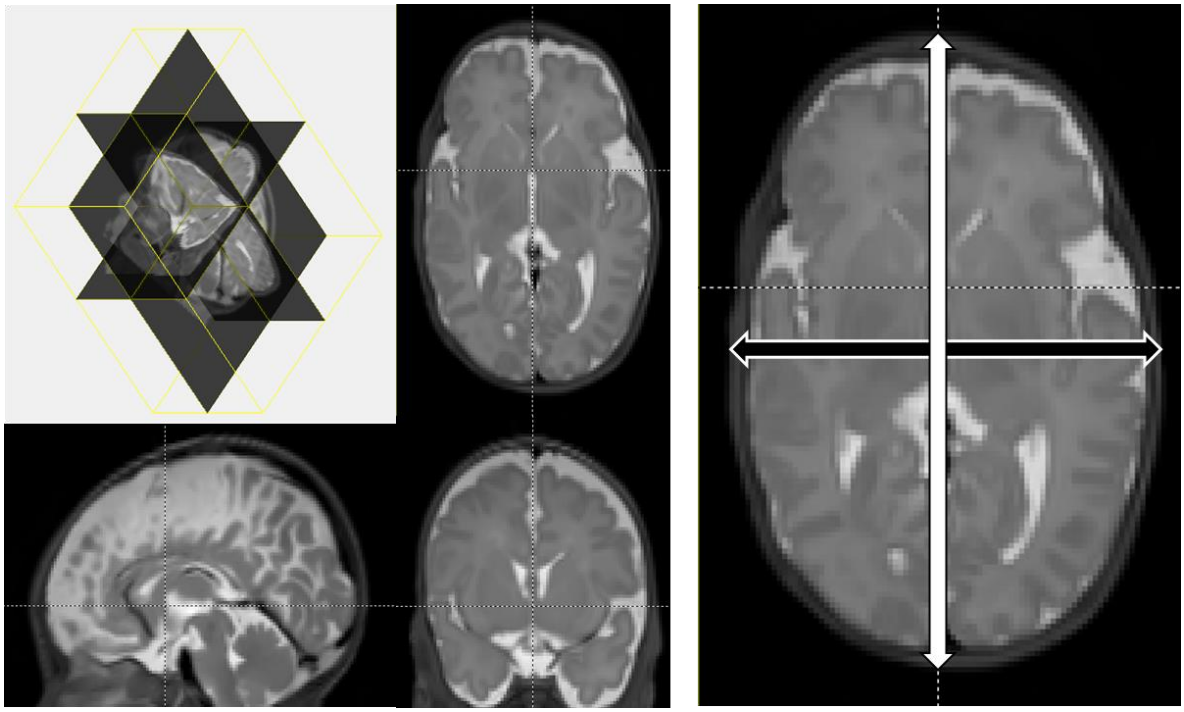

Fig. 1. Evaluation of the degree of dolichocephalic deformation using the minor diameter to major diameter ratio (MRI-based cranial index).

Black arrow: minor diameter, White arrow: major diameter

MRI scan acquisition

MRI scans were performed on a 3-T Siemens Magnetom Verio (Siemens Medical Solutions, Erlangen, Germany). Before the MR examination, infants were fed and sedated with oral monosodium trichorethyl phosphate syrup (0.5-1.0 mL/kg), swaddled, and placed in a Vac Fix (Radiation Products Design Inc., Albertville, MN) beanbag designed to keep the infant still and supported in the MR scanner. The Sampling Perfection with Application optimized Contrasts using different flip angle Evolutions (SPACE) sequence was used to obtain the three-dimensional T2-weighted image, with the following parameters: repetition time, 3200 ms; echo time, 409 ms; slice thickness, 1 mm; and image matrix, 256 × 256.

Image processing

The SPACE images were realigned to adjust the head position based on the anterior- and posterior-commissure line, using the Analyze11.0 software package (Mayo Clinic, Mayo Foundation, Rochester, MN, USA). The intracranial space and the cerebrospinal fluid (CSF) space, which includes the ventricles, were semi-automatically delineated by intensity thresholds followed by manual touch-up performed by a neonatologist (Y.K.) using the ROIEditor software (www.mristudio.org) (Fig. 2). After the intracranial volume (ICV) and the CSF measurements, the total brain volume (TBV) was calculated by the equation: TBV = ICV - CSF. The precision of the TBV volume measurements was evaluated based on a randomly selected five images. The inter-rater reproducibility was measured by the interclass correlation coefficient (ICC) obtained from two independent raters (Y.K. and A.H.), which yielded r = 0.97. The intra-rater reproducibility was measured based on one rater (Y.K.) at two separate time points at intervals of approximately two months. The ICC coefficient was r = 0.95.

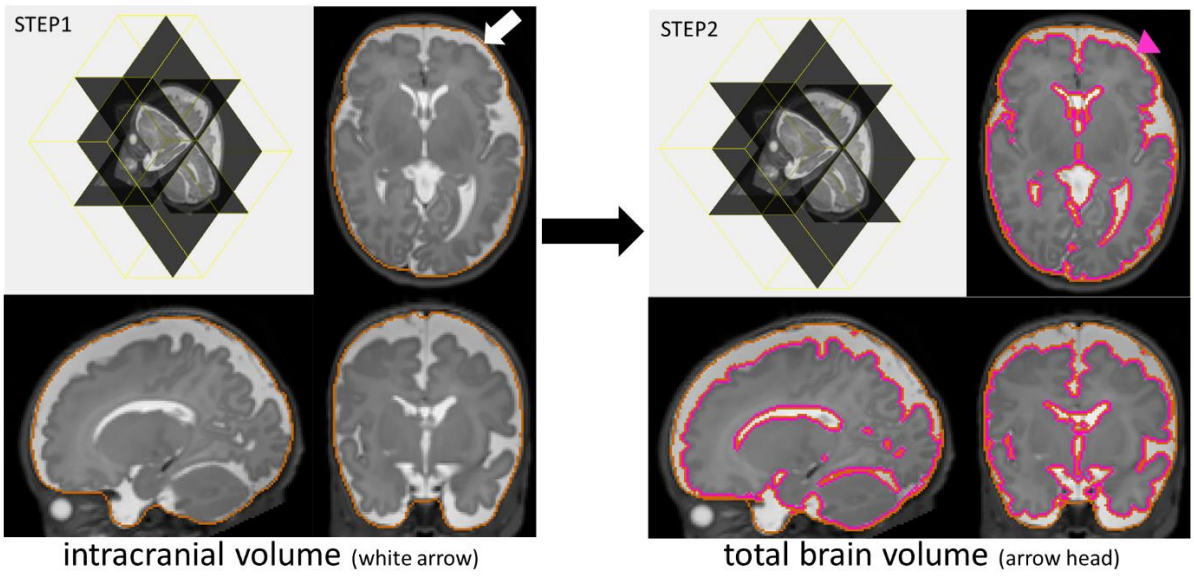

Fig. 2. Procedure for image processing

Lines show the contour of the intracranial space (left, white arrow) and the brain parenchyma (right, arrowhead).

Statistical analysis

To investigate the relationship between HC and TBV on scans, a linear regression model was applied. It has been reported that the HC and the TBV correlate well with PMA.[17,21] Female sex [21] and multiple births[22] are related to smaller HC and TBV. Therefore, the HC and TBV measures were adjusted for sex, PMA, and multiple births using the method proposed in[23] and were then converted to z-scores (zHC and zTBV, hereafter). The resultant regression function, $zTBV_{predicted} = \alpha * zHC_{measured} + \beta$, in which α represents the slope and β represents the intercept, were used to calculate the predicted zTBV ($zTBV_{predicted}$) from the measured zHC ($zHC_{measured}$). The Pearson correlation coefficient was calculated from the measured zTBV ($zTBV_{measured}$) and the $zTBV_{predicted}$ to investigate how well the $zTBV_{predicted}$ agreed with the $zTBV_{measured}$.

To investigate the effects of neurodevelopmental risk factors on the HC-TBV relationship, the correlation between 14 factors, listed in Table 2, and the TBV/HC ratio, were evaluated using Spearman's rank moment correlation coefficient. For the factors that correlated with the TBV/HC, I further investigated the risk for the overestimation of TBV, which was defined by a $zTBV_{measured}$ of 1.5 or smaller than the $zTBV_{predicted}$.[24] Sensitivity and specificity were used for the evaluation. For the continuous variables (e.g., BBW, gestational age at birth, or mCI), the maximum value of the Youden's Index on the receiver operating characteristic (ROC) curve[25] was used to define the cut-off value for calculating the sensitivity and specificity. If the number of children categorized into the high-risk group was less than half the children categorized into the low-risk group, the low-risk group was further partitioned into two bins, each with an equal number of participants, which were called the close-to-threshold and far-from-threshold groups. The risk-ratio, defined by the (number of underestimated children)/ {1 – (number of underestimated children)} was calculated for each group and the difference between groups was examined by the Fisher's exact test. The significance was defined by $p < 0.05$. R (version 3.4.4) software was used for the analysis.

Table 2. Bivariate correlation with total brain volume / head circumference

	r	p value
Gestational age at birth	0.2645	0.1137
Birth body weight	0.3773	0.0213*
Apgar score (1 min.)	0.2536	0.1299
Apgar score (5 min.)	0.2635	0.1151
Received antenatal corticosteroids	0.1532	0.3654
Duration of intubation	-0.0640	0.7067
Duration of oxygenation	-0.3122	0.0600
Presence of bronchopulmonary dysplasia	0.0661	0.6976
Postnatal corticosteroid use	-0.1383	0.4144
Treated patent ductus arteriosus (PDA)♯	-0.2696	0.1066
Treated retinopathy of prematurity	-0.2050	0.2235
Neonatal infection	0.2008	0.2335
Days of stable enteral nutrition	-0.2832	0.0894
MRI-based Cranial Index	0.4634	0.0068*

min = minute(s)

♯PDA was medically treated without surgical intervention.

*$p < 0.05$

Results

The mean gestational age at birth was 29.0 weeks (range: 23.1–33.8 weeks), and the mean body weight at birth was 1116 g (range: 426–1494 g). The mean postmenstrual age (PMA) at the MRI scan was 38.6 weeks. The mean HC measurement at scanning (37.8 – 41.8 weeks PMA) was 33cm (-0.1SD), which was age-appropriate (Table 1). The mean TBV was 355.4 ± 40.3 ml, the mean ICV was 438.7 ± 41.1 ml, and the mean CSF was 83.2 ± 23.3 ml.

There was a positive correlation between the $zHC_{measured}$ and the $zTBV_{measured}$ ($r = 0.58$, $p = 0.000168$; Fig. 3(A)). There were four infants with an overestimated TBV (Fig. 3 (B)). The correlations between the clinical factors and the TBV/HC ratio are demonstrated in Table 2. Two clinical factors, the BBW ($r = 0.38$, $p = 0.02$) and the

mCI (r = 0.46, p = 0.006), were identified as factors that were correlated with the TBV/HC ratio. A positive correlation was observed between BBW and TBV (r = 0.43, p = 0.007), and between mCI and TBV (r = 0.37, p = 0.02), but not between BBW and ICV (r = 0.13, p = 0.43), or between mCI and ICV (r = 0.19, p = 0.24) (Fig. 4).

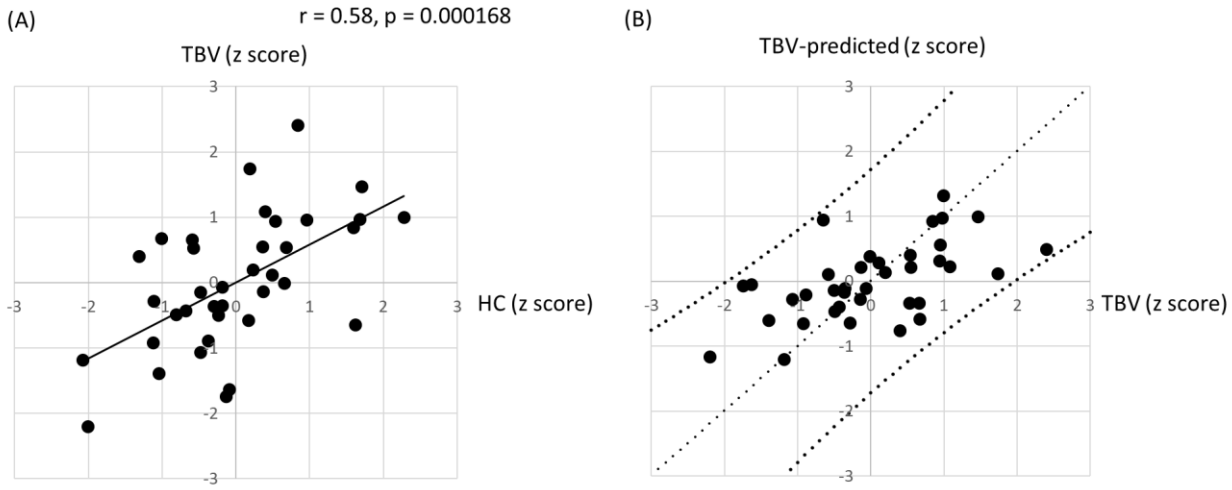

Fig. 3.

(A)The relationship between the head circumference (HC) z score and the total brain volume (TBV) z score at scanning (r = 0.58; p = 0.000168).

(B) The relationship between TBV z score and TBV-predicted z score. The dotted line indicates 95% prediction interval.

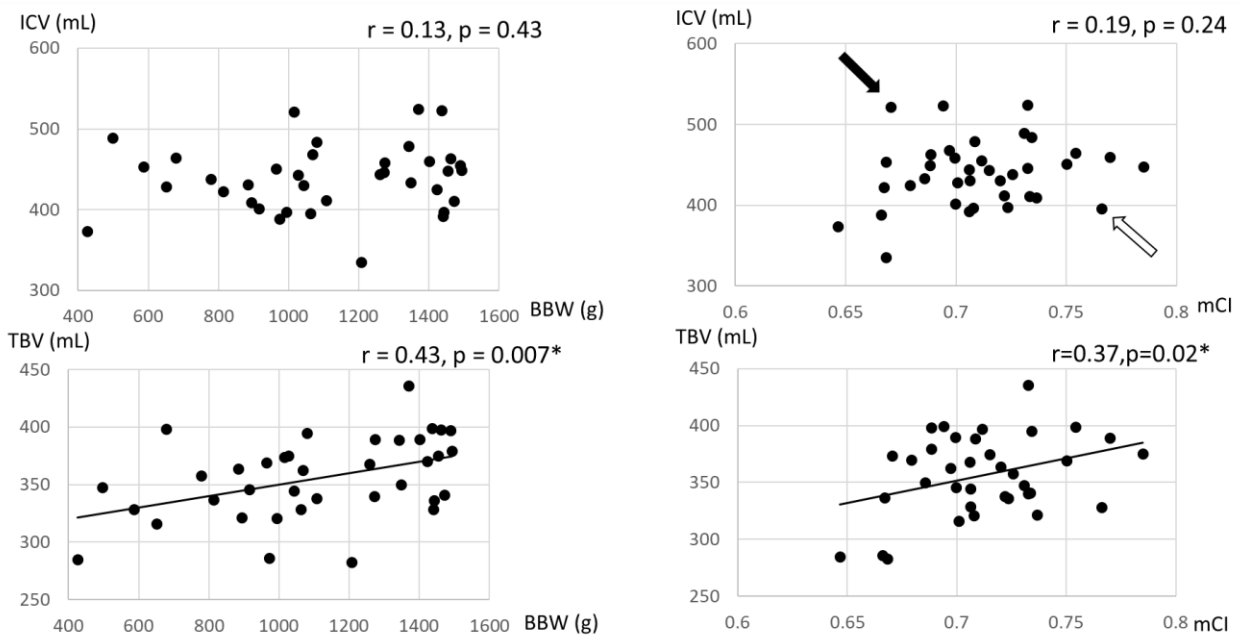

Fig. 4. The relationship between volumes (intracranial volume (ICV) and total brain volume (TBV)) and clinical factors (birth body weight (BBW) and the MRI-based Cranial Index (mCI)). A positive correlation was observed between BBW and TBV ($r = 0.43$, $p = 0.007$), and between mCI and TBV ($r = 0.37$, $p = 0.02$). *$p < 0.05$

White and black arrows indicate two outliers.

For the BBW, the cut-off value to define the high-risk group was approximately 600g (Fig. 5A), and the remaining children were further categorized into close-to-threshold and far-from-threshold groups. The sensitivity and specificity of a BBW < 600g in predicting the overestimation were 0.67 and 0.94, respectively (Table 3). The risk-ratio of each group was 0.67 (high-risk group), 0.06 (close-to-threshold group), and 0.06 (far-from-threshold group) (Fig. 6). The difference in the risk-ratio between the high-risk group and the other two groups was significant ($p = 0.04$ and 0.04, Fig. 6A). There was no difference between the close-to-threshold and far-from-threshold groups. For the mCI, the cut-off value was approximately 0.68 (Fig. 5B), and the remaining children were further categorized into close-to-threshold and far-from-threshold groups. The sensitivity and specificity of the mCI < 0.68 to predict the overestimation were 0.43 and 0.97, respectively (Table 3). The risk-ratio of each group was 0.43 (high-risk group), 0 (close-to-threshold group), and 0.07 (far-from-threshold group). The difference between the ratio of the high-risk group and the

close-to-threshold group was significant (p = 0.02), but the differences were not significant between the high-risk and the far-from-threshold groups, or between the close-to-threshold and the far-from-threshold groups.

The number of infants with a BBW < 600 or an mCI < 0.68, or both, was eight. The correlation between the $zHC_{measured}$ and the $zTBV_{measured}$ calculated without these infants was r = 0.63, which was close to the reported value of r = 0.68.

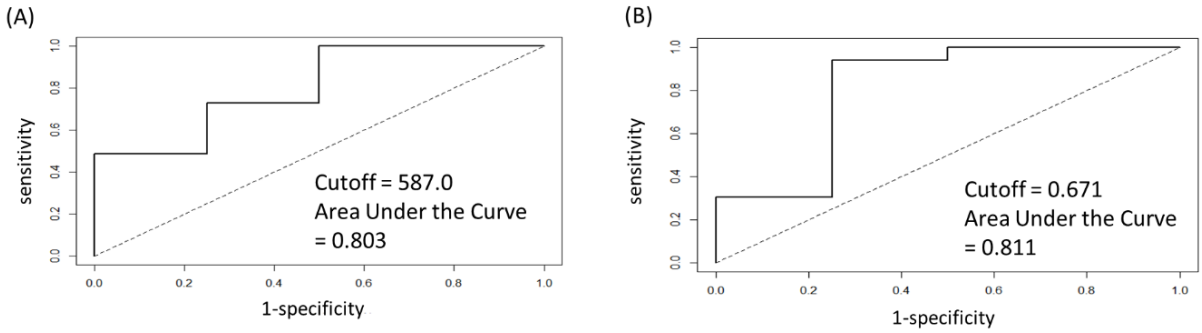

Fig. 5. Receiver operating characteristic curves of (A) birth body weight and (B) MRI-based Cranial Index

Table 3. The confusion matrices

	$zTBV_{measured}$ < -1.5	$zTBV_{measured}$ ≧ -1.5	Total
BBW < 600	2	1	3
600 ≦ BBW	2	32	34
Total	4	33	37

	$zTBV_{measured}$ < -1.5	$zTBV_{measured}$ ≧ -1.5	Total
mCI < 0.68	3	4	7
0.68 ≦ mCI	1	29	30
Total	4	33	37

TBV = total brain volume, BBW = birth body weight, mCI = MRI-based Cranial Index

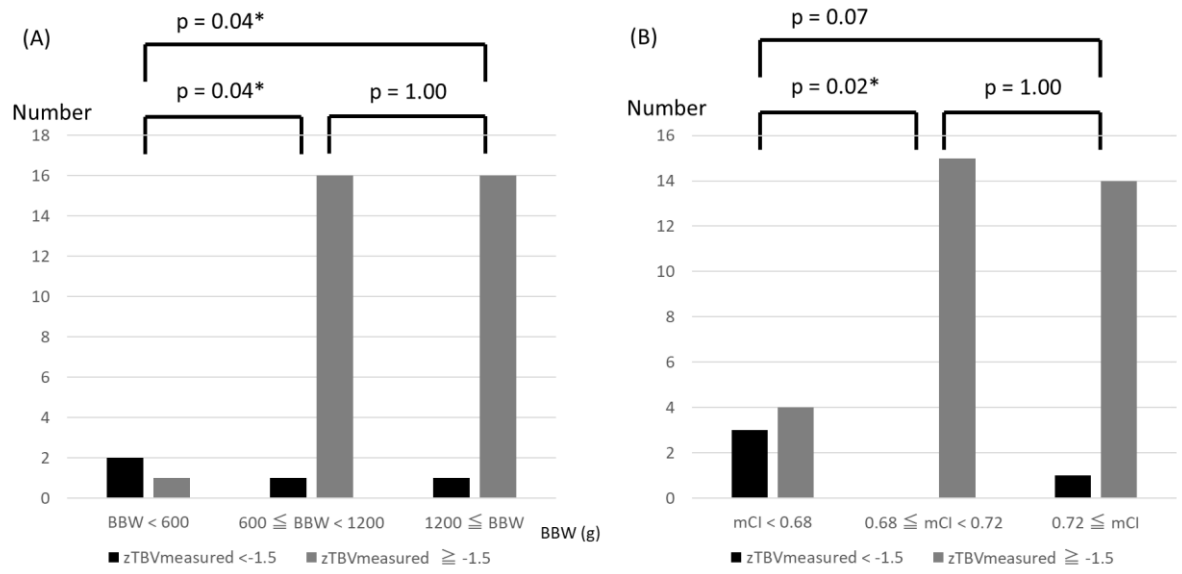

Fig. 6.

(A) Number of infants with a total brain volume (TBV) less than predicted ($zTBV_{measured}$ < -1.5, black bar) or appropriately predicted ($zTBV_{measured}$ ≧ -1.5, gray bar) that belong to each bin of the birth body weight (BBW).

(B) Number of infants with a TBV less than predicted ($zTBV_{measured}$ < -1.5, black bar) or appropriately predicted ($zTBV_{measured}$ ≧ -1.5, gray bar) that belong to each bin of the MRI-based Cranial Index (mCI).

Discussion

The HC and TBV were positively correlated in my VLBW population, which was congruent with a previous study.[11] However, the correlation (r = 0.58) was weaker than that observed in that study (r = 0.68).[11] One of the major differences between the previous study and my study is the proportion of the small-for-gestational-age (SGA) infants within the study populations. In my study, 16.2% of the study population was SGA, while 9.3% was SGA in the previous study by Cheong JL et al.[11] Since I identified the BBW as a factor that affects the relationship between HC and TBV, as detailed below, the greater rate of SGA in my cohort might have resulted in the lower correlation coefficient. Indeed, the correlation coefficient calculated from a subgroup of infants with a BBW > 600 g and an mCI > 0.68 was closer (r = 0.63) to that reported by Cheong JL et al.

The novel finding was the effect of the BBW and the mCI on the TBV/HC ratio. The results indicated that the TBV could be overestimated in infants with lower BBW

(less than 600 g), or in infants with dolichocephaly (mCI less than 0.68), when the HC is used as the source of estimation. The comparison between close-to-threshold and far-from-threshold groups indicated that the infants with a BBW more than 600g or an mCI more than 0.68 could be regarded as a group at low-risk for overestimation. The BBW correlated well with the TBV ($r = 0.43$, $p = 0.007$), but there was no correlation between the BBW and the ICV ($r = 0.13$, $p = 0.43$). This indicated that the BBW did not affect the growth of the skull and scalp, but the lower BBW negatively affected the growth of the brain. Indeed, growth alteration in brain structures, such as the cerebral cortex, myelinated white matter, and deep nuclear structures, has been reported in VLBW.[26,27] The discrepancy between altered brain growth and normal skull-scalp growth might be a source of the inaccuracy in predicting the TBV from the HC.

The low CI, which is often seen in infants born preterm or with VLBW, is called non-synostotic dolichocephaly. The position of the head, which is often fixed in a sideways position in preterm-born or VLBW infants, is believed to cause non-synostotic dolichocephaly.[18] In preterm-born infants, the occurrence of dolichocephaly is related to the degree of prematurity and lower BBW.[26] Based on the isoperimetric inequality in mathematics, I expected that, if the perimeter (= HC) were identical, the higher mCI would be better at storing more content (= brain), compared to the lower mCI. Although the mCI was correlated with the TBV ($r = 0.37$, $p = 0.02$), there was no correlation between the mCI and the ICV ($r = 0.19$, $p = 0.24$), which was unexpected. The scatterplots (Fig. 4, upper right) indicated that there were two infants (marked by white and black arrows) who confounded the correlation. The infant marked by the black arrow had the largest head volume among the study population, with an age-appropriate brain volume, and the infant marked by the white arrow had a flat-shaped head (the top-to-bottom length was shorter than the left-right length). These observations suggested that a discrepancy in growth between the brain and the tissue outside the brain (skull, scalp, and soft tissue), and the shape of the head observed in both the axial and coronal orientations, are the factors that can affect the accuracy of the TBV prediction.

Several limitations should be noted. First, the MRI data and non-image information were from a single institute with a fixed protocol for neonatal care and

interventions. Therefore, whether the results obtained from this study are generalizable to infants cared for with different protocols at other institutes needs to be investigated. Second, the cohort size was relatively small and might not be large enough to detect clinical factors with minor effects on the TBV/HC ratio. Third, since my study was explorative, I did not correct the p value after multiple comparisons to avoid false-negative findings. Although my study provided the hypothesis that the BBW and the mCI affect the prediction of TBV, a confirmative study is still needed. Fourth, the threshold used to define infants with the overestimated TBV ($zTBV_{predicted}$ - $zTBV_{measured}$ $\leqq$ 1.5) was arbitrary and might have affected the results. Finally, the clinical factors included in this study were limited; known clinical factors that affect later neurodevelopment, such as years of maternal education and socioeconomic status, were unavailable. Further studies with larger cohort size should identify additional clinical correlates, such as serum albumin, bilirubin, and CRP that may influence the relationship between HC and TBV. Such data could be factored into a predictive algorithm, and further clarify the key contributors to brain development.

In summary, although HC has predictive value for TBV in VLBW infants, care should be taken in infants with a BBW less than 600 g or in infants with dolichocephaly (mCI less than 0.68), which were related to overestimation of the TBV.

Acknowledgments

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Chapter 2

Brain-Derived Neurotrophic Factor Val66Met variant on brain volumes in infants

Abstract

Objective: The brain-derived neurotrophic factor (BDNF) has many important roles in neurogenesis and neuronal health. BDNF is also involved in learning and memory. Individuals with BDNF-Val66Met variant (Met+) are at higher risks for neuropsychiatric disorders and have smaller hippocampi and amygdalae compared to those without this variant (Met-). Whether these smaller brain volumes are already present at birth is unknown and were evaluated.

Methods: 66 newborn infants were genotyped for BDNF-rs6265 and had brain MRI scans. The T1-weighted images were automatically parcellated into intracranial volume (ICV), total brain volume, total gray and white matter, hippocampus and amygdala, using a multi-atlas label fusion method, implemented in the MRICloud (https://braingps.anatomyworks.org). The segmented brain volumes were normalized to the ICV for group comparisons.

Results: The two infant groups were not different in their demographics and birth characteristics. However, compared to Met- infants, the Met+ infants had smaller hippocampi ($p = 0.013$), smaller amygdalae ($p = 0.041$), and less steep age-related declines in total brain volume and % white matter volume.

Conclusion: The smaller hippocampal and amygdala volumes in Met+ infants suggest that the Met+ genotype affected prenatal developmental processes. In addition, the slower age-dependent declines in the relative total brain and white matter volumes of the Met+ group suggest the BDNF-Val66Met variant has ongoing negative influence on the postnatal developmental processes.

Introduction

The brain-derived neurotrophic factor (BDNF) plays important roles in neurogenesis, neuronal survival, formation of new synapses, dendritic branching, modulation of neurotransmitters, and is abundant in various brain structures; furthermore, reduced levels of BDNF are often found in patients with psychiatric or neurodegenerative disorders.[28] The role of BDNF is particularly evident in the hippocampus, and is involved in learning and memory.[29-31] Interestingly, the activity-dependent secretion of BDNF varies with the polymorphism of a single nucleotide polymorphism (SNP) on the BDNF gene, the Val66Met variant, with substitution of a valine (Val) by a methionine (Met) at codon 66.[30] This BDNF-Met substitution in cultured hippocampal neurons led to lower depolarization-induced secretion of BDNF, as well as fewer and shorter labeled neuronal processes.[30] Similarly, a transgenic mouse model that expressed BDNF-Met/Met showed smaller hippocampal volumes, defective BDNF secretion and lesser dendritic arbor complexity in the hippocampal neurons, as well as poorer memory and greater stress-induced anxiety behaviors.[32] Human subjects with the Val66Met variant also showed abnormally greater hippocampal activation on functional MRI, and lower levels of hippocampal N-acetylaspartate on MR spectroscopy compared to those with the Val/Val variant.[30] Clinical studies demonstrated an association between the Val66Met variant with higher risks for psychiatric disorders (e.g., major depressive disorder, bipolar disorder, anxiety disorders and schizophrenia) [33] and poorer outcomes in neurological disorders (e.g., Alzheimer's disease, Parkinson's disease, epilepsy).[28] To date, findings from morphometry studies of the BDNF-Val66Met on hippocampal volumes are inconsistent. Three meta-analysis studies of the different BDNF variants found Met+ carriers to have smaller hippocampal volumes than Val/Val homozygotes,[34-36] while another meta-analysis in healthy individuals found no association between this BDNF polymorphism and hippocampal volumes.[37] This discrepancy may be due to the inclusion of participants with a variety of neuropsychiatric disorders, or with children and elderly subjects. Inclusion of such wide age ranges might have confounded the analyses since the hippocampal volume has a U-shaped developmental trajectory that peaks in adolescence.[38,39] In addition to the hippocampus, the BDNF-Val66Met variant was also associated with smaller right

amygdala[40] and lesser volumes in bilateral dorsolateral prefrontal cortices[41] in healthy adults. To minimize postnatal environmental, hence epigenetic influences that might confound the results, I compared the whole brain volumes, total gray and white matter volumes, as well as hippocampal and amygdala volumes of newborn infants with and without the BDNF-Val66Met variant. Based on prior studies in adults and children,[40,42] I hypothesized that the Met+ (Met homozygotes (Met/Met) and Val/Met heterozygotes) neonates would have smaller total brain volumes, hippocampal and amygdala volumes, relative to the intracranial volumes (ICV), compared to the Val/Val neonates.

Methods

Participants

66 healthy newborn infants (36 boys, 30 girls) underwent an MRI scan at 37.9 – 47.6 postmenstrual weeks and were evaluated for the BDNF genotype. The participants were part of a larger neuro-development study, which was approved by the Cooperative Institutional Review Board of the Queen's Medical Center, the University of Hawaii and the Johns Hopkins University. All parents of the infants signed the IRB-approved informed consent forms before the study procedures. 37 infants were categorized as Met+, including 15 heterozygous for Met (Met/Val) and 22 homozygous for Met (Met/Met), and 29 infants were categorized as Met- (Val/Val). The Met allele frequency within the general population ranges from ~ 4% in African Americans to ~ 30% in Caucasians and ~ 50% in Asians[43-45]; the 56% with Met+ in my primarily mixed-race infants reflects the high proportion with Asian ancestry.

BDNF genotyping

Buccal swabs were obtained from these infants. Genotyping for BDNF-rs6265 was done using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) followed by electrophoresis on a 4% agarose gel. AflIII restriction enzyme was used resulting in 2 bands of 77 and 59-bp for the G allele and 2 bands of 143- and 59-bp for the A allele.

MRI data acquisition procedures and quality control

All MRI scans were performed at the Queen's Medical Center MR Research Center in Honolulu, HI. The whole-head 3D T1-weighted images were acquired using a 3.0 Tesla Siemens TIM Trio scanner (Siemens Medical Solutions, Erlangen, Germany) equipped with a 12-channel phased-array RF coil for parallel imaging. The infants were scanned without sedation. A vacuum immobilization mat (Noras MRI Products, Hoechberg, Germany) was used to minimize infant motion, and earmuffs were used to attenuate the scanner noise. A pulse oximeter was used to monitor the heart rate and oxygen saturation of each infant. A skilled MRI investigator (S.B) visually inspected the MRI scans immediately after each acquisition, and images with artifacts were rescanned within the same session. The total study visit time (including consenting, neurological evaluation, scan, and time for neonates to fall asleep) varied between 4-8 hours. In case some or all the sequences failed, the families were brought back for rescans, typically within a few days. However, some infants were unable to complete the study (~15%) due to their inability to hold still for the DTI, or the families' inability to return for the repeat scans. In the current paper, I included only those who had successful and usable MRI scans.

Since the scans were performed during natural sleep, the MRI facility had a dedicated room for the mother to nurse the infant. This nursing room was adjacent to the MRI scanner room; both rooms were kept quiet and dimmed. The infants were nursed by the mothers in the nursing room until they naturally fell asleep. The infants were then wrapped and swaddled in a vacuum immobilization mat to minimize motion. Infant earmuffs were then placed along with custom-sized headphones to attenuate the scanner noise. The infants were placed gently on the scanner table and moved into the scanner bore. If the infants moved or woke up during a scan, the acquisition was paused until they stopped moving and went back to sleep, or were taken out for feeding and diaper change before another attempt at the scan after the infant fell asleep again. A skilled research staff remained in the scanner room to observe the infant and monitor the sleep status and motion. Structural images were acquired with three-dimensional (3D) magnetization-prepared rapid gradient-echo (MPRAGE) sequence, with TE/TI/TR of 4.15/1400/3200 ms, a flip angle of 7°, an imaging matrix of 176 × 256 × 160, and 1-mm isotropic resolution. These MRI scans were originally acquired for a larger study and the

quality control procedures were also reported[46] and only those that had paired genotype data and had good quality were included in this current study.

Image analysis

All brain MRIs were read by a pediatric neuroradiologist (D.L.) as normal. The MRIs were automatically parcellated into intracranial volume (ICV), total brain volume, total gray and white matter, hippocampus and amygdala (Fig. 1A-C) using a multi-atlas label fusion method, implemented in the MRICloud (https://braingps.mricloud.org)[47] for the volume measurements. The UH-JHU-neonate multi-atlas repository[48] was used as the anatomical reference. The resultant parcellation maps for each MRI were reviewed by a neonatologist (Y. K.) for quality control. Only one scan had a segmentation error in the brain surface after automatic processing, which was corrected manually. The segmented hippocampal and amygdala volumes, as well as the total brain volume, total gray matter volume, total white matter volume, were normalized to the ICV of each participant. The left and right hippocampal and amygdala volumes were averaged since I did not find a hemispheric difference in this small cohort of infants. Furthermore, BNDF genetic polymorphisms were associated with laterality of regional cortical brain volume differences in some studies,[40,49] but not for the hippocampal volumes[50] or for the amygdala.[49]

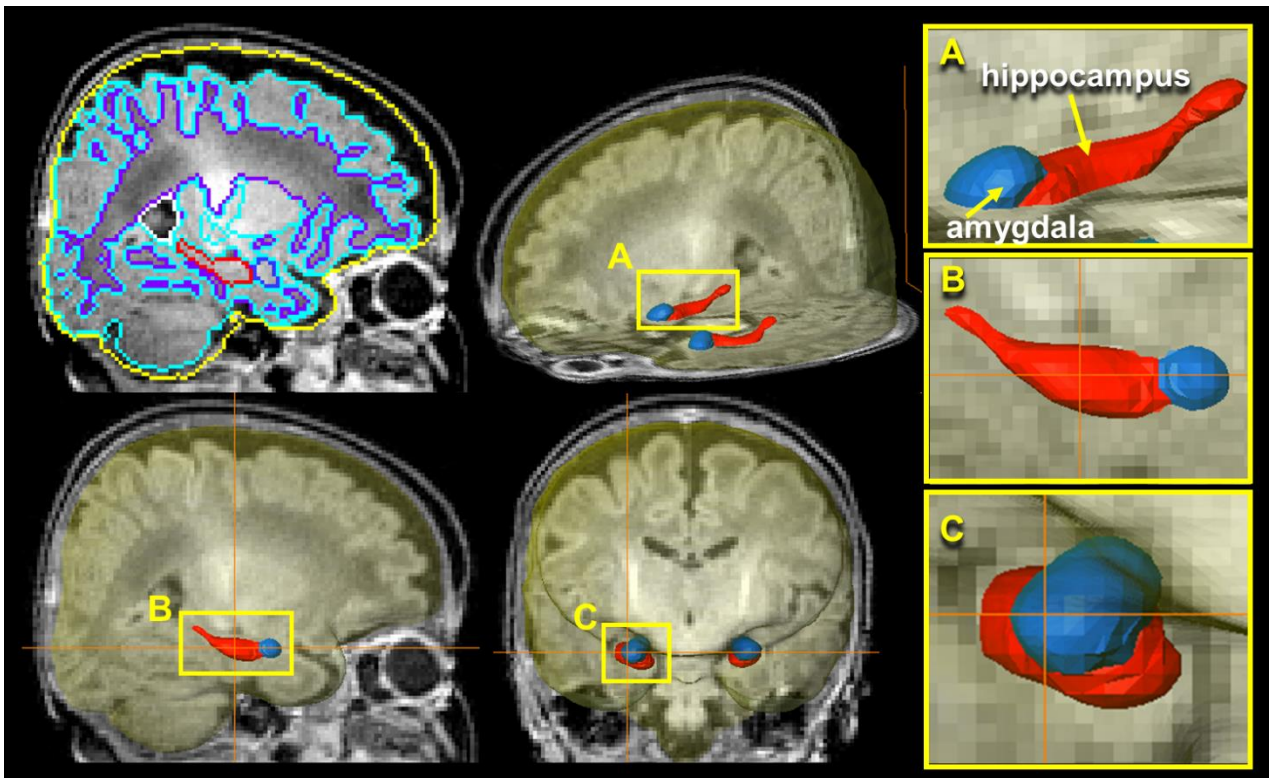

Fig. 1: Automated segmentation of brain structures, showing the total gray matter (green outline), the total brain volume (yellow outline), and the total intracranial volume (transparent yellow overlay on MRI), the hippocampus (red) and amygdala (turquoise color), on a T1-weighted MRI scan from a neonate. The inlays A, B and C show enlarged versions of hippocampus and amygdala for better visualization.

Statistics

The genotype effects, Met+ (Met/Met or Met/Val) vs. Met- (Val/Val) on ICV and % volume (Table 1) of the two anatomical structure of interest hippocampus, and amygdala), and the total brain, gray matter, white matter volumes, relative to ICV were investigated using analysis of covariance, with PMA and genotype (2 levels) as independent variables. I started with the following basic model to evaluate for possible interactions between the genotype status and the PMA on the brain volumes:

Model A <- lm (volume ~ Met group * PMA weeks).

However, if the Met group * PMA weeks interaction was not significant, the following simplified model was used to evaluate each of the brain volumes above:

Model B <- lm (volume ~ PMA weeks + Met group).

The final models included the PMA × genotype interaction only when its p-value was

<0.05. Sex showed no effects and was therefore not included in the final model. Since my hypotheses for genotype were directional, single-sided tests were performed for the genotype effects. Significance was defined at $p < 0.05$. The R software (version 3.6.2) was used for the analysis.

Table 1. Effects of BDNF Genotype on The Segmented Brain volumes

Brain Structure	Volume or % (Mean ± S.D.)		P-values*		
	Met-	Met+	PMA	Met+ Effect	PMA x Met+ status
Global or Regional Volume (mL)					
Intracranial (ICV)	477.9 ± 69.9	478.8 ±63.1	**< 0.0001**	0.465	0.465
Total Brain Volume	393.6 ± 52.7	394.8 ± 50.6	**< 0.0001**	0.494	0.326
Total Gray Matter	236.0 ± 39.5	235.9 ± 35.3	**< 0.0001**	0.412	0.439
Total White Matter	106.4 ± 11.8	107.2 ± 12.3	**0.007**	0.411	0.080
Hippocampus**	1.84 ± 2.78	1.79 ± 2.36	**< 0.0001**	0.136	0.451
Amygdala**	0.71 ± 0.11	0.69 ± 0.09	**< 0.0001**	0.125	0.284
Volume % Relative to ICV					
Total brain volume	82.5 ± 2.0	82.5 ± 1.8	**0.001**	**0.256 (NM)**	**0.025**
Gray matter	49.2 ± 1.6	49.1 ± 1.1	**< 0.0001**	0.320	0.450
White matter	22.5 ± 2.9	22.5 ± 2.2	**< 0.0001**	**0.030 (NM)**	**0.030**
Hippocampus	0.386 ± 0.020	0.376 ± 0.017	**0.009**	**0.015**	0.350
Amygdala	0.150 ± 0.012	0.145 ± 0.010	**0.008**	**0.040**	0.325

* P-values for the Met+ effect reflect single-sided post-hoc tests. The final models included the PMA x genotype interaction only when the interaction p-value was <0.05 (i.e., for %TBV and %WM relative to ICV).

** Hippocampal and amygdala volumes were averaged from left and right hemispheres.
NM= Not Meaningful – due to the interactions, these main effect p-values are not meaningful.
All p-values <0.05 are bolded.

Results

Participant Demographics and Characteristics (Table 2)

The Met+ and Met- groups had similar clinical characteristics, with no group differences in gestational age (38.9 ± 1.5 vs. 39.2 ± 1.2 weeks), sex or racial proportions. APGAR scores at birth were also similar at 1 minute and at 5-minutes.

The two groups also had similar weight, lengths, and head circumferences, both at birth and at the time of the scans.

Table 2. Demographic Characteristics of The Two Neonatal Groups (Mean ± SD)

	Met- (Val/Val)	Met+ (Met/Val or Met/Met)	P-value*
Sample size	n=29	n=37 (Met/Val:22 Met/Met:15)	
Gestational age at birth (weeks)	38.9 ± 1.5	39.2 ± 1.2	0.39
Sex, boy (%)	17 (58.6%)	19 (51.3%)	0.55
Apgar score at 1 minute (0-10)	7.6 ± 1.5	7.5 ± 1.7	0.82
Apgar score at 5 minutes (0-10)	8.8 ± 0.3	8.9 ± 0.2	0.41
Birth body weight (kg)	3.2 ± 0.4	3.2 ± 0.4	0.92
Length at birth (cm)	50.6 ± 2.7	50.6 ± 2.6	0.98
Head circumference at birth (cm)	34.1 ± 1.2	33.5 ± 3.8	0.40
Post-menstrual age at scan (weeks)	41.8 ± 2.4	41.9 ± 2.3	0.90
Weight at scan (kg)	3.8 ± 0.8	3.8 ± 0.6	0.85
Length at scan (cm)	52.7 ± 3.5	52.7 ± 0.0	0.95
Head circumference at scan (cm)	35.8 ± 1.9	35.8 ± 1.9	0.99
Race/Ethnicity			
Asian	2	3	
Native Hawaiian / Other Pacific Islander	2	8	0.38
White	0	1	
More Than One Race	25	25	

* P-values are from un-paired t-tests or chi-squared test (for Sex and Race proportions)

Polymorphism of Val66Met on global and regional brain volumes (Fig. 2)

As expected, all brain regions assessed showed age-dependent increases without normalization to the ICV (Fig. 3). The ICV and the % total gray matter volume relative to ICV were not different between the two groups (Fig. 2A, B), and both groups showed similar age-dependent increases in these variables (ICV: PMA-p < 0.0001, r = 0.82; % total gray matter volumes: PMA-p < 0.0001, r = 0.68). However, compared to the Met- group, the Met+ group had less steep age-dependent declines in relative total brain volumes (interaction-p = 0.025, Fig. 2C) and in relative white matter volumes (interaction-p = 0.003, Fig. 2D). Furthermore, the relative hippocampal volumes of the Met+ group (0.377 ± 0.003%) were significantly smaller than those of the Met- group (0.386 ± 0.003%; ANCOVA-p = 0.013, Fig. 2E).

Similarly, the relative amygdala volumes were also smaller in the Met+ compared to the Met- group (p = 0.041, Fig. 2F). Both groups also showed similar age-dependent decreases in relative hippocampal volumes (PMA-p = 0.009, r = -0.29) and relative amygdala volumes (PMA-p = 0.008; r = -0.29) (Fig. 2E, 2F).

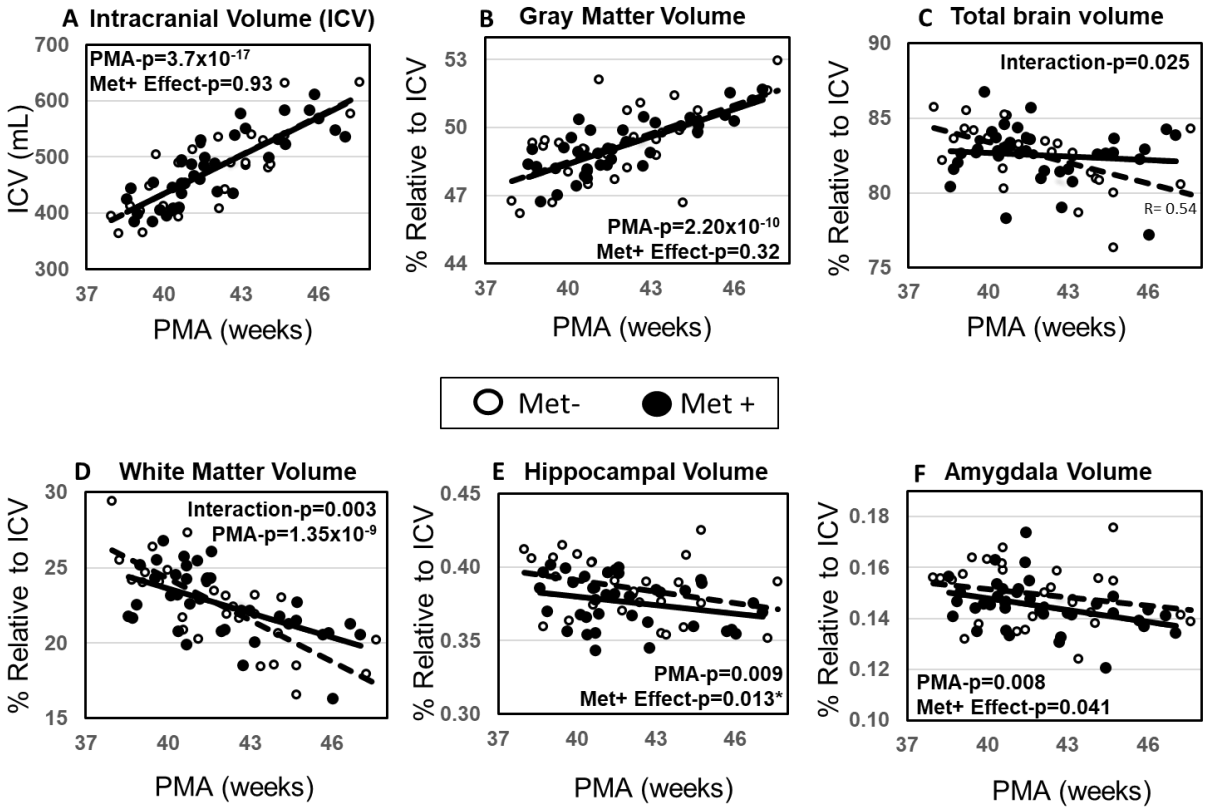

Fig. 2. Scatterplots comparing the morphometric measures of global and regional brain volumes in the Met- (open circles, dotted lines) and the Met+ (black circles, and solid lines) infants. Both groups showed no difference in age-dependent increase in intracranial volumes (ICV) (**A**) or in % total gray matter volumes relative to ICV (**B**). However, compared to Met-, the Met+ group showed less steep age-dependent decreases in % total brain volume relative to ICV (**C**) and in % white matter volume relative to ICV (**D**). Met+ subjects also showed smaller % hippocampus volumes relative to ICV (**E**), and smaller % amygdala volumes relative to ICV (**F**).

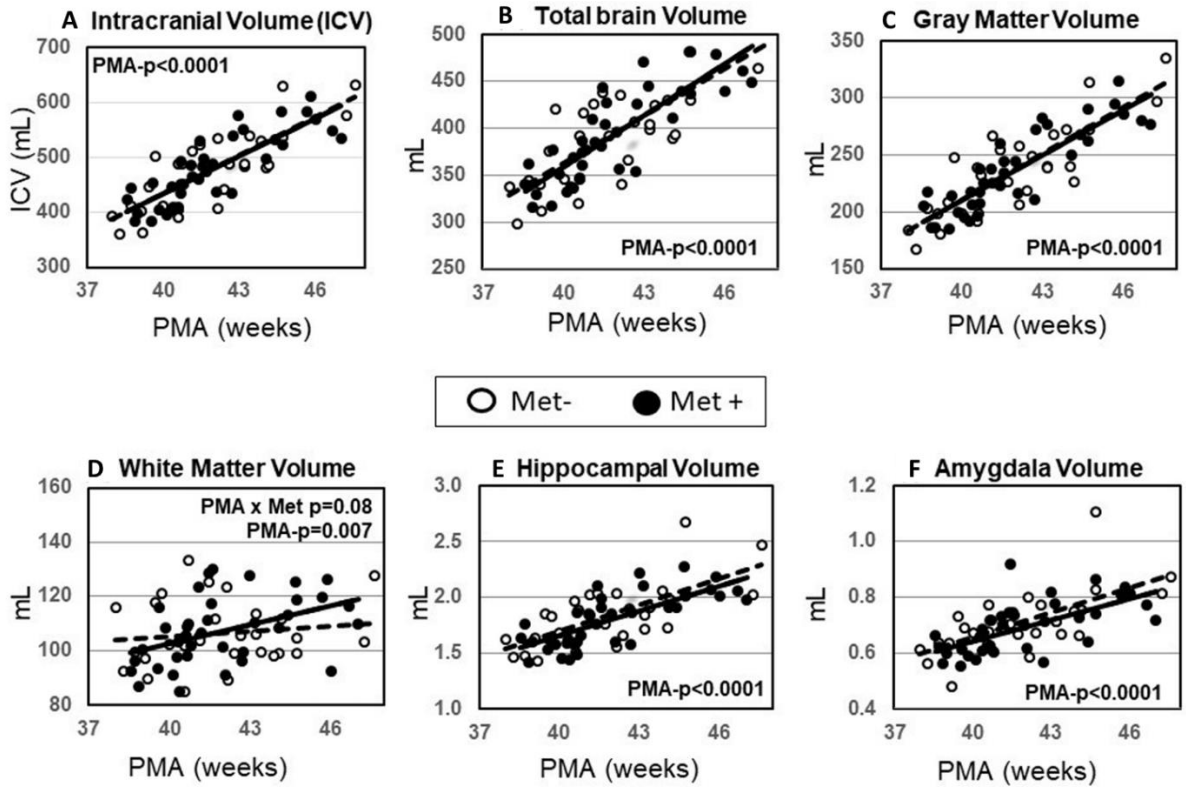

Fig. 3. Scatterplots similar to those in Fig. 2 comparing the two subject groups (Met-: open circles, dotted lines; Met+: black circles, solid lines), are shown for the volumes in each brain regions assessed, except these were the actual measurements without normalization to ICV. All brain volumes, globally or regionally, showed the expected age-dependent increase across the PMA studied.

Discussion

In my healthy neonatal cohort, the Met+ group had smaller %hippocampi, smaller %amygdalae, and slower age-dependent decline in %total white matter volumes, and hence slower age-dependent decline in %total brain volumes, compared to the Met- group. These findings are consistent with findings from rodent models with haplo-insufficient BDNF,[30] and from prior brain morphometry studies in participants with the BDNF-Val66Met variant, including healthy adults[40,41] or infants from parents with psychiatric history,[42] or were exposed to maternal anxiety.[51] My results also indicate that the smaller hippocampi and amygdalae represent prenatal phenotypic expression of the Met+ allele, rather than atrophy due to neurodegeneration or slower development after birth. However, the apparently

slower age-dependent declines in the relative total brain and white matter volumes of the Met+ group also suggest a negative effect of the BDNF-Val66Met variant on the postnatal developmental process; future longitudinal studies are needed to validate these cross-sectional observations.

Although the influence of Val66Met on hippocampal development remains unclear, mice with BDNF-Val66Met demonstrated defective regulated secretion of BDNF from hippocampal neurons despite normal constitutive secretion, and decreased dendritic arbor complexity in dentate gyrus.[32] Therefore, one possible mechanism is the reduced levels of BDNF secretion in the Met+ group that might affect the hippocampal and amygdala development in humans. Further studies are needed to investigate the possible interactive relationships between the Val66Met variant and other potentially negative prenatal influences, such as maternal anxiety on methylome [51] or prenatal substance exposure[46] (e.g., maternal tobacco, marijuana or alcohol use) on neonatal brain volumes.

The Val66Met variant is a known risk factor for the onset of neuropsychiatric disorders,[52] and both smaller hippocampi and amygdalae were commonly reported in individuals with neuropsychiatric disorders.[37] Whether the smaller hippocampal volume in the Met+ group also predicts future development of neuropsychiatric disorders is yet to be investigated.

This study has several limitations. First, the cohort size was relatively small which did not allow us to compare the hippocampal volumes of Met+/Met+ versus Val/Met+, in order to investigate the dose effect of the Met+ allele. Second, the majority of the participants were of mixed race; future larger study should include the genetic ancestry factor as a co-variate in the comparison, or to evaluate the possible racial contribution on the expression of this variant. Third, since I quantified global anatomical structures (gray and white matters and total brain) and included only amygdala and hippocampus as regional structures, the effect of Val66Met on other brain regions remains to be investigated. I did not correct the p-values after multiple comparisons of six anatomical regions, given the hypothesis-testing nature of the study, and to avoid false-negative findings. Although the findings support my hypothesis that the effects of BDNF-Val66Met genotype on relative volumes of the hippocampus and amygdala would be evident even at birth, a larger confirmative study would strengthen my conclusions.

In summary, the BDNF-Val66Met variant was associated with smaller hippocampal and amygdala volumes in newborn infants, suggesting that the Met+ genotype affected prenatal developmental processes. Lastly, the apparent slower age-dependent declines in the relative total brain and white matter volumes of the Met+ group suggest a negative effect of the BDNF-Val66Met variant on the postnatal developmental process, which needs to be validated in future longitudinal studies

Ethics approval

This study was approved by the Cooperative Institutional Review Board of the Queen's Medical Center, the University of Hawaii and the Johns Hopkins University.

Consent to participate

All parents of the infants signed the IRB-approved informed consent forms before the study procedures.

Acknowledgments

I thank all the families for their willingness to participate in this study.

Summary

In this study, early postnatal brain volume was measured using 3D-MRI images, and found the following results;

1) In very low birth weight infants, measuring not only the head circumference but also the brain volume helps predict the neural prognosis, and leads to therapeutic education and additional medical care in high-risk infants.
2) BDNF-Val66Met variant has ongoing negative influence on the postnatal developmental process.

A detailed assessment of early postnatal brain volume using 3D-MRI images can minimize acquired effects. It is a useful evaluation method for predicting clinical and genetic effects on neurodevelopment in children. Longer longitudinal studies are warranted to complimentarily evaluate an association of these clinical and genetical effects with future developmental abilities in children.

References

1. Kawasaki Y, Yoshida T, Matsui M, et al. Clinical Factors That Affect the Relationship between Head Circumference and Brain Volume in Very-Low-Birth-Weight Infants. J Neuroimaging 2019;1: 104-110.
2. Kawasaki Y, Oishi K, Hernandez A, et al. Brain‐derived neurotrophic factor Val66Met variant on brain volumes in infants. Brain Structure and Function 2021; 226:919–925.
3. Harris SR. Measuring head circumference: Update on infant microcephaly. Can Fam Physician 2015;61:680-684.
4. Vinchon M, Rekate H, Kulkarni AV. Pediatric hydrocephalus outcomes: a review. Fluids Barriers CNS 2012;9:18.
5. Woods CG, Parker A. Investigating microcephaly. Arch Dis Child 2013;98:707-713.
6. Cooke RWI, Lucas A, Yudkin PLN, et al. Head circumference as an index of brain-weight in fetus and newborn. Early Human Development 1977;1:145-149.
7. Bartholomeusz HH, Courchesne E, Karns CM. Relationship between head circumference and brain volume in healthy normal toddlers, children, and adults. Neuropediatrics 2002;33:239-241.
8. Ivanovic DM, Leiva BP, Perez HT, et al. Head size and intelligence, learning, nutritional status and brain development. Head, IQ, learning, nutrition and brain. Neuropsychologia 2004;42:1118-1131.
9. Spittle AJ, Orton J. Cerebral palsy and developmental coordination disorder in children born preterm. Semin Fetal Neonatal Med 2014;19:84-89.
10. Marlow N, Wolke D, Bracewell MA, Samara M, Group EPS. Neurologic and developmental disability at six years of age after extremely preterm birth. N Engl J Med 2005;352:9-19.
11. Cheong JL, Hunt RW, Anderson PJ,et al. Head growth in preterm infants: correlation with magnetic resonance imaging and neurodevelopmental outcome. Pediatrics 2008;121:e1534-540.
12. Spittle A, Orton J, Anderson PJ, et al. Early developmental intervention programmes provided post hospital discharge to prevent motor and cognitive impairment in preterm infants. Cochrane Database Syst Rev 2015:CD005495.
13. Wood NS, Costeloe K, Gibson AT, et al. The EPICure study: associations and

antecedents of neurological and developmental disability at 30 months of age following extremely preterm birth. Arch Dis Child Fetal Neonatal Ed 2005;90:F134-140.
14. van Vliet EO, de Kieviet JF, Oosterlaan J, et al. Perinatal infections and neurodevelopmental outcome in very preterm and very low-birth-weight infants: a meta-analysis. JAMA Pediatr 2013;167:662-668.
15. Linsell L, Malouf R, Morris J, et al. Prognostic factors for poor cognitive development in children born very preterm or with very low birth weight: a systematic review. JAMA Pediatr 2015;169:1162-1172.
16. Papile LA, Burstein J, Burstein R, et al. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr 1978; 92:529-534.
17. Itabashi K, Miura F, Uehara R, Nakamura Y. New Japanese neonatal anthropometric charts for gestational age at birth. Pediatr Int 2014;56:702-708.
18. Morisaki N, Belfort MB, McCormick MC, et al. Correction: Brief parenteral nutrition accelerates weight gain, head growth even in healthy VLBWs. PLoS One 2015;10:e0143984.
19. McCarty DB, Peat JR, Malcolm WF, et al. Dolichocephaly in preterm infants: prevalence, risk factors, and early motor outcomes. Am J Perinatol 2017;34:372-378.
20. Wilbrand JF, Schmidtberg K, Bierther U, et al. Clinical classification of infant nonsynostotic cranial deformity. J Pediatr 2012;161:1120-1125.
21. Barbier A, Boivin A, Yoon W, et al. New reference curves for head circumference at birth, by gestational age. Pediatrics 2013;131:e1158-1167.
22. Buckler JM, Green M. A comparison of the early growth of twins and singletons. Ann Hum Biol 2004;31:311-332.
23. Dukart J, Schroeter ML, Mueller K, Alzheimer's Disease Neuroimaging I. Age correction in dementia--matching to a healthy brain. PLoS One 2011;6:e22193.
24. Adams-Chapman I, Heyne RJ, DeMauro SB, et al. Neurodevelopmental impairment among extremely preterm infants in the neonatal research network. Pediatrics 2018;141.
25. Youden WJ. Index for rating diagnostic tests. Cancer 1950;3:32-5.
26. Inder TE, Warfield SK, Wang H, et al. Abnormal cerebral structure is present at

term in premature infants. Pediatrics 2005;115:286-94.
27. Solsnes AE, Sripada K, Yendiki A, et al. Limited microstructural and connectivity deficits despite subcortical volume reductions in school-aged children born preterm with very low birth weight. Neuroimage 2016;130:24-34.
28. Lima Giacobbo B, Doorduin J, Klein HC, et al. Brain-Derived Neurotrophic Factor in Brain Disorders: Focus on Neuroinflammation. Mol Neurobiol 2019; 56:3295-3312.
29. Cunha C, Brambilla R, Thomas KL, et al. A simple role for BDNF in learning and memory? Front Mol Neurosci 2010; 3:1.
30. Egan MF, Kojima M, Callicott JH, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 2003; 112:257-269.
31. Park H, Poo MM, et al. Neurotrophin regulation of neural circuit development and function Nat Rev Neurosci 2013; 14:7-23.
32. Chen ZY, Jing D, Bath KG, et al. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 2006; 314:140-143.
33. Harrisberger F, Smieskova R, Schmidt A, et al. BDNF Val66Met polymorphism and hippocampal volume in neuropsychiatric disorders: A systematic review and meta-analysis. Neurosci Biobehav Rev 2015; 55:107-118.
34. Hajek T, Kopecek M, Höschl C, et al. Reduced hippocampal volumes in healthy carriers of brain-derived neurotrophic factor Val66Met polymorphism: meta-analysis World J Biol Psychiatry 2012; 13:178-187.
35. Kambeitz JP, Bhattacharyya S, Kambeitz-Ilankovic LM, et al. Effect of BDNF val(66)met polymorphism on declarative memory and its neural substrate: a meta-analysis Neurosci Biobehav Rev 2012; 36:2165-2177.
36. Molendijk ML, Bus BA, Spinhoven P, et al. A systematic review and meta-analysis on the association between BDNF val(66)met and hippocampal volume--a genuine effect or a winners curse? Am J Med Genet B Neuropsychiatr Genet 2012;159b:731-740.
37. Harrisberger F, Spalek K, Smieskova R, et al. The association of the BDNF Val66Met polymorphism and the hippocampal volumes in healthy humans: a joint meta-analysis of published and new data. Neurosci Biobehav Rev 2014; 42:267-278.

38. Giedd JN, Rapoport JL Structural MRI of pediatric brain development: what have we learned and where are we going? Neuron 2010; 67:728-734.
39. Wierenga L, Langen M, Ambrosino S, et al. Typical development of basal ganglia, hippocampus, amygdala and cerebellum from age 7 to 24. Neuroimage 2014; 96:67-72.
40. Montag C, Weber B, Fliessbach K, et al. The BDNF Val66Met polymorphism impacts parahippocampal and amygdala volume in healthy humans: incremental support for a genetic risk factor for depression. Psychol Med 2009; 39:1831-1839.
41. Pezawas L, Verchinski BA, Mattay VS, et al. The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. J Neurosci 2004; 24:10099-10102.
42. Knickmeyer RC, Wang J, Zhu H, et al. Common variants in psychiatric risk genes predict brain structure at birth. Cereb Cortex 2014; 24:1230-1246.
43. Shimizu E, Hashimoto K, Iyo M Ethnic diference of the BDNF 196G/A (val66met) polymorphism frequencies: the possibility to explain ethnic mental traits. Am J Med Genet B Neuropsychiatr Genet 2014;126B:122–123.
44. Pivac N, Kim B, Nedic G, et al. Ethnic diferences in brain-derived neuro-trophic factor Val66Met polymorphism in Croatian and Korean healthy participants. Croat Med J 2009; 50:43–48.
45. Petryshen TL, Sabeti PC, Aldinger KA, et al. Population genetic study of the brain-derived neurotrophic factor (BDNF) gene. Mol Psychiatry 2010; 15:810–815.
46. Chang L, Oishi K, Skranes J, et al. Sex-Specific Alterations of White Matter Developmental Trajectories in Infants with Prenatal Exposure to Methamphetamine and Tobacco. JAMA Psychiatry 2016; 73:1217-1227.
47. Tang X, Oishi K, Faria AV, et al. Bayesian Parameter Estimation and Segmentation in the Multi-Atlas Random Orbit Model. PLoS One 2013;8: e65591.
48. Otsuka Y, Chang L, Kawasaki Y, et al. A Multi-Atlas Label Fusion Tool for Neonatal Brain MRI Parcellation and Quantification. J Neuroimaging 2019; 29:431-439.

49. Hashimoto T, Fukui K, Takeuchi H, et al. Effects of the BDNF Val66Met polymorphism on gray matter volume in typically developing children and adolescents. Cereb Cortex 2016; 26:1795–1803.
50. Bueller JA, Aftab M, Sen S, et al. BDNF Val66Met allele is associated with reduced hippocampal volume in healthy subjects. Biol Psychiatry 2006; 59:812–815.
51. Chen L, Pan H, Tuan TA, et al. Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism influences the association of the methylome with maternal anxiety and neonatal brain volumes. Dev Psychopathol 2015; 27:137-150.
52. Chen L, Lawlor DA, Lewis SJ, et al. Genetic association study of BDNF in depression: finding from two cohort studies and a meta-analysis. Am J Med Genet B Neuropsychiatr Genet 2008;147b:814-821.