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Altered Brain Functional Connectivity Varies by Form of Craniosynostosis

A Thesis Submitted to the Yale University School of Medicine
In Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

by
Alexander Haosi Sun
2018
ALTERED BRAIN FUNCTIONAL CONNECTIVITY VARIES BY FORM OF CRANIOSYNOSTOSIS. Alexander H. Sun, Jeffrey Eilbott, Carolyn Chuang, Jenny F. Yang, Eric D. Brooks, Joel Beckett, Derek M. Steinbacher, Kevin A. Pelphrey, John A. Persing. Section of Plastic and Reconstructive Surgery, Department of Surgery, Yale School of Medicine, New Haven, CT.

This study uses functional MRI (fMRI) to study long-term neurocognitive sequelae of nonsyndromic craniosynostosis (NSC), and understand if these aberrations vary by form of synostosis. Twenty adolescent participants with treated NSC (10 sagittal (SSO), 5 right unilateral coronal (UCS), 5 metopic (MSO)) were matched to controls by age, gender, and handedness. A subgroup of MSO was classified as severe metopic synostosis (SMS) based on the endocranial bifrontal angle. Resting-state fMRI was acquired in a 3T Siemens TIM Trio scanner, and data was motion-corrected, cluster-corrected with nonparametric permutation tests, and analyzed with BioImage Suite. SSO had decreased intrinsic connectivity compared to controls in the superior parietal lobules and the angular gyrus (p=0.071). UCS had decreased intrinsic connectivity throughout the prefrontal cortex (p=0.031). The SMS subgroup had significantly decreased connectivity among multiple subcortical structures. SSO had changes in regions associated with visuomotor integration and attention, while UCS had changes in circuits crucial in executive function. Finally, severity of metopic synostosis may influence the degree of neurocognitive aberration. This study provides neurologic evidence of long-term sequelae of NSC that varies by suture type, which may underlie different phenotypes of neurocognitive impairment.
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INTRODUCTION

Craniosynostosis is the premature fusion of one or more skull sutures in early development. Nonsyndromic craniosynostosis (NSC) typically only affects a single suture at a time, and does not include extracranial malformations. On the other hand, syndromic disease contains extracranial findings and typically affects multiple sutures. NSC has an incidence as high as 0.4 to 1 per 1000 live births, with sagittal synostosis (SSO) accounting for the highest percentage of cases.\textsuperscript{1,2} In recent years, the rates of metopic synostosis (MSO) have risen for unknown reasons, and have overtaken unilateral coronal synostosis (UCS) as the second most common form of nonsyndromic craniosynostosis.\textsuperscript{2-4} Depending on the suture that has prematurely fused, calvarial growth is affected in specific patterns that lead to characteristic cranial base and calvarial deformities.\textsuperscript{5} Therefore, craniosynostosis is surgically-corrected to normalize skull morphology.

It has been unclear, however, how the calvarial and skull base changes in craniosynostosis affect neurodevelopment in patients. Early on, restrictions in cranial development during periods of rapid brain growth were believed to primarily damage the brain through local increases in intracranial pressure (ICP).\textsuperscript{6} In early studies, there appeared to be a correlation between mental level and the type of head shape, with a majority of patients with scaphocephaly and plagiocephaly having normal intellect.\textsuperscript{7} Patients with multiple sutures fused had greater rates of impaired mental level, and worse intellectual outcome correlated with intracranial pressure changes for certain
head shapes, but not others.\textsuperscript{7} Other studies could not find a significant difference in mental development index based on whether patients have been operated on or not, which supported the belief that surgical correction of skull deformity in nonsyndromic craniosynostosis might be a primarily cosmetic procedure.\textsuperscript{8} A limitation to understanding the neurocognitive effects of craniosynostosis is that very few tests exist for infant neurocognitive testing, and several of the early studies focused on methods that have been demonstrated to be poor predictors of future cognitive impairment.\textsuperscript{9}

In recent years, neurocognitive testing in adolescents has begun to elucidate the neurocognitive burden of disease in nonsyndromic craniosynostosis with improved granularity. These studies have found that while there may not be dramatic intellectual impairment in patients with NSC, patients tend to have subtle neurocognitive deficits that persist in the long-term. These include higher rates of learning disorders and behavioral problems.\textsuperscript{10-14} In one cohort of sagittal synostosis patients, up to 50\% had a learning disorder, which can only be diagnosed in the setting of normal intellectual quotient.\textsuperscript{10} Other studies have found that while the differences between NSC patients and unaffected controls were small, NSC patients still performed worse than controls on achievement and intelligence quotient testing.\textsuperscript{11} Kapp-Simon et al. found that measures of math achievement and full-scale intelligence quotient were particularly different between NSC patients versus controls.\textsuperscript{11} In addition to achievement, several studies have sought to characterize executive function and behavior in patients with nonsyndromic craniosynostosis.\textsuperscript{13-15}
Due to limitations in testing, most behavioral studies are performed using parental and clinical questionnaires. A retrospective review by Becker et al. found that patients with nonsyndromic craniosynostosis had higher rates of documented behavioral or neurocognitive issues compared to the general population, with sagittal synostosis patients having the lowest rates among all suture types. Additionally, a study by Collett et al. directly compared a cohort of NSC patients to a cohort of unaffected controls, and found that while NSC patients tended to have more behavioral issues than unaffected controls, these differences were small with the exception of inhibitory control.\textsuperscript{15} Speltz et al. similarly concluded that NSC patients had higher reported behavioral problems compared to controls; but when segregated by suture types, sagittal synostosis patients had the lowest percentage with behavioral problems compared to other forms of NSC.\textsuperscript{14}

While neurocognitive and behavioral testing have indicated that patients with craniosynostosis have long-term aberrations in function, there has been no conclusive evidence of the etiology of this neurocognitive dysfunction in NSC patients. There are several theories about how craniosynostosis affects the brain. Previous studies with three-dimensional magnetic resonance imaging (MRI) of the brain have demonstrated that patients with NSC have altered cortical and subcortical neural organization.\textsuperscript{16} This aberrant neural organization can then affect the white matter tracts in the brain, which can lead to downstream neurocognitive effects.\textsuperscript{17} In 2011, a study by Florisson et al. used diffusion tensor imaging (DTI), a form of magnetic resonance imaging, to examine the microarchitecture of the white matter tracts in the
brains of patients with syndromic craniosynostosis. This study found that patients with syndromic craniosynostosis had discrete alterations in their white matter integrity, which is hypothesized to underlie some of the neurocognitive abnormalities that can be seen in these syndromes. However, the cause of the white matter disorganization is unclear. Traditionally, it was believed that skull constriction on brain growth during crucial periods of brain development led to altered brain morphology. Alternatively, because brain growth also governs skull growth, such as in the natural growth of the brain and fusion of the sutures, a primary brain malformation can possibly lead to secondary skull deformity. Florisson et al. concluded that because anisotropic changes were found in the whole brain in patients who had already been surgically treated, white matter alterations in syndromic craniosynostosis may be due to a primary brain disorder. Further supporting this theory is that there are known genetic mutations involved with syndromic forms of craniosynostosis that also directly affect brain parenchyma. Thus, neurocognitive pathology and calvarial pathology may not necessarily occur in a causative relationship, as aberrant genes can concurrently lead to downstream effects in multiple tissue types. There have not yet been conclusive studies looking at diffusion tensor imaging in the nonsyndromic craniosynostosis population. In 2014, Beckett et al. had preliminary findings of statistically significant alterations in mean diffusivity in certain regions of white matter in patients with nonsyndromic sagittal synostosis; however, future studies will need to examine this in a larger cohort of patients.
In addition to understanding the morphologic, anatomic sequelae of craniosynostosis, it is also necessary to understand how the functional networks of the brain are altered. Functional MRI (fMRI) has been an imaging modality used for over twenty years, with capabilities of spatially localizing brain activity and functional connectivity under various states. Functional MRI operates by characterizing the hemodynamic response, or blood-oxygen-level-dependent (BOLD) contrast signal, at each voxel in the brain for different neural states, including the resting state. By examining the resting brain in a task-independent setting, fMRI can identify how different regions of the brain fluctuate in BOLD contrast signal in patients with craniosynostosis compared to controls. Additionally, by performing these tests in adolescents, this study can ascertain whether any significant differences in neural activity between craniosynostosis patients and typically-developing controls persist in the long-term.

It has been previously demonstrated that there are alterations in intrinsic connectivity in the resting-state fMRI of nonsyndromic sagittal synostosis patients. These included decreased activation differences in the left angular gyrus and left superior parietal lobule (Brodmann’s Areas (BA) 7, 39, and 40), as well as increased activation differences in the cerebellum and medial frontal cortex (BA 8) in SSO patients compared to controls. These were determined by a more liberal threshold of \( p < 0.1 \) and cluster size \( (k = 150) \). Since that study, there has been a paradigm shift in the field of neuroimaging of how to best process clusterwise inference data. The previously used parametric methods of cluster correction tend to have increased rates of false positives, while nonparametric permutation tests can best control for these
false positives.\textsuperscript{21} The aim of this study is to compare functional connectivity changes in patients with nonsyndromic sagittal synostosis, right unilateral coronal synostosis, and metopic synostosis to assess for long-term changes in these patients using a nonparametric permutation method for cluster correction.
STATEMENT OF PURPOSE

Recent evidence has demonstrated that patients with nonsyndromic craniosynostosis (NSC) have greater rates of neurocognitive and behavioral abnormalities that persist into childhood and adolescence even after surgical correction of the deformity in infancy. The etiopathogenesis of these impairments is unclear, but may be due to either skull constriction during crucial periods of early brain development or due to a primary brain defect that has not yet been identified. Functional magnetic resonance imaging is a form of neuroimaging that seeks to elucidate any functional changes that may occur in nonsyndromic craniosynostosis.

**Aim 1:** Determine if there are statistically significant functional differences that persist in the long term in patients who have been surgically-treated for NSC compared to typically-developing controls.

**Rationale:** Craniosynostosis is becoming increasingly recognized as a condition with relatively significant neurologic sequelae. While our group has previously published a cohort of eight sagittal NSC patients with alterations in their white matter microstructure and with functional connectivity aberrations, these results were only approaching significance.²⁰
**Hypothesis:** It is hypothesized that patients with NSC will have regions of the brain with significant differences in resting-state functional connectivity compared to typically-developing controls.

**Aim 2:** Determine if differences in resting-state functional connectivity vary depending on the site of premature suture fusion.

**Rationale:** There are several forms of NSC, named by the site of suture fusion. These three forms of synostosis may not be etiologically or mechanistically similar, and may not have similar effects on long-term neurocognitive function. Previous studies have mainly focused on sagittal NSC, as this is the most common form of NSC.

**Hypothesis:** It is hypothesized that there may be discrete differences in functional connectivity patterns in different forms of NSC depending on site of suture fusion.

**Aim 3:** Understand how nonsyndromic craniosynostosis influences specific connections in the brain by performing a region of interest analysis. This analysis will focus on Brodmann Areas 7, 39, and 40 in the left hemisphere in this preliminary study.

**Rationale:** Previous literature has demonstrated aberrant connectivity in these specific areas for sagittal synostosis patients. These are areas that are involved with
language and visuospatial processing, and may be significantly affected in other populations of nonsyndromic craniosynostosis as well.

**Hypothesis:** It is hypothesized that Brodmann Areas 7, 39, and 40 are significantly altered on region of interest analysis for nonsyndromic craniosynostosis patients, but the specific connectivity differences may vary based on the type of synostosis.
METHODS

This was an IRB-approved prospective cohort study. Patients (7-15 years old) with nonsyndromic sagittal synostosis (SSO), right unilateral coronal synostosis (UCS), and metopic synostosis (MSO) were recruited from the Yale Craniofacial Center, and typically-developing controls were recruited at the Yale Child Study Center. Patient recruitment was performed by authors AS, JE, CC, JY, EB, and JB. Since surgery itself may affect the brain, all patients with gross changes to the brain seen on postoperative computed tomographic (CT) scans were excluded from this study. Craniosynostosis patients were individually matched to controls by age, gender, and handedness.

The subgroup of severe metopic synostosis (SMS) was determined based on the degree of deformity of the endocranial bifrontal angle (EBA).\textsuperscript{22} To calculate the EBA, the digital imaging and communications in medicine format (CT-DICOM) for CT data was retrieved for preoperative CT scans for all patients with metopic synostosis. Patients without available preoperative CT imaging were excluded from the SMS subgroup. Three-dimensional reconstruction of the CT-DICOM data was performed in Materialise Mimics 20.0 (Leuven, Belgium). The endocranial bifrontal angle was then calculated at the level of the most superior point of the crista galli, with the vertex of the angle located at the midline of the endocranial side of the frontal bone, and the end points at the lateral border of the orbital aperture on each side. Patients with an EBA of less than 124 degrees were included in the SMS subgroup. This
cutoff has been used in previous literature discussing severe metopic synostosis.\textsuperscript{3,22}

Three-dimensional reconstruction and calculation of the EBA was performed by author AS.

All test subjects underwent magnetic resonance imaging in a 3T Siemens TIM Trio scanner (Erlangen, Germany) with a 32-coil polarized head coil. Subjects were awake in the scanner and underwent a localizing scan, an MP-RAGE scan for anatomical detail (160 slices, 1.0 mm thick, FOV 256 mm, TR 1900 msec, TE 2.96 msec), and then resting state functional MRI (34 slices, 4.0 mm thick, FOV 220 mm, matrix size 64 x 64) using a T1-weighted sequence (TR 270 msec, TE 2.46 msec, FOV 220 mm, matrix size 256 x 256, flip angle 60 degrees). Resting state fMRI was acquired in a dark room isolated from any visual or auditory distractions to minimize aberrant stimuli. Subjects wore ear plugs and noise-cancelling headphones and were instructed to focus on a black digital display with a 1-inch white plus sign visible inside the scanner. Test subjects were old enough to understand directions and staff ensured that subjects were not asleep or moving during scans. Scans were conducted by staff from the Yale Magnetic Resonance Research Center, who also prepared patients for the scans. Scans were supervised by authors AS, JE, CC, JY, EB, or JB. These authors were also responsible for consenting patients and their families. Patients were given $100 for their participation in the study.

After the scan, subjects with gross anatomical aberrations, such as arachnoid cysts or other evidence of structural neurologic pathology, were excluded from the data
analysis. All scans were individually inspected for head motion and underwent nuisance regression with three translation (x, y, z) and three rotation motion (pitch, roll, yaw) parameters to correct for small movements using SPM (University College London, London, UK). Data then underwent cerebrospinal fluid and white matter signal regression and was registered to Montreal Neurological Institute (MNI) space. These steps were performed by author JE. Group degree analysis was used to generate output correlation maps, which were then smoothed to account for individual differences in registration and localization. This step and further analysis was performed by author AS with technical assistance from author JE. BioImage Suite (Yale School of Medicine, New Haven, CT) was used to analyze whole-brain intrinsic connectivity by generating four-dimensional group outputs for patients and controls. These resulting group-level t-maps were cluster-corrected using nonparametric permutation tests in FSL (FMRIB, Oxford, UK) with up to 5,000 permutations. Cluster-based thresholding was corrected for multiple comparisons by using the null distribution of the maximum cluster size with a voxel-level threshold of p<0.05. This then generated corrected p-value maps, and significance was set to alpha equals 0.05.

MNI coordinates of areas with significant findings were converted to Brodmann Areas based on a previously-defined atlas. Additionally, MNI coordinates were input into neurosynth.org to locate relevant literature and studies pertaining to those locations. Figures were generated by visualizing corrected p-value maps in BioImage Suite, with red-and yellow-colored overlays representing areas that have increased
intrinsic connectivity in patients compared to controls, and blue-colored overlays representing areas with decreased intrinsic connectivity in patients compared to controls. P-values were generated by recording the lowest-possible threshold that would show a difference in intrinsic connectivity in the corrected p-value map. These steps were performed by author AS.

Next, a region-of-interest (ROI) analysis was performed for seeds based on left hemisphere Brodmann Areas (BA) 7, 39, and 40. These regions were selected for their involvement in language processing and visuomotor attention, as well as their suggested implication in nonsyndromic craniosynostosis by a previous study. The ROIs were generated in MNI space in accordance to the same region that was defined by Beckett et al. in order to represent the defined Brodmann Areas of interest. Analysis and figure generation was then performed by author AS as above with intrinsic connectivity.
RESULTS

Twenty-four participants with surgically-treated NSC (11 SSO, 7 UCS, 6 MSO) were scanned. One patient with metopic synostosis was excluded because there was no left-handed control who could be used to match for the patient’s gender and age. Two patients with unicoronal synostosis were excluded because one could not be matched to a same-handed control, and the second was found to have a hematoma upon completing the MRI. One patient with sagittal synostosis was excluded after an arachnoid cyst was discovered upon completing the MRI. In total, twenty patients (10 SSO, 5 UCS, 5 MSO) were included in the study, and subject demographics for patients and matched controls are shown in Table I. On average, patients were between ten and twelve years of age. All patients and controls were right-handed. Three of the metopic synostosis patients had EBAs that were classified as the severe metopic synostosis (SMS) subgroup, which had an average EBA of 116.77±1.53 degrees.

Intrinsic Connectivity

On intrinsic connectivity analysis, sagittal synostosis (SSO) patients demonstrated areas of decreased connectivity compared to controls. Notably, these areas were localized in the bilateral Brodmann Areas 7, which is the superior parietal lobule, and the left BA-39, which is the angular gyrus component of the inferior parietal lobule (Figure 1, p=0.071). The unilateral coronal synostosis (UCS) patients demonstrated significant areas of decreased connectivity as well. These were localized in the
bilateral BA-11, right BA-38, and right BA-47 (Figure 2, p=0.031). BA-11 is the orbitofrontal cortex, which is the medioventral portion of the frontal lobe. BA-38 is the temporal pole, which is the most anterior point of the temporal lobe. BA-47 is a portion of the inferior frontal gyrus in the frontal cortex, and is located next to BA-11 and the orbitofrontal cortex. The metopic synostosis (MSO) patients did not demonstrate any significant areas of increased or decreased connectivity up to a threshold of alpha equals 0.100.

*Left Brodmann Area 7 Seed*

On seed-based analysis, the left BA-7 region of interest did not demonstrate any areas of increased or decreased connectivity in SSO at an alpha of 0.100. The UCS patients demonstrated areas of increased connectivity with the left BA-7 seed (Figure 3). These areas included the right BA-8, left BA-24, bilateral BA-10, bilateral BA-11, and bilateral BA-32 (p=0.065). BA-8 is a portion of the prefrontal cortex, and is a part of the frontal cortex that is directly anterior to the premotor cortex. BA-24 is a part of the anterior cingulate gyrus, located around the genu of the corpus callosum. BA-10 is the anteriormost portion of the prefrontal cortex, and includes parts of the superior and middle frontal gyri. BA-32 is also a portion of the cingulate cortex surrounding the outside of the anterior cingulate gyrus.

MSO patients also demonstrated areas of increased connectivity with right BA-44, right BA-45, the right insula, the right putamen, right BA-22, and right BA-47; however, these differences were observed at p=0.090 and may not be statistically
reliable (Figure 4). Brodmann Areas 44 and 45 are parts of the inferior frontal gyrus of the frontal cortex, which comprises Broca’s area in the dominant hemisphere. BA-22 is part of the superior temporal gyrus.

*Left Brodmann Area 39 Seed*

The left BA-39 seed did not demonstrate any significant differences between patients and controls in any group (SSO, UCS, or MSO) to an alpha of 0.100.

*Left Brodmann Area 40 Seed*

The left BA-40 seed did not demonstrate any significant differences between patients and controls for the SSO or MSO groups up to an alpha of 0.100. In the unilateral coronal synostosis patients, there was increased connectivity between this region of interest and several areas (Figure 5). This included bilateral BA-6, bilateral BA-8, bilateral BA-9, left BA-32 (p=0.050), as well as right BA-7 and right BA-39 (p=0.077). BA-6 is the premotor cortex in the frontal lobe, and BA-9 contributes to the dorsolateral and medial prefrontal cortices.

*Severe Metopic Synostosis Subgroup*

On intrinsic connectivity analysis, the severe metopic synostosis (SMS) subgroup demonstrated several areas with significantly decreased intrinsic connectivity (Figure 6). These were primarily localized in the bilateral caudate lobes, the left thalamus, the left putamen, the left insula, and the right hypothalamus (p=0.041). For the region of interest analysis, the left BA-7 seed demonstrated significant areas of decreased
connectivity throughout the left hemisphere, including BA-6, 8, 9, 10, 20, 21, 22, 44, 45, 46, 47, as well as the bilateral fusiform gyri, the right hippocampus, and the right parahippocampus (p=0.050, Figure 7). The left BA-39 seed also demonstrated significant areas of decreased connectivity with the bilateral caudate lobes, the bilateral hypothalami, the left thalamus, the left putamen, and the left amygdala (p=0.050, Figure 7). Finally, the left BA-40 seed had decreased connectivity with the bilateral visual association cortices and the bilateral primary visual cortices, the right BA-19, 20, 23, 31, the fusiform gyrus, and the parahippocampus (p=0.100, Figure 7).
DISCUSSION

In recent literature, several studies have begun to better characterize the neurocognitive changes that may develop in patients with single-suture craniosynostosis. Chieffo et al. demonstrated that patients with sagittal synostosis had visuospatial defects and visual memory recall deficits. Additionally, 17.1% of patients with sagittal synostosis had selective and sustained attention deficits, and approximately 30% of patients with unicoronal synostosis had issues with verbal fluency. Smaller percentages of the unicoronal patients also demonstrated issues with working memory and visual-attention skills. In 2016, Kapp-Simon et al. further corroborated these findings and demonstrated that patients with unilateral coronal synostosis performed worse on verbal comprehension, working memory, and language compared to controls. While these neurocognitive studies have begun to elucidate some of the findings in school-age patients, there are no studies that have linked these results to neuroimaging findings in the brain. This study is the first to demonstrate changes in brain functional connectivity that may underlie the long-term neurocognitive changes in nonsyndromic craniosynostosis even after surgical correction in infancy. Additionally, this study demonstrates that these changes in neural connectivity vary between different forms of NSC, depending on the original suture of fusion.

In the past, neuroimaging studies have found that in cases of syndromic craniosynostosis, such as Apert syndrome and Saethre-Chotzen syndrome, significant
changes in white matter microarchitecture can exist.\textsuperscript{18} These findings as well as others have suggested that the known mutations causing syndromic forms of craniosynostosis may also be directly causing a primary disorder in brain development that is not secondary to skull deformity or intracranial pressure.\textsuperscript{26} While mutational drivers of nonsyndromic craniosynostosis have been elucidated in some cases, the vast majority cases are still unknown in etiology.\textsuperscript{27,28} Therefore, it is unclear whether the neurocognitive changes seen in nonsyndromic craniosynostosis are also reflected in changes in neural activity or tissue microarchitecture.

Infant neuroimaging studies in recent years have suggested that due to highly plastic nature of the infant brain, many neuropsychiatric diseases may have origins during \textit{in utero} or neonatal brain development.\textsuperscript{29,30} Additionally, changes in functional connectivity have been correlated with early measures of cognitive performance.\textsuperscript{31,32} Currently, however, there have been no studies performed in infants with craniosynostosis. Beckett et al. demonstrated that brain connectivity and white matter structure may be altered in adolescents with nonsyndromic sagittal synostosis, although these results were preliminary and an expanded study and sample size is needed. In addition to examining the white matter microarchitecture, Beckett et al. found that patients with sagittal synostosis had altered functional connectivity in several regions of the brain. One limitation, however, is that this study only included a cohort of eight patients with SSO and eight matched controls. Additionally, in an effort to reduce the number of false positives in fMRI data, cluster correction methods have changed in the past several years. Based on recent neuroimaging literature, this
study aims to use a method of nonparametric permutations for cluster correction to reduce the number of false positives.21 As nonsyndromic craniosynostosis is a heterogeneous condition with a variety of etiologies and phenotypes, the neurocognitive effects may be just as variable. As a result, this study also seeks to examine cohorts separately based on the initial suture of fusion by including patients with metopic synostosis and unicoronal synostosis. In order to limit any confounding factors from sidedness of disease, only right-sided unilateral coronal synostosis was included in the UCS group.

Based on these resting-state fMRI results, there are long-term changes in brain connectivity in patients with nonsyndromic craniosynostosis that persist into adolescence, despite treatment for skull deformity in infancy. Additionally, the effects on resting-state connectivity vary based on the original suture of fusion. On intrinsic connectivity analysis, the SSO cohort demonstrated decreased connectivity mostly in the parietal lobe, in BA-7 and 39. These are the superior and inferior parietal lobules, which are associated with visuomotor attention and coordination, higher-level processing and language use, and memory retrieval and attention.33-36 The UCS cohort demonstrated significant areas of decreased intrinsic connectivity as well; but in contrast to the sagittal synostosis patients, these changes occurred mostly in the prefrontal cortex. Specifically, these areas were in the orbitofrontal cortex and the ventrolateral prefrontal cortex. These are areas associated with decision making, complex behavior planning, reasoning, and social behavior.37-39 Several of these regions are involved in disorders of executive function.40 Currently, there are limited
means to evaluate behavior other than relying on questionnaire data. A greater percentage of patients with nonsyndromic craniosynostosis have been demonstrated to have psychologic and behavioral abnormalities compared to the general population, although sagittal synostosis patients are the least likely of all forms of NSC to have abnormalities. While many behavioral abnormalities could be attributed to altered functional connectivity in the prefrontal cortex, future studies are needed to evaluate this relationship.

In this study, the metopic synostosis cohort did not demonstrate any significant alterations in intrinsic connectivity in the resting-state fMRI. The severe metopic synostosis subgroup, however, had significant areas of decreased connectivity found primarily in the insular cortex and subcortical areas such as the basal ganglia and thalamus. These subcortical structures serve as relay stations for the brain that are crucial in brain development, and connectivity changes may affect cognitive performance in early life. This study provides further evidence that the phenotype of metopic synostosis may be associated with the degree of neurocognitive impairment, which has been suggested in the past based on studies of auditory processing in infants. This may additionally serve as a basis for affecting operative decision-making in these patients. It is not clear, however, whether the severity of trigonocephaly directly impacts neurocognitive outcome, or if primary genetic factors separately govern both phenotype and neurocognitive outcome.
While functional neuroimaging in the resting-state provides a baseline for understanding how the brain is affected in the absence of stimulus, future studies will need to assess these patients using task-based paradigms that can be performed in the scanner. Tasks that will be especially relevant include spatial memory tasks and a go/no-go task to study behavioral inhibition.\(^{39}\) Paradigms such as these can then provide specific information about attention, visuospatial processing, and inhibitory control that cannot fully be captured by parental and clinician questionnaires. By correlating which regions of the brain are more or less active in the task-based setting, neuroimaging studies can begin to understand how the brain is affected in settings that may be relevant to real-world situations and academic achievement. This study focuses on the resting-state, which provides the first baseline analysis of connectivity changes in NSC in the absence of stimulus, and can serve as a comparison for future task-based fMRI studies.

One limitation of this study is the absence of preoperative data to serve as a comparison. Because of the relative clinical novelty of functional MRI, there are no preoperative scans available to serve as an internal longitudinal comparison for these patients. Additionally, because brain networks are not fully mature in infancy, preoperative fMRI data may not serve as an adequate baseline for adolescent scans. Currently, parcellation of the infant brain for fMRI is still not clearly delineated, but it is known that in the first two years of life, the infant brain undergoes dramatic, non-linear developmental changes in local subdivision.\(^{42}\) Specifically, while primary networks may already be developed in infancy, higher order networks have not yet
finished development in neonatal life. In addition to the maturation of higher order networks in the infant brain, synaptic pruning in early life leads to the reorganization of existing brain networks, which further complicates comparison studies in infants. Because of the drastic changes the brain undergoes from infancy into adolescence and the current understanding of the field, adult parcellations cannot be used in infants, and infant fMRI data cannot serve as a direct comparison to adolescent data. Additionally, it is also not feasible to use adolescent patients with untreated NSC as comparisons, although this is the ideal. As a result, this study used age-, gender-, and handedness-matched typically-developing controls as comparisons. In the future, studies will be able to correlate functional MRI throughout late adolescence and adulthood with long-term neurocognitive data. As neurocognitive testing begins to clarify neurologic and behavioral impairments with further granularity, functional neuroimaging data can be used to better correlate and understand what neural networks underlie the observed deficiencies.

This study is additionally limited by the small sample sizes per cohort. While the cohort sizes are small, all reported changes are significant at p<0.05. These conclusions are not necessarily definitive, but these preliminary findings suggest differences may indeed exist between the cohorts. In order to overcome this limitation, this study used a highly rigorous control selection methodology, and also performed cluster-correction using a nonparametric permutation method that has been demonstrated to be highly conservative in reducing false-positive rates compared to prior methods. In literature, prior parametric methods of cluster-correction can have
false-positive rates as high as 70%, while nonparametric methods can produce the expected 5% false-positive rate. In the interest of developing working hypotheses for all to test against, it is appropriate to present this information to stimulate further analysis.

The resting-state results in this study demonstrate that there are significant areas of functional connectivity in the brain that are altered in the long-term, and suggest that neural activity in patients with nonsyndromic craniosynostosis may not completely normalize despite treatment of the skull deformity in infancy. While the affected areas are known to be associated with certain functions, these changes in functional neuroimaging must be correlated to clinical findings by neurocognitive and behavioral testing. This will allow for a better understanding of the basis for neurocognitive impairment in these patients, and better tailoring of both operative and other supportive management for these patients.
CONCLUSION

While patients who have been surgically-treated for nonsyndromic craniosynostosis tend to perform normally on intelligence quotient testing, parents often report that there are subtle changes in behavior or neurocognitive status. In the past several years, several forms of detailed neurocognitive testing have been employed to understand the specific deficits that occur in nonsyndromic craniosynostosis. It is also critical, however, to understand how neural networks are functionally altered. This study has used functional MRI to demonstrate that patients with nonsyndromic craniosynostosis still have long-term effects that can be detected on functional neuroimaging. These changes may persist into adolescence despite early correction of the skull deformity in infancy. Additionally, the alterations in neural networks appears to vary by the initial suture of fusion and head shape deformity. Sagittal synostosis patients tended to have decreased connectivity in regions of the parietal cortex associated with spatial cognition, visuomotor integration, and attention. Right unilateral coronal patients demonstrated significantly decreased intrinsic connectivity in the prefrontal cortex, which plays a crucial role in executive function, as well as increased connectivity between the prefrontal cortex and the right parietal cortex. Of note, the metopic synostosis cohort did not demonstrate any significant changes on intrinsic connectivity analysis. However, the severe metopic synostosis subgroup had significant areas of decreased connectivity in the subcortical structures. Additionally, the region of interest analysis in this study has begun to elucidate how connectivity between different regions of the brain is specifically altered. Future directions for this
study will be to comprehensively analyze connectivity changes in the various brain networks, and also to perform more task-based functional imaging. Additionally, as neurocognitive testing begins to understand the neurocognitive impairments in craniosynostosis with further granularity, future studies will seek to understand how neuroimaging findings may underlie different phenotypes of impairment.
REFERENCES


8. Kapp-Simon KA, Figueroa A, Jocher CA, Schafer M. Longitudinal assessment of mental development in infants with nonsyndromic


Figure 1. Intrinsic connectivity analysis for sagittal synostosis patients. Axial slices represent MNI z=46, 54, 62, and 70. Blue areas represent areas of decreased activation in SSO subjects compared to controls (p=0.071).

Figure 2. Intrinsic connectivity analysis for right unilateral coronal synostosis patients. Axial slices represent MNI z=−31, -25, -19, and -13. Blue areas represent areas of decreased activation in UCS subjects compared to controls (p=0.031).
Figure 3. Seed-based analysis for right unilateral coronal synostosis patients for the left BA-7 seed. Axial slice represents MNI z=4. Red areas represent areas of increased connectivity with the BA-7 seed in UCS subjects compared to the same regions in controls (p=0.065).
Figure 4. Seed-based analysis for metopic synostosis patients for the left BA-7 seed. Axial slice represents MNI z=3. Red areas represent areas of increased connectivity with the BA-7 seed in MSO subjects compared to the same regions in controls (p=0.090).
**Figure 5.** Seed-based analysis for right unilateral coronal synostosis patients for the left BA-40 seed. Axial slice represents MNI z=40. Warm-colored areas represent areas of increased connectivity with the BA-40 seed in UCS subjects compared to the same regions in controls (red: $p=0.077$, yellow: $p=0.050$).
Figure 6. Intrinsic connectivity analysis for severe metopic synostosis patients. Axial slices represent MNI z=-18, -8, 2, and 12. Blue areas represent areas of decreased activation in SMS subjects compared to controls (p=0.041).

Figure 7. Seed-based analysis for severe metopic synostosis for the (a) left BA-7 seed (slice represents MNI z=-15, with blue representing areas of decreased connectivity with the BA-7 seed in SMS subjects compared to the same regions in controls at p=0.050), (b) left BA-39 seed (slice represents MNI z=13, with blue representing areas of decreased connectivity with the BA-39 seed in SMS subjects compared to the same regions in controls at p=0.050), and (c) left BA-40 seed (slice
represents MNI z=7, with blue representing areas of decreased connectivity with the BA-40 seed in SMS subjects compared to the same regions in controls at p=0.100).
**Table I. Patient Demographics**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Gender</th>
<th>Age (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sagittal Synostosis (SSO)</td>
<td>10</td>
<td>8 M, 2 F</td>
<td>11.9±2.3 years</td>
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<tr>
<td>SSO Matched Controls</td>
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<td>8 M, 2 F</td>
<td>12.6±2.2 years</td>
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<tr>
<td>Right Unilateral Coronal Synostosis (UCS)</td>
<td>5</td>
<td>4 M, 1 F</td>
<td>11.9±2.4 years</td>
</tr>
<tr>
<td>UCS Matched Controls</td>
<td>5</td>
<td>4 M, 1 F</td>
<td>11.9±2.6 years</td>
</tr>
<tr>
<td>Metopic Synostosis (MSO)</td>
<td>5</td>
<td>3 M, 2 F</td>
<td>10.8±2.4 years</td>
</tr>
<tr>
<td>MSO Matched Controls</td>
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<td>3 M, 2 F</td>
<td>11.1±2.2 years</td>
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<tr>
<td>Severe Metopic Synostosis (SMS)</td>
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<td>10.2±1.0 years</td>
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<tr>
<td>SMS Matched Controls</td>
<td>3</td>
<td>3 M, 0 F</td>
<td>10.4±0.8 years</td>
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