Neurodevelopmental Outcomes in Craniosynostosis Repair

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Abstract

Context: Craniosynostosis is a common craniofacial abnormality which can be associated with various clinical syndromes. Though it has been established that children with craniosynostosis score lower on certain developmental tests, the effect of craniosynostosis and cranioplasty surgery on the neural circuitry and brain development is less well known or understood.

Objectives: The purpose of this study is to describe the effect of cranial vault remodeling in children with craniosynostosis on white matter tracts with tractography and Diffusion tensor imaging (DTI), functional MRI, and neurodevelopmental tests, before and after surgery as compared to age-matched controls.

Study Design: This will be a prospective study of patients diagnosed with craniosynostosis and who are going to have open or endoscopic cranial vault remodeling (CVR).

Study Measures: The study will measure MRI sequences before and after surgery and at set time intervals to quantify the effect of white matter tract maturity. Parallel to this, neurodevelopmental tests will be administered at these same intervals.

Background and Rationale

Introduction

Craniosynostosis, the premature ossification and fusion of one or more cranial sutures, is a common craniofacial abnormality occurring in 1 of 2,000 live births. [1 2] Craniosynostosis can lead to increased intra-cranial pressure in between 12-50% of those affected which may have delayed neuro developmental implications.

The crucial neurodevelopment occurs in the first years of life and is most rapidly progressing during the first year of life. Premature closure of the cranial sutures decrease the intracranial volume and space available for the rapidly developing brain. Cranial vault remodeling (CVR) is the current standard of care to mitigate any possible developmental delay secondary to craniosynostosis and also improve the head shape. CVR is a combined surgery between the neurosurgeon who takes off the cranial bone/bones, and the craniofacial surgeon who assists and reconstructs the cranium with absorbable plates and screws.

After surgery, although there is no doubt that there is an improvement in head shape, the craniofacial skeleton and the soft tissues, but there is less data, and virtually no functional imaging information available addressing the effect of CVR surgery on the growing brain.

The main objective of this project would be to begin to explore the relationship of CVR and it requisite effects on the growing brain with a cohort of patients who are set to undergo CVR, while performing a battery of neurodevelopmental and imaging studies pre- and post-operatively and compare with normative controls. This is one part of a three-center study collaborating with craniofacial units at Yale University and Wake Forest University in which similar, if not identical protocols will be carried out. We hope that analyses of these data will provide better insight into and greater definition of the effect of CVR and the secondarily available increase in intra-cranial volume on the growing brain in the clinical setting of craniosynostosis.

Relevant Literature and Data

Cranial sutures are dense fibrous connections between adjacent intramembranously-formed bones which permit minor movement to take place. Sutures have several functions:

a) cranial molding allowing passage through a narrow birth canal by the combined process of sutural overlap and parietal bone deformation,
b) serving as shock absorbers, (c) permitting brain growth to take place, and
c) preventing the separation of bones. [3 4 5] Regardless of the underlying etiology, the early fusion of a suture abolishes further growth of the abutting bones in a direction perpendicular to the suture.

As a consequence, continued enlargement of the brain promotes compensatory overgrowth at other sutures, leading to progressive distortion in the skull shape. Multiple complications can arise because of raised intracranial pressure, facial deformities affecting vision, breathing, and dentition, and other features such as hearing loss, intellectual disability or behavioral issues that might be caused by the underlying gene defect or alternatively might occur secondary to craniosynostosis. [5 6 7 8 9]

Craniosynostosis may be characterized as simple (involving 1 suture) or complex (involving two or more sutures), primary (caused by an intrinsic defect in the suture) or secondary (premature closure of normal sutures because of another medical condition such as deficient brain growth), and isolated or nonsyndromic (occurring without other anomalies) or syndromic (accompanied by other dysmorphic features or developmental defects). All subclassifications of craniosynostosis can be genetic. [2] Craniosynostosis of the sagittal suture is the most commonly affected suture and shows a strong male predominance (male:female ratio of 3.5:1). It accounts for 40% to 58% of all cases of craniosynostosis . [2 6]

Nonsyndromic craniosynostosis (NSC) is usually an isolated abnormality, classified according to the involved suture. Early studies of the epidemiology of simple craniosynostosis noted a predominance of isolated sagittal synostosis, accounting for

more than half of all reported cases. Approximately 85% of cases are believed to be nonsyndromic with no identifiable gene mutation.[2] Syndromic craniosynostosis, on the other hand, can be associated with various dysmorphic features involving the face, skeleton, or nervous system and may be accompanied by developmental delay.

For specifics of development of neural circuitry, i.e., about 50% of adolescents with NSC experienced some sort of significant cognitive and/or executive planning dysfunction. Abnormal growth can also cause abnormal brain development, particularly in the white matter tracts of the brain. There is also known decreased synaptic branching and myelination in children with syndromic craniosynostosis, as evidenced by Diffusion tensor imaging (DTI).[10]

Other groups have begun to look at DTI as a methodology to examine the changes in the brain in children with craniosynostosis, but in older children [9 11], this group found that, "Although these patients have a normal fiber organization, increased diffusivity parameters suggest abnormal microstructural tissue properties of the investigated white matter tracts." Thus far, only animal studies demonstrate the effect of the CVR in a rabbit model of craniosynostosis, which suggests enhanced white matter tracts following intra-cranial expansion [12].(12) As this is a pilot study, we will plan to recruit our own control subjects, age matched to our test subjects, but also plan to compare them with de-identified publicly, available datasets, i.e., the Baby Connectome project once they are made available.[13] Our hypothesis is that CVR has a positive effect on white matter tract development and our proposed study seeks to perform the first human study of the relevant patient population.

Study Aims

Aim 1: We will *establish* pilot data to establish a normative curve of white-matter tract development in normal children under 4 years of age using (DTI). Fractional anisotropy (FA) (a main DTI parameter sensitive to white matter integrity) maps will be created to achieve a normative "white matter change curve" with from infancy to pre-school age.

Aim 2: Will *quantify* the change in white matter tract organization after cranial vault remodeling (CVR) surgery in patients with craniosynostosis by analyzing pre- and post-operative DTI data. This experimental cohort will also be examined in comparison to age-matched controls as compiled in Specific Aim #1, pooled with other centers and compared with published data sets of normal babies from the Baby Connectome Project. . The imaging will be repeated at three intervals after surgery leading up to approximately 4 years of age.

Aim 3: Will *assess* neurodevelopmental test results in craniosynostosis patients and seek to *correlate* these findings with white matter tract changes.

Study Population

This will be a prospective, longitudinal observational study. Prospective subjects will come from those patients seen as they present to the plastic and/or neurosurgery clinics and a diagnosis of craniosynostosis is suspected. Subsequently, if surgery is indicated, these patients will be approached and considered for inclusion the study. Of note, regardless of study participation it is our practice to follow children with CVR annually until the age of skeletal maturity, i.e., between 16-18 years of age. Annual visits may include but are not limited to the clinic exam, imaging, neurodevelopmental testing and or laboratory blood work, all of which are part of the standard of care for children with craniosynostosis.

We estimate that the duration of this study to be completed by December 31, 2030. We intend to include up to 500 subjects diagnosed with craniosynostosis and up to 60 normal control subjects in this study. The normal control subjects will undergo only a single MRI without sedation, using previously published calming techniques [13] and a single Bayley and/or Mullen Scales (age appropriate) administered just prior to the MRI. If the child is deemed within normal limits, they can proceed to the MRI imaging.. We plan to recruit normal, age-matched controls with an awarded UAMS Research Scholar Pilot grant. We will recruit control patients from our neurosurgery and plastic surgery clinics. These patients will be chosen amongst healthy children as stated in the inclusion /exclusion criteria. We expect these patients to be mostly patients who are referred to our clinic with abnormal head shapes due to position but does not have craniosynostosis or any related health problems.

Inclusion Criteria

- Up to 500 children, for all subjects, age of initial study entry: one test group between 3-6 months old (endoscopic assisted), and the other 6-24 months of age (open CVR)) once enrolled, children will be included until 4 years of age.
- Diagnosed with craniosynostosis, single or multiple suture
- Babies with craniosynostosis syndrome including but not limited to Pfieffers, Aperts, Cruozons, Muenkes.
- Approximately 60 age-matched **controls** (3 in each group) not diagnosed with craniosynostosis syndrome, stratified by age groups starting at 3 months, 5 months, 7 months, 9 month 11 months, 13 months, 15 months, 17 months, 19 months, then every 3 months, 22 months, 25 months, 28 months, 31 months, 34 months, 37 months,40 months,43 months,46 months,49 months.

Exclusion Criteria

- Children not meeting the inclusion criteria
- Children with traumatic brain injury, brain cancer, or VP shunt that could affect development.
- Children who have already undergone CVR repair
- **Developmentally Normal children who cannot undergo or cannot be still for an awake or asleep non-sedated MRI**

Study Design and Procedures

This observational study intends to collect all available patient data, including but not limited to:

- Age
- Date of birth
- Sex
- Race
- Ethnicity
- Physical Examination
- Personal Medical History
- Family Medical History
- Presenting clinical status
- Craniosynostosis type
- Present surgical history and procedures
- Inpatient Progress notes
- Discharge summaries
- Clinic notes
- Other interventions
- All Laboratory Results
- All Imaging Results
- Neurodevelopmental Assessments

Radiological Component

Standard-of-care at ACH and at other institutions is initial imaging to confirm the diagnosis of craniosynostosis and identify and any other intra-cranial abnormalities. This initial imaging can be in the form of either a low-dose radiation computed-tomography (CT) of the head or a MRI. Post-operative imaging, either CT or MRI, is based on clinical indicators and not routinely performed on all patients. Once the patient is identified and consented for the study, we will implement an MRI-based protocol to look at both the bone and the brain, thus obviating the need for a CT scan, however, in some cases a CT scan may need to be obtained or have been obtained at an outside facility.

Presurgical MRI will be done on a 3T MRI (Prisma Siemens MRI scanner) or 1.5T (Achiva, Phillips MRI scanner). The protocol for patients less than 18 months of age will be Sagittal T1, Axial inversion recovery (IR), T2, fluid-attenuated inversion recovery (FLAIR), susceptibility weighted image (SWI), diffusion weighted images (DWI), diffusion tensor imaging (DTI), resting state fMRI, and sagittal 3D GRE / FFE (on 1.5 T scanners) or 3D T1 VIBE (on 3T scanner).

The MRI protocol for patients over 18 months of age will be: Sagittal T1, coronal T2, Axial T2, fluid-attenuated inversion recovery (FLAIR), susceptibility weighted image (SWI), diffusion weighted images (DWI), diffusion tensor imaging (DTI), resting state fMRI, and sagittal 3D GRE / FFE (on 1.5 T scanners) or 3D T1 VIBE (on 3T scanner).

Post-processing of the acquired 3D GRE, FFE and T1VIBE sequences will be done on vitrea station. The acquired images will be reviewed by US board-certified pediatric neuroradiologists for single or multi suture craniosynostosis. Intracranial volume, ventricular size and sulcal crowding will be accessed. The DTI data will be analyzed on a password protected workstation by MRI physicist (Co -investigator, Dr. Ou and his research assistant, Xiaoxu Na) for evaluation of white matter microstructural integrity. Other imaging data from the same MRI sequences may also be exported to the workstation for additional quantitative analysis.

The expected time for MRI image acquisition is approximately 60 minutes for control subjects. If this process is prolonged greater than 2 hours due to control subject's lack of cooperation or discomfort then it will be aborted and will be re-scheduled. If the parents do not wish to proceed with a rescheduled time then the control subject will be removed from the study.

The MRI protocols will be identical for the control subjects and scheduled at the parent's convenience, but falling within one week on either side of their requested age. Because scheduling of the MRI may not allow this degree of control, control patients will be stratified by the age when they actually undergo the study not the age of recruitment. We will not plan to sedate the normal control subjects, however, sedation *may be* a

component for test subjects who, for developmental or behavioral reasons, could not successfully complete an imaging study necessary for diagnosis and completion of the study. We will carefully detail the risks of performing the MRI under sedation as expanded in the risks section below. One Pre-operative MRI is clinically indicated to establish and confirm the diagnosis and the three post-operative MRI's will be done for research purposes.

Surgical Component (Plastic Surgery & Neurosurgery)

Once patients are identified and surgery is planned, the standard of care procedures fall into two categories based on age of presentation and clinician judgement and family preference all other things being equal. Both the endoscopic-assisted craniectomy and open CVR are standard of care procedures performed at every major craniofacial center. The ideal candidate for endoscopic-assisted craniectomies are infants with confirmed craniosynostosis (typically sagittal) who present at an age in which they can be operated on within 3-6 months of age. The ideal candidate for open CVR is any patient with confirmed craniosynostosis in which surgery can be performed after 6 months old. It has been our experience that these patients self-select into these groups solely by what age they present to our clinic. None of the surgery performed nor any aspect of the surgery is experimental.

Neurodevelopmental Assessment Component

Under direction of Dr. Tiffany Howell and her teams routinely examine patients with craniosynostosis both pre-operatively and post-operatively as part of American Cleft palate craniofacial Association (ACPA) guidelines and standard of care. Thus, these examinations are part of the normal clinical exam and part of the medical record. The Bayley Scales of Infant and Toddler Development are used up to and including age 4 and the Mullen Scales of Early Learning are used from birth to 68 months.

Control Group Neurodevelopmental Assessment

Control subjects will be tested by ACH but if published MRI and developmental testing are readily available and sufficient for data analysis that will be used instead of enrolling control patients.

Schedule of Imaging and Neurodevelopmental Assessment

1. **PRE-OP** Within 30 days of the surgery date (all 3 components may occur on the same day or different days for family convenience):

- pre-anesthesia testing (45-minute duration) & pre-operative phlebotomy including, type and screen (2-4ml), complete blood count (CBC) (0.5ml), and possibly basic metabolic panel (0.5ml), and coagulation panel (2-4ml)
- initial neurodevelopmental testing (Bayley and Mullen Scales – 1 hour duration performed by Dr. Dr. Tiffany Howell)
- preoperative MRI functional sequences (60 minute duration) (research-related)

2. **Surgery Day:** Two cohorts:
   - endoscopic-assisted group (age range at surgery between 3- and 6-months old) and
   - Open cranial vault remodeling group (age range at surgery between 6- and 24-months old).
3. Six (6) to 6-months post-op:
   - clinic visit,
   - Neurodevelopmental testing (research-related)
   - repeat MRI Imaging(60 minute duration) (research-related)
4. Twelve (12-) to18-months post-op:
   - clinic visit,
   - Neurodevelopmental testing (may be repeated earlier at 9-15 months if clinically indicated) (research-related)
   - Repeat MRI imaging(60 minute duration) (research-related)
5. Pre-school age (4 years old and younger):
   - clinic visit
   - Neurodevelopmental testing (research-related)
   - repeat MRI Imaging(60 minute duration) (research-related)

Risks and Benefits

One potential risk to study participants is the potential for loss of confidentiality. Measures to protect the confidentiality of study participants will be implemented as described in the Data Handling and Recordkeeping section below. Another potential small risk is the additional anesthesia for the research related MRI's both in terms of the subjective experience of the test subjects themselves and risk of administering

sedation, if and only if non-pharmacologic techniques do not produce a state in which the radiologist (Dr. Raghu) thinks a high-quality image can be generated. Our team will attempt the non-sedation techniques first and if a child does need sedation to successfully complete the imaging study, the following risks will apply and be explained in the consent to the parents including: administration of medications for anesthesia including inhaled sevoflurane, intravenous propofol which have known side effects of nausea, vomiting, tachycardia, respiratory obstruction, hemodynamic instability, cough or laryngospasm. In addition other known complications of using a controlled airway, i.e., laryngeal mask airway (LMA) for children > 1 year old and endo-tracheal tube for < 1 year old, include tooth or mucosal injury, malignant hyperthermia, allergic reaction to an agent given.

There is also the risk assosciated with putting in performing intravenous (IV) access and these risk are typically infiltration of saline or anethestic agents such as propofol into the skin or hematoma and/or bruising. There is also a risk of mulitple sites puncture wounds, if a child if intra-venous access proves difficult. If for whatever reason the imaging study can not be performed within a reasonable parental expectation then the child will not be included in the study, but continue clinical care regardless.

All these common risks will be explained to the parents and if a side-effect or complication occurs, all treatment will be administered by the anesthesia team and the study coordinator and PI's will be notified and the IRB through our data-safety monitor within 24 hours of occurrence.

The potential risk of the MRI itself is the risk of causing anxiety in the patients and families and we plan to adopt the following measures borrowed from the techniques described from the investigators of the NIH funded Baby Connectome Project [13] for non-sedated MRI included but not limited to a tour of the MRI area prior to the scheduled MRI to familiarize the child and parents with the scan experience, prepping the parents with the headphones the child will wear, use of attenuating foam on the scanne, sandbags to reduce vibrations, dim lights, and pre-selected movies for the awake scans for the child.

Regarding neurodevelopment as we are NOT planning to sedate normal children the issue becomes the risk of the inhalational agents used (in this case sevoflurane) on neurodevelopment outcomes for the up to three additional post-op MRI's for the test subjects. Although the FDA issued a statement of the risks to neurodevelopment in children under 3 specifically " repeated or lengthy use of general anesthetic during surgeries or procedures in children under 3... may affect development of their child's brain" (http://www.fda.gov/Drugs/DrugSafety/ucm532356.html) However, many of these concerns were actually extrapolated from pre-clinical animal studies. In our and others

[15] examination of the human trials evidence—there is scant evidence for clinically significant detriment to neurodevelopment in children.

In his excellent and balanced review paper Davidson et.al [15] counsels that in some of the best studies that DO show a statistical difference between exposed children and age-match un-exposed cohorts, the difference in school grades was 0.41% lower and IQ 0.97% lower in the exposed cohort. [14] In the MASK study, one exposure of anesthetic was associated with a 1.3 IQ point lower in the exposed cohort, but this was not statistically significant. [16] In the PANDA trial published in JAMA with a mean duration of 80 minutes of anesthesia, IQ's were not statistically different when compared with age- matched controls, moreover their study did not show any difference in memory, learning language visuospatial reason and executive functioning. [17] In Davidsons randomized control trial of patients to spinal anesthesia vs general anesthesia with a median exposure time of 54minutes, there was no difference in measureable neurodevelopmental outcomes. [18]

Regarding multiple exposure risk, much of this data comes from the Olmstead County birth cohort (1976-1982) in which about 88% of the cohort received halothane for a mean of 133 min and median of 75 minutes, the authors showed a 2.4% increased incidence of attention deficit hyperactivity disorder in the multiple-exposure cohort.

All these studies must be considered with the following factors. For one thing, it is impossible to separate some of the confounding factors to isolate the causative effect of anesthesia from others, most notably the surgery itself. Two, some other factors such as sex and maternal education have been shown to have a much stronger influence on all measurable neurodevelopmental outcomes than the measurable associations of anesthetic. Many of the other health and social factors that would necessitate multiple surgeries (and anesthetics) for whatever reason, may contribute to a larger extent to these measurable outcomes. Thus, there is mixed evidence at best, that there is a significant risk to neurodevelopmental outcomes of the exposure to general anesthetic in this patient population, particular in this study with a maximum of three additional exposures totaling approximately 90 minutes. This is all the more important to consider in the context that it will be impossible to state whether the child's potential developmental problems, were from the craniosynostosis, the anesthesia or any subsequent anesthetic exposures. It is important to note that the duration of anesthesia in the test subjects undergoing surgery will be anywhere from 2 hours to 5 hours and thus 4-10 times longer than any MRI with sedation they might have after. Moreover, if those patients need sedation they will receive it an older age (i.e. post-op) where there is arguably less change of any further anesthetic exposure affecting development.

As stated, our team will attempt non-pharmacologic calming methods with ALL patients first but will clearly explain to parents the small additional risk of additional MRI's if sedation is necessary. There is a benefit of avoiding the routine use of radiation to diagnose the craniosynostosis, as we would elect to obtain the 'black-bone MRI' pre-operatively which has windowing that emulates the look of a CT scan, enabling easier interpretation.

There is also another potential benefit of identifying neurodevelopmental abnormalities not readily identified with standard CT scans, for both subjects and control subjects. In the event that unexpected neurodevelopmental abnormalities are noted, the subject and parents will be referred to the appropriate clinic for follow-up. In addition, the assurance of a negative scan (i.e., normal) is an added benefit to reassure parents.

Handling of Adverse Events (AE)

ADVERSE EVENTS

Definitions

An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as AEs. Abnormal results of diagnostic procedures are considered to be AEs if the abnormality:

- results in study withdrawal;
- is associated with a serious AE;
- is associated with clinical signs or symptoms;
- leads to additional treatment or to further diagnostic tests; or
- is considered by the investigator to be of clinical significance.

Serious AE (SAE) means any untoward medical occurrence that at any dose: An event is "serious" if it involves considerable detriment or harm to one or more persons (who may or may not be participants), or required intervention to prevent one or more persons from experiencing considerable detriment or harm. SAEs include:

- Death
- Life-threatening experience – Disease or condition where the likelihood of death is high unless the course of the disease/condition is interrupted or diseases/conditions with potentially fatal outcomes where the end point of the clinical trial analysis is survival
- Inpatient hospitalization or prolongation of hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect in participant's offspring

- Any other important medical event that, based upon appropriate medical judgment, may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, the development of drug dependency or drug abuse, suicidal ideation or attempts, or the unintentional revealing of some genetic information to insurers.

Related

An event is "related" if more likely than not it was caused by the research activity.

Unexpected

An event is "unexpected" when its specificity, nature, severity or incidence is not accurately reflected in the consent form, protocol, or investigator's brochure previously reviewed and approved by the IRB. Examples include a lower rate of response to treatment or a side effect that is more severe that initially expected.

Study Period

All Adverse Events (AE) will be recorded by the Investigator from the time of study enrollment through the end of the designated follow-up period. All AEs will be recorded within the research database. All relevant historical medical conditions that are known/diagnosed prior to the study enrollment are to be recorded to establish the subjects' baseline condition.

Abnormal Laboratory Values Defined as AEs

An abnormal laboratory value is considered to be an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Requires treatment, modification of study procedures, or any other therapeutic intervention
- Is judged by the Investigator to be of significant clinical impact/importance
- Grade 3 or Grade 4 lab abnormalities regardless of significance

If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be recorded as an AE. If the laboratory abnormality was not a part of a diagnosis or syndrome, then the abnormality should be recorded as the AE.

Monitoring, Recording, and Reporting of AEs

All AEs occurring during the study period will be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined

that the study procedures or participation is not the cause. SAEs that are still ongoing at the end of the study period will be followed up for up to 30 days to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study procedures or study participation should be recorded and reported immediately to the Sponsor.

All subjects will be monitored for AE's during the study. Assessments may include monitoring the participant's clinical symptoms; laboratory, pathological, radiological, or surgical findings; physical examination; or other appropriate tests and procedures.

AE's from all study sites will be pooled and evaluated on a regular basis so that any unlikely safety issues can be more readily detected.

AE data collection and reporting, which are required as part of every study, are done to ensure the safety of subjects enrolled in the studies and those who will enroll in future protocols. AEs are to be reported in a routine fashion at scheduled times during the trial, such as with the annual continuing review to the IRB. Certain AEs must be reported in an expedited fashion to allow for more-timely monitoring of subject safety and care. The reporting of these events depends on the characteristics of the event:

1. Seriousness (grading of event)
2. Relatedness to study procedures
3. Expectedness

Steps to Determine if the Event Requires Expedited Reporting:

1. Identify the type of event
2. Grade the severity of the event according to the following scale:
    1. Grade 1 – Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; no intervention indicated
    2. Grade 2 – Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)
    3. Grade 3 – Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
    4. Grade 4 - Life-threatening consequences; urgent intervention indicated.
    5. Grade 5 – Death related to AE
3. Determine whether the adverse event is related to the study procedures. Attribution categories are as follows:
    1. Unrelated
    2. Unlikely

3. Possible
4. Probable
5. Definite

4. Determine expectedness of event. An adverse event is considered unexpected when the type or severity of the event is not listed in:
    1. The investigator's brochure
    2. Consent form

Note: This includes all events that occur within 30 days of the last study procedure. Any event occurring more than 30 days after the end of study that is possible, probably, or definitely attributable to the study procedures should be reported according to the instructions above.

Expedited Reporting of SAEs

Only adverse events meeting the UPIRTSO (Unanticipated Problem Involving Risks to Subjects or Others) will need to be reported to the UAMS IRB within the expedited 10-day period of being notified of the event. UPIRTSO requires that an unanticipated problem meet the following qualifications: a) unanticipated or unexpected; b) related to the research; and c) involves new or increased risk to the subject(s). All other adverse events should be recorded and reported to the UAMS IRB at continuing review.

Specific to this study the most likely adverse events may relate to unexpected findings on pre-operative imaging or neurodevelopmental testing, complications related to anesthesia, surgery or the ability to undergo a non-sedated MRI.

Reporting of AEs and Findings to the Participants' Parents /Legal Guardian.

Any adverse event and/or abnormal findings (including the control patients and craniosynostosis patients) will be disclosed to the subjects' parents or legal guardian at the time that it occurs or noted who consented for the study involvement. The disclosure will be documented in patients' EMR under the study protocol.

Data Handling, Recordkeeping, and Data Safety Monitoring

The following measures will be undertaken in maintaining primary records (source documents) and entering the study data into any computerized systems. Doctors Ocal, Ramakrishnaiah, and Ou are UAMS faculty. Subject medical record data in the radiography system and EPIC are on password-protected databases. Each study subject will be assigned a unique five-digit study identification number (IDN). Study data will be entered into a password-protected database file under the subject IDN. The study database file will not contain subject names. Arkansas Children's will use REDCap, a secure web platform, as our data collection tool. Moreover, as this study is being performed at ACH, our study coordinator will enter the subject's information in the password protected ACHRI Clinical Trials Management System.

A master key list of study subject identifiers including names, MRNs, and five-digit study IDNs will be maintained on a password protected computer. As this is a study at ACH, we will also be registering test and control subjects into the Clinical Trials Management System. The study subject identifiers will not be linked to the study data. All study subject identifiers in the master list will be destroyed after publication. De-identified study data will be backed up by maintaining a copy on the computer of the principal investigator Eylem Ocal and co-investigator Raghuhr Hosahalliramakrishnaiah MBBS, with the original on the ACH password-protected shared drive server. De-identifiable study data will be retained indefinitely.

Regarding the data from other sites, only de-identified, data will be pooled for analysis, ie quantitative data from the DTI analysis and neurodevelopmental results. Each outside site, will be responsible for de-identifying its own data set for pooling with ours. UAMS will be the repository for the data analysis, and the central database we will keep will ONLY contain the 5-digit IDN. Each institution will retain its key for the IDN and corresponding patient identifiers.

The data safety monitor(DSM) will be an appointed clinical team member, (Samantha Snavely, PA) who will NOT be responsible for recruiting patients into the study but rather have the role of communicating with the PI's mentioned above (Ocal Hosahalliramakrishnaiah) and study coordinator about unanticipated problems, deviations from protocols and/or modifications. If an AE or SAE occurs as defined in the section above to any subject in the study, the study coordinator will relay this information to the IRB in the appropriate time frame but also will notify the DSM to make note in routine reports to the IRB on study progress.

Data Analysis

Primary and Secondary Endpoints

1) Understand to a previously unreported level of detail the brain function in children with craniosynostosis using DTI-MRI sequences as compared with age-matched controls
2) Determine the effect of CVR on brain development in comparing pre and post-operation DTI-MRI sequences at several intervals post-operatively
3) Identify the relationship between the brain development changes as seen on DTI with cognitive testing results

Note that from this master group, multiple cohorts will be grouped for analysis, i.e., single suture and multiple suture craniosynostosis, syndromic craniosynostosis. We

plan to analyze data both for quality control and preliminary analysis every 6 months while the study is on-going.

Statistical Methods

For the complex analysis of large amounts of MRI data on the same patient and comparing with other patients, we plan to employ biostatistical support baseline and demographic characteristics will be summarized by standard descriptive summaries (e.g., means and standard deviations for continuous variables such as age and percentages for categorical variables such as gender).

Data will be presented as frequencies, medians with ranges, or ± SD, as appropriate. Tests of group differences will include the paired t-test, $\chi 2$, and z-test, as appropriate. Repeated measures analysis will also be performed to on pre- and post-operative data for the same patient.

DTI parameters will be computed by standard software and will be compared between patients and controls or between different ages using standard statistical methods. Of note, for each MRI session, whether or not the participant was sedated will be a variable including in analysis.

Ethical Considerations

This study will be conducted in accordance with all applicable government regulations and Arkansas Children's Hospital research policies and procedures. This protocol and any amendments will be submitted and approved by the UAMS Institutional Review Board (IRB) to conduct the study.

The formal consent of each caregiver for their child, using the IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. The caregivers of all subjects for this study will be provided a consent form describing this study and providing sufficient information in language suitable for subjects to make an informed decision about their participation in this study. The person obtaining consent will thoroughly explain each element of the document and outline the risks and benefits, alternate treatment(s), and requirements of the study.

The consent process will take place in a quiet and private room, and caregivers may take as much time as needed to make a decision about their participation. Participation privacy will be maintained and questions regarding participation will be answered. No coercion or undue influence will be used in the consent process. This consent form must be signed by the subject or legally authorized representative and the individual obtaining the consent. A copy of the signed consent will be given to the participant, and the informed consent process will be documented in each subject's research record. If

assent is required, include a statement that assent will be obtained and that assenting minors will be consented if they reach the age of majority during the study.

Dissemination of Data

Results of this study may be used for presentations, posters, or publications. The publications will not contain any identifiable information that could be linked to a participant. Once all de-identified information has been stripped, the data from this study may be pooled for statistical analysis with the data from our collaborators, Wake Forest, Yale University Departments of Plastic Surgery, and Vanderbilt Department of Plastic Surgery.

Compensation of study participants (including new and existing subjects and controls):

1. Given the changing economical resources of our participants due to pandemic and related other financial hardships, active study participants will receive a $100 gift card after the completion of each research MRI study (4 cards total up to $400).
2. The control group will receive a one time $50 gift card for their participation in the study

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