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The Effect of Hypocalcaemia and Hypoxia on Risk of Autism in Patients with 22q11 Deletion Syndrome

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The Effect of Hypocalcaemia and Hypoxia on Risk of Autism in Patients with 22q11 Deletion Syndrome

By

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B.S.
University of Georgia
2008

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An abstract of
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Abstract

The Effect of Hypocalcaemia and Hypoxia on Risk of Autism
in Patients with 22q11 Deletion Syndrome
By Meghan Muldoon

Background: The importance of copy number variants (CNV) in complex pediatric disorders is of growing interest. 22q11 deletion syndrome (22q11DS) is a CNV disorder that has a diverse clinical presentation including congenital heart defects, palatal abnormality, immunodeficiency, hypocalcaemia, language and learning disabilities, and psychiatric disorders. Many patients with 22q11DS present with signs of Autism Spectrum Disorders (ASD), which manifest as impairments in social interaction and communication, repetitive behaviors, and idiosyncratic interests. The physiological mechanisms that link 22qDS with ASD are unknown. This study explores the influence of hypocalcaemia in 22q11DS on the risk and severity of social communication delays, which can be associated with ASD.

Methods: In a retrospective cohort study testing the association of physiological variables with social and communication abilities in infants and toddlers from Children's Healthcare of Atlanta 22q11 clinic, we abstracted medical and laboratory records for the earliest and lowest serum albumin-adjusted calcium level (n=151). Multiple childhood psychological assessments were used to detect the presence of ASD symptoms. The models controlled for age at assessment, age at calcium draw, and gender.

Results and Discussion: On average, the calcium level was taken contemporaneously with CSBS. There was a significant relationship between the lowest calcium value and CSBS Social Score (R^2=0.25, p=0.05), CSBS Speech Score (R^2=0.32, p=0.04), CSBS Symbolic Score (R^2=0.31, p=0.02), and overall CSBS Total Score (R^2=0.28, p=0.04). This relationship between low calcium and deficits in CSBS was also seen at the trend level in models using the earliest calcium value (p=0.08-p=0.11). Earliest calcium value was significantly associated with CDIP social scores (p=0.05). Finally, there appears to be a significant association between hypocalcaemia and the level of infant hypoxia (p=0.007).

Conclusions: Lower calcium level associates with impaired social communication development in patients with 22q11DS. Further studies are needed to elucidate the relationship between hypoxia and hypocalcaemia.Early peripheral risk factors such as hypocalcaemia may impact neuropsychological outcomes in 22q11DS patients. Calcium dysregulation affects neuroplasticity, and studies are needed to explore the influence of hypocalcaemia, and the role of calcium management, on early and later neurodevelopmental and psychiatric outcomes.

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INTRODUCTION

Autism Spectrum Disorder (ASD) is a range of developmental disabilities that has a variety of clinical presentations. Individuals can have significant behavioral, communication, and social challenges. In the past two decades, the prevalence of ASD has nearly doubled, increasing from 1 in 150 live births to 1 in 88 live births. Furthermore, Autism is thought to be four times more likely to occur in males than females. Severity of symptoms ranges from mild presentation to severe developmental delay. Despite this increase and interest in research to determine the cause, the etiology of ASD has not yet been determined. Family history is known to be associated with increased risk of individual to be diagnosed with Autism (Newschaffer et al).

Most researchers agree that the cause of ASD is heterogeneous, with an interaction of both environmental and genetic factors. Genes that have been associated with ASD are expressed in other peripheral tissues such as endocrine glands. Individuals with ASD have been shown to have altered neuronal organization, cortical connectivity, neurotransmitter pathways, and brain growth (Grafodatskaya, et al. 2010). In addition, many individuals with ASD have significant co-morbidities. Environmental factors have been known to range from prenatal, perinatal, and postnatal factors. Risk factors for ASD include hypoxia, endocrine disruptions, or congenital heart defects. These environmental factors are believed to interact with neuronal development and result in increased risk of ASD. The interaction of environmental factors on a genetically susceptible population may modify the phenotypic expression and severity of symptoms. A better understanding of peripheral physiological processes involving endocrine mediators and hypoxic conditions may shed light on the cellular and molecular pathways

leading to disruptions in neuronal development. Due to the complexity of studying disease with both genetic and environmental factors, one approach has been to study individuals with a well defined chromosomal disorder. Since ASD is known to be more prevalent in individuals with various chromosomal disorders, there is an opportunity to study how genetic abnormalities have a bidirectional interaction with environmental factors that may increase the risk of Autism.

One such chromosomal disorder is 22q11 deletion syndrome (22q11DS), also known as DiGeorge Syndrome or velocardiofacial syndrome. This disorder is one of the most common chromosomal disorders, with an incidence of approximately 1 in 3,000 live births (Fomen et al., 2010). This disorder is caused by micro-deletions on the long arm on chromosome 22, specifically the 11.2 region. Deletions in this region have a variable phenotype, affecting multiple systems in the body. Common presentations of patients with 22q11DS include congenital heart defects, palatal abnormalities, immunodeficiency, and parathyroid dysfunction. Parathyroid dysfunction can lead to difficulties regulating calcium levels within the body. Calcium is essential for neuronal function, and it is a critical mediator of neuroplasticity, and therefore calcium dysregulation could affect neuronal development. Congenital heart defects are currently being used as a proxy indicator for hypoxia levels. Oxygen is known to affect brain function during all stages of development. Furthermore, patients commonly have delayed psychosocial development and have an increased risk of psychiatric illness, including ASD. The study will examine the effect of environmental factors on a relatively stable genetic background. Specifically, this study aims to understand the effect of peripheral factors on the risk and severity of ASD within patients with 22q11DS.

BACKGROUND/ LITERATURE REVIEW

22q11 Deletion Syndrome

Genetic mutations have become a common topic for research for their influence on medical outcomes. One well-studied genetic syndromes is 22q11 deletion syndrome, also known as DiGeorge Syndrome, which has an estimated prevalence of 1 in 3000 births (Fomin AB, 2010). 22q11 deletion syndrome (22q11DS) is caused by a submicroscopic deletion of the large arm of chromosome 22 in an area called DiGeorge chromosomal region (McDonald-McGinn DM E. B., 2005). Deletions in this region have been shown to cause defects in the third and fourth pharyngeal pouches during embryonic development, which is characterized by absent or hypoplastic parathyroid glands and conotruncal cardiac malformations (Driscoll DA, 1992).

Patients often present in infancy with immune system dysfunction or cardiac defects; these symptoms often suggest the possibility of a genetic syndrome. However, a diagnosis of 22q11DS is confirmed by the use of fluorescent in situ hybridization, or FISH testing. FISH allows for the clinician to both confirm a 22q11 diagnosis, as well as determine the size of the deletion. While the majority of patients with 22q11DS obtain their genetic mutation *de novo*, the mutation may be inherited, in which case there is a fifty percent chance of a parent passing the mutation onto children (McDonald-McGinn DM E. B., 2005). While over forty genes have been associated with the DiGeorge chromosomal region, two of those genes are of particular interest: *TBX-1* and *PRODH*.

The *TBX1* gene, which encodes the TXB1 protein, has been shown to be involved in cardiac development and parathyroid gland development (TBX1 T-box 1 [Homo sapiens], 2011). Specifically, it has been postulated that deletions in the *TBX1*

gene cause improper migration of neural crest cells resulting in the pharyngeal pouch abnormalities (Merscher S, 2001). A second gene that has been found in the DiGeorge chromosomal region is the *PRODH* gene. The *PRODH* gene encodes proline oxidase, an enzyme with prominent expression in the brain, liver, and kidneys. A recent study suggested a decrease in expression of PRODH in lymphoblasts in 22q11DS-related ASD (Nord AS, 2011). While the molecular mechanisms are largely undefined, patients with 22q11DS have a genetic susceptibility to Autism spectrum Disorders, or Autism.

Clinical Manifestations of 22q11DS

The deletions within 22q11DS produce a wide range of phenotypic expression. Consequently, a wide range of clinical manifestations have been observed in patients with 22q11DS including congenital heart defects, palatal abnormalities, learning difficulties, abnormal immune function, and psychiatric illness. This study focuses on three specific manifestations of 22q11DS: congenital heart defects, parathyroid related functions, and psychiatric illness.

Approximately seventy five percent of individuals with 22q11DS present with congenital heart disease, which also is found to be the major cause of mortality (McDonald-McGinn DM E. B., 2005). Considering those patients with a cardiac defect, twenty percent of patients are diagnosed with Tetralogy of Fallot (McDonald-McGinn DM T. M.-C., 2001). Tetralogy of Fallot is comprised of four distinct cardiac defects: right ventricular hypertrophy, overriding aorta, pulmonary stenosis, and ventricular septal defect. Other common cardiac defects include interrupted aortic arch, ventricular septal defect, and truncus arteriosus. An important feature of these cardiac defects is there

effect on proper circulation of oxygenated blood. The American Heart Association has linked congenital heart defect to hypoxemia, or low oxygenation levels. For those defects that are most frequently seen in 22q11DS, Tetralogy of Fallot, truncus arteriosus most or all of patients experienced hypoxemia (Mahle WT, 2009).

This study also examines hypocalcaemia, defined as low calcium levels in the circulation. While hypocalcaemia is often one of the first clinical symptoms, causing seizures in infants, it is present in approximately fifty percent of individuals with 22q11DS. Deregulation can be explained by the altered expression of the *TBX1* gene, and subsequent alteration in the organogenesis of the parathyroid gland. In normal calcium homeostasis, the decreases in plasma calcium concentrations cause the parathyroid to secrete parathyroid hormone, or PTH. PTH increases calcium levels by increasing bone resorption calcium reabsorption in the kidney, and calcium absorption in the intestine. In patients with 22q11DS, deregulated calcium can alter neuronal development. Calcium has been shown to be intricately involved in excitability of neurons; this activity is essential to proliferation, migration and differentiation of neurons (Spitzer, 2006). Furthermore, recent studies indicate that circulating levels of calcium could influence nerurite outgrowth via interaction with the extracellular calcium-sensing receptor, which is present on the parathyroid gland as well as developing neurons (Vizard TN, 2008).

Autism Spectrum Disorder and 22q11DS

Autism Spectrum Disorder (ASD) is an increasingly common psychological disorder with an unclear etiology. According to the Centers for Disease

Control and Prevention, current prevalence rates of ASD are estimated to be 1 in 88 children, with more male diagnosed than females (Data and Statistics, 2012). The spectrum includes autistic disorder, pervasive developmental disorder – not otherwise specified, and Asperger syndrome. Patients present with symptoms and signs including impairments in social interaction, and communication. These patients also frequently have repetitive and stereotyped behaviors. Typical onset occurs prior to three years of age with the appearance of at least one of the symptoms listed previously. Past studies of ASD have attempted to elucidate factors that are involved in etiology. However, ASD has proved to be a complex disease involving both genetic and environmental factors. Recent studies suggest ASD has been associated with *de novo* and inherited copy number variants, which can include 22q11DS (Nord AS, 2011). Studies with 22q11DS patients reveal the presence of Autism, or ADHD, or both to be at least forty percent (Niklasson L, 2009). The presence of Autism both in the general population and at higher rates within specific genetically-affected populations further supports a complex genetic link to Autism.

Importance of Current Project and Hypotheses

The present study explores the influence of two important clinical presentations of the 22q11DS, hypocalcaemia and cardiac defects, on the risk and severity of ASD in our genetically susptible population. Calcium is essential for both neuronal development and neuronal function. Articles have recognized the importance of calcium in many aspects of early neuronal development (Spitzer, 2006). Furthermore, studies have suggested that individuals with Autism have altered neuronal organization, cortical connectivity,

neurotransmitter pathways, and brain growth (Grafodatskaya D, 2010). Therefore, 22q11DS patients could have altered neuronal development due to poorly regulated calcium that could lead to increased risk of Autism.

The second indicator that will be examined is hypoxia. Considering the large percentage of patients with 22q11DS present with a congenital cardiac defect, this study will use the classification of the cardfic lesion as a proxy measure for hypoxia. Cardiac defects have been shown to place patients at an increased risk of psychiatric disorders, including ASD (Olsen M, 2011). Supporting this connection, researchers have indicated that excess risk of Autism has been found in males who were hypoxic at birth (Burstyn I, 2011). As such, 22q11DS patients could also have an increased risk of Autism if they are exposed to hypoxic environments early in childhood development.

While many hypotheses have been proposed for risk factors of Autism, no studies have provided answers for why the 22q11 population is at such greatly enhance the risk. The current study aims to provide insights into the causes for increased risk of Autism based on the impact of hypocalcaemia and hypoxia within a 22q11DS genetic background. There will be three main hypotheses explored:

Primary 1: Is hypocalcaemia associated with elevated risk for Autism symptoms, measured by psychological assessment scores?

Primary 2: Are early hypoxic insults associated with elevated risk for Autism symptoms, measured by psychological assessment scores? Do hypoxic insults before the age of 3 cause a greater risk of Autism?

Secondary: Do patients with combined hypocalcaemia and hypoxia indices have a greater risk for Autism symptoms than individuals with one insult? Is there interaction between hypocalcaemia and hypoxia?

METHODS

Recruitment, Study Sites, and Participants

Recruitment for the current study was made possible through the coordination of multiple researchers and sites. Patients were recruited by researcher at Children's Healthcare of Atlanta (Karlene Coleman, RN, MN, CGC), Emory Children's Center (Lisa Kobrynski, MD, MPH), Marcus Autism Center and Emory Autism Center (Opal Ousley, PhD; Joseph Cubells, MD, PhD; Samuel Fernandez-Caribba, PhD), Sibley Heart Center (Matthew Oster, MD, MPH), and the Emory Department of Genetics (Joseph Cubells, Karlene Coleman). Together these researchers established a 22q11DS Multidisciplinary Clinic in 2005; these patients are the source of recruitment for the current study. The South East Regional Phenotypic database for patients with 22q11DS (SERPh22 database) was created by Dr. Pearce and his collegues and used for this analysis. Currently, the SERP22 database contains information on 628 unique patients.

For those individuals in the SERPH22 database with a medical record number, Children's Healthcare of Atlanta supplemented demographic data, calcium data, and albumin data (we are grateful to Tal Senior RN for invaluable support in marshaling this data). This resource comprised over 5,000 calcium data points on 162 individuals with a medical record number from the SERPH22 database. Sibley Heart Center provided diagnoses for individuals within the SERPH22 database, which was subsequently used to determine hypoxia status. Finally, the Marcus Autism Center and Emory's Autism Center provided access to cognitive and psychosocial development of 22q11DS patients.

The combination of these sources allow for the investigation of environmental impacts on genetic susceptibility for Autism.

Age and Gender of Full Database

The age range (n=627) for patients was from 0.4 years to 57.64 years. The median age was 16.5 years. Approximately 48% of the individuals are male, and 48% are female, with 4% missing information on gender. For the subset of patients included in the current study (n=361), 53% of the individuals are male, and 45% of the individuals are female, with a mean age of 11.60 years (Table 1).

Calcium Homeostasis

Information regarding calcium levels was obtained from electronic medical records at Children's Healthcare of Atlanta. Calcium levels were obtained through previous blood samples for 162 individuals. Simultaneously, we obtained albumin blood levels. Albumin is a blood marker that is often used to indicate protein levels in the blood. Importantly, if the protein level is low within the blood, there is a possibility that it could cause the calcium level to appear low. Therefore, calcium levels were used only if it could be correct with an albumin level taken at the same time. If an albumin level was available, it was used to calculate a corrected calcium level (Calcium Correction for Hypoalbuminemia Medical Calculator, 2012). This produced a corrected calcium value for 151 individuals. Furthermore, for each individual, the earliest and lowest calcium value were determined and set aside for analysis.

Cardiac Factors

The SERPH22 database contains diagnoses of cardiac defects from the Sibley Heart Center. For the patients in the study with a cardiac defect, the study determined the percentage of subjects that had specific cardiac defects, as well as number of individuals with no cardiac defect. Overall, 328 individuals had cardiac data within the SERPH22 database. This was used to identify hypoxia levels by type of defect. For those individuals with a cardiac defect that has a priori been targeted for screening due to known risk of hypoxia and complications, they received a hypoxia scale score of 2.

Defects that have been targeted include: hypoplastic left heart syndrome, pulmonary atresia, tetralogy of Fallot, transposition of the great arteries, tricuspid atresia, truncus arteriosus, total anomalous pulmonary venous return, coarctation of the aorta, double outlet right ventricle, Ebstein's anomaly, interrupted aortic arch, and single ventricle. Other known cardiac defects received a score of 1; an example of such a defect could be an atrial septal defect. Finally, if an individual had no known cardiac defect, he or she was given a hypoxia scale value of 0. The date of surgery was also obtained for 178 individuals to account for repair of cardiac defects.

Neuropsychological Assessment

Developmental and psychological assessments were obtained by psychologists (Dr. Ousley and Fernandez-Carriba), psychiatrists, and experts and behavioral health under their supervision at the Marcus Autism Center and Emory Autism Center. Psychological evaluations were performed on patients through direct clinical

assessments, parental checklists, or parent interviews. In addition, developmental evaluations were given at Children's Healthcare of Atlanta. These assessment values were used as outcome variables in this current analysis. Raw scales were used to best assess the presence of autism symptoms within individuals. Detailed information concerning topics, scoring, and references for each assessment are found in Table 2. I also supplemented this data with a parent phone interview as described below. Brief descriptions of the individual assessments used in this study are as follows:

Aberrant Behavior Checklist (ABC)

This scale is composed of 58 items designed to evaluate childhood development. The assessment can be given to individuals ages 6-54. The scale is completed by a caregiver or another adult who knows the child well. There are five subscales, which are used to characterize manifestation of mental disorders. These include irritability, agitation and crying, lethargy and social withdrawal, stereotypic behavior, and hyperactivity and non-compliance. Scores from these five subsets are added to obtain a total score. The higher the score on an ABC evaluation, there is an indication of impairment in behavioral domains.

Autism Diagnostic Interview-Revised (ADI-R)

The ADI-R is an interview-based assessment used to evaluate autism in children and adults. For children, the clinician questions the caregiver about the child's behavior and developmental history. There are 93 specific items covering three major areas: language and communication, reciprocal social interaction, and finally restricted,

repetitive, and stereotyped behavior. Furthermore, the interview assesses early developmental highlights, social development and interaction, and current language and communication skills. To be diagnosed with autism, an individual must exceed a threshold level in all three sections.

Autism Diagnostic Observation Schedule (ADOS)

As opposed to parent checklists, the ADOS uses semi-structured play sessions with clinicians to evaluate communication, social interaction, and play. Typical sessions consist of standardized activities that allow the clinician to identify behavior patterns over approximately thirty to forty five minutes. The observation is designed to provide information for the diagnosis of autism or other pervasive developmental disorders. Clinicians specifically evaluate stereotypic behaviors and restricted interest that are of note in autistic behavior. Higher scores indicate greater presence of symptoms relating to autism.

Autism, Tics, ADHD and other Co-morbidities Inventory (A-TAC)

To examine the feasibility of further follow up studies, I used the SEPRh22 database to contact parents of children in the database for the phone follow-up with the help of Jessie Preslar. The A-TAC is a unique parent telephone interview used for epidemiologic research on child and adolescent mental health (Larson T, 2010). It is used as a screening tool for autism spectrum disorders, attention deficit/hyperactivity disorders, and learning disorders among others. The interview is arranged into different modules each containing gate questions and follow-up questions. The full version

contains 96 gate items, with 163 corresponding follow up questions. Scores for each section are totaled, and are combined to achieve scores for autism, learning disorders, and other psychiatric problems. Higher scores correspond to higher presence of symptoms for each psychiatric problem.

Childhood Development Inventory Profile (CDIP)

The childhood development inventory profile is used to indicate delays in social and language development. The inventory is given to children, ages 15 months to six years of age. There are 270 statements that are used to describe behaviors in the first years of life. Those statements are grouped into scales, including social, self-help, gross motor, fine motor, expressive language, language composition, and general development among others. Each score is determined based on age appropriate norms. Scores below the age appropriate mean are considered to be indicative of developmental delays, shown by scores below 25 percent of the mean.

Communication and Symbolic Behavior Scales- Developmental Profiles (CSBS)

This tool is often used by psychiatrists to evaluate communication of children between the ages of 6 and 24 months. Initially, an infant-toddler checklist is administered. The checklist is composed of 24 questions that have three main aspects: social, speck, and symbolic composites. The scores from these three sections are added to obtain a total score. If scores on this checklist are of concern, a caregiver questionnaire and behavior sample is obtained. Furthermore, if concern is noted the CSBS can be given again to follow a child's communication development. Children who

have scores in the concern range on any composite or the total score may validate the need for further tests to determine if the child has specific language impairments, more general developmental delays, or autism spectrum disorders (CSBS resource). The higher the score on a CSBS, the less likely there is concern for developmental delays or communication disabilities.

Child Behavior Checklist (CBCL)

The Child Behavior Checklist is a parent questionnaire to evaluate the child's behavior and emotions; it is targeted at children ages 2 to18. The scale is composed of 112 items that assess internalizing and externalizing behavior. Internalizing behaviors include anxious, depressive, and over controlled behaviors; externalizing behaviors include aggressive, hyperactive, noncompliant, and under controlled behaviors. Answers to the CBCL are summarized and are compared to a normal range. If out of range, a clinician may suggest further testing.

Study Population

Despite the variety of clinical information collected in the SERPH22 database, this thesis focuses on cardiac, calcium, and neuropsychological data. Only those individuals with a medical record number were able to be included. Furthermore, the medical record number was linked to cardiac data records, as well as surgery date and diagnosis; medical record number was also used to obtain calcium data. These subgroups were used to determine the current study's sampling design (Figure 1).

Specific sections of each of the psychological assessments were chosen based on the number of assessments taken and the ability to correlate with ASD diagnosis. Assessment were carried forward for further analysis if at least eight individuals at assessment data, and the assessment res relevant to ASD. Furthermore, separate scales were used based on the recommendation of a psychologist (Dr. Ousley) within the current research team. Since many children with Autism also possess developmental delays and language development delay, CSBS overall score, CDIP language score, and DAS verbal standard score were independently used as primary outcomes. Specific to behavioral outcomes, general development was measured using CBCL internalizing and externalizing behavioral problems were used independently as primary outcomes. Finally, the ADOS scores and the aberrant behavioral checklist are also used to assess Autism outcomes. A complete list of psychological scales initially evalueated are listed in the Appendix under Table 2. If the individual number of assessments did not contain enough individuals or if initial correlations were not statistically significant, no further anlaysis was completed.

While all the previous assessments were taken prior to the current study, we chose to follow up with individuals in the SERPH22 database using the A-TAC telephone interview. In order to qualify for this analysis, individuals needed to have a MRN to link to exposure variables, as well as home addresses and telephone numbers. Of the SERPH22 database, 185 individuals met the above criteria and were asked to participate in a 30 minute telephone interview. Of those individuals, we were unable to contact 154 individuals of which 71 phone numbers had been disconnected and the remainder did not

return a message. Fourteen individuals refused to participate (45% of those individuals who we spoke to) and 13 individuals completed the interview (42% participation rate).

Study data is maintained in the REDCap (Research Electronic Data Capture), which is an Emory University, secure, web-based application. Using this interface, the SERPH22 database is able to contain both demographic data and clinical data, all while validating data entry, creating audit trails for data changes and exporting procedures, and allowing for seamless import or export of data from the database.

Statistical Methods

Initial exploratory analyses were used to become familiar with the dataset and ensure there are no erroneous values. For categorical variables, the study first explored frequency distributions. For continuous variables, the mean, standard deviation, skewness, and kurtosis statistics were explored to evaluate normality for model assumption. These descriptive statistics were obtained for all predictive and outcome variables contained in the SERPH22 database (Table 1, Table 3). Since the study population was selected based on availability of medical record number, the same descriptive statistics were determined for only those individuals who possessed a medical record number. Finally, descriptive statistics of those individuals with medical record number and calcium value (Table 4), and those individuals with medical record number and hypoxia scale (Table 5). The previous two tables allowed us to determine if the study population was distributed in the same fashion as the entire SERPH22 database and eliminate the concern for selection bias.

To explore possible confounding, the correlation of covariates of interest with psychological outcomes were assessed (Table 6). Covariates were considered to be controlled for in further analysis if the p-value was ≤ 0.10. Covariates of interest that were considered as possible confounders include the patient's age at calcium blood draw, age of surgery correction, gender, and deletion size. In addition to exploring correlations between possible confounders and outcomes, correlations were determined between main predictors of a priori interest and outcome variables (Table 7). As with covariates, predictors were considered to be relevant for further analysis if the p-value was ≤ 0.10.

Data Analyses

Primary analysis

Primary analysis had three main components that explored the first two hypotheses. The first hypothesis is to determine if hypocalcaemia is associated with elevated risk for ASD, determined by psychological assessment scores. To complete this analysis, linear regression was used to model psychological assessment scores. Two main predictors were used to explore this hypothesis: low calcium and early calcium. Forward selection regression models were used and included each main predictor, age at calcium blood draw, age at assessment, and gender. This process was completed twice: once for an individual's lowest calcium, and again for an individual's earliest calcium.

Using SAS, the forward selection begins with only the main predictor and main outcome in the model. For each independent variable in the process, an F statistic is calculated that reflects the variable's contribution to the model. In addition, a corresponding p-value is used to determine significance at a predetermined alpha

significance value. If none of the F statistics has significance value less than the alpha level, the forward selection stops. Otherwise, variables are added to the model and F statistics are recalculated. The process is repeated until no remaining variables produce a significant F statistic. A variable remains in the model once added, even if additional variables cause it to no longer be significance. For this analysis, the predetermined alpha level to enter the model is 0.10.

In addition, backward selection was also used to help ensure the best model was chosen. Using this method, predictors and all covariates are originally entered into the model. Similar to forward selection, F statistics are calculated for each model. If all variables are found to be significant, backward selection stops and all variables are included. However, if a variable is determined to have a p-value greater than the predetermined alpha level, it is removed from the model. If more than one variable is not significant, the variable with the largest p-value is selected for removal. The model regression is run again with a reduced variable number to recalculate F statistics. This process is repeated until all variables are significant in the model. For this analysis, the predetermined alpha level to remain in the model is 0.10. For each model in both selection processes, collinearity was assessed using Variance Inflation Factor (VIF) cutoff value of 10. All best fit models had VIF values of less than 5. Therefore collinearity was not abaove the preset limits within the current study. The above methods were conducted using SAS Version 9.3 software (SAS Institute Inc., Cary, NC). For all overall models, a cutoff level of p-value ≤ 0.10 was the predetermined alpha significance level.

Secondary analysis

Finally, we examined the combined effect of hypocalcaemia and hypoxia on psychological risk outcomes. Due to the low number of patients with calcium data, hypoxia data, as well as psychological assessment, we were unable to perform regression analysis. However, initial correlations between calcium and hypoxia revealed a potential complex relationship that warrents further exploration.

Ethics

The Institutional Review Board at Emory University, Atlanta, Georgia, approved the story (IRB00045086). Furthermore, Children's Healthcare of Atlanta officially approved the study. Each individual provided informed consent as per each individual study sites' protocol.

RESULTS

Descriptive Analysis

Predictor Variables

The SERPH22 database contains 617 individuals who have a combination of both physiologic and psychiatric data. My analysis began with those individuals with a medical record number that could be linked to predictor variables (n=361). Demographics for those individuals were first determined (Table 1). The average age for those patients was 11.60 years (SD 7.54, Range: 0.30-50.24 years). We had three different ages of assessment, depending on the psychological assessment given. For those individuals who received the CSBS, the average age at assessment was 1.23 years (SD 0.41, Range: 0.46-2.07). The average age at CDIP assessment was 3.88 years (SD 5.84, Range: 2.11-38.35). Finally, all other assessments had an average age of 9.83 years (SD 2.33, Range: 6.22-11.95). We had three main predictor variables: lowest calcium value, earliest calcium value, and hypoxia scale.

If an individual only had 1 calcium value present, it was counted as the lowest and earliest calcium value. This occurred in 61 individuals of the 151 with calcium values. The lowest calcium value, corrected previously with albumin to account for protein, had an average value of 8.32 mg / dL (SD 1.07, Range: 4.20-11.18) with an approximate normal distribution. The age at which the lowest calcium was taken had an average of 2.48 years (SD 4.56, Range: 0-19.46). The earliest calcium value, corrected previously with albumin to account for protein, had an average value of 9.08 mg / dL (SD 1.03, Range: 5.78-11.18) with an approximate normal distribution. The age at which the lowest calcium was taken had an average of 2.03 years (SD 4.56, Range: 0-19.46). While

ages at assessment and calcium blood draw were skewed, all data points were within expected ranges so were not transformed for analysis.

The final predictor, hypoxia scale was determined for all individuals with a confirmed cardiac examination. Of those 628 individuals in the SERPH22 database, 230 individuals did not have a confirmed cardiac exam that was sufficient for coding 9% of individuals had no cardiac defect (n=58), 17% had a hypoxia scale value of 1 (n=105), and 37% had a hypoxia scale of 2 (n=235). These values are consistent with population studies of 22q11DS. Only 270 individuals had a medical record number for the current analysis and confirmed cardiac diagnosis (figure 1). Not counting those individuals who are missing a cardiac diagnosis, the distribution of cardiac defects are as follows: 15% of individuals had no cardiac defect (n=58), 26% had a hypoxia scale value of 1 (n=105), and 59% had a hypoxia scale of 2 (n=235).

Outcome Variables

To determine developmental outcomes, and possible link to autism, multiple childhood neuropsychiatric assessments were administered (Table 2). All outcome variables, represented by child psychological assessment scores, had a normal distribution for patients in the SERPH22 Database (Table 3). For those individuals with a medical record number, thirty-seven individuals had a Communication and Symbolic Behavior Scale (CSBS). The average total score for CSBS was 30.54 (SD 10.40, Range: 11.00-48.00). The average CSBS Social Total score was 17.05 (SD 5.47, Range: 8.00-26.00). The average CSBS Speech Total score was 5.59 (SD 2.66, Range: 0.00-10.00). The average CSBS Symbolic Total score was 7.92 (SD 3.49, Range: 2.00-14.00).

The next child assessment used in the analysis is the Child Development Inventory Profile (CDIP), which was used on thirty-six individuals. The average CDIP Social score was 23.83 (SD 9.86, Range: 4.00-40.00). The average CDIP Expressive Language score was 24.25 (SD 16.37, Range: 1.00-48.00). The average CDIP Language Composition score was 26.47 (SD 15.64, Range: 0.00-50.00). The average CDIP Gross Motor score was 18.67 (SD 7.02, Range: 4.00-29.00).

In addition, a few other psychological assessments were used in this analysis. The Child Behavior Checklist (CBCL) was also administered on twenty-one individuals. The average CBCL Externalizing Problems Score was 11.81 (SD 9.35, Range: 0.00-35.00). The average CBCL Internalizing Problems Score was 12.43 (SD 6.31, Range: 3.00-25.00). The average CBCL Social Score was 5.73 (SD 2.76, Range: 1.00-13.50). The Autism Diagnostic Observation Schedule Repetitive Behavior and Social Affect Total Score was assessed on thirteen individuals, with an average score of 7.15 (SD 3.74, Range: 1.00-13.00). The A-TAC was also used in telephone interviews to assess Autism symptoms; the average A-TAC score was 18.64 (SD 12.75, Range: 0.00-47.00). Finally, the Autism Diagnostic Interview - Revised Social Score (ADI-R) was performed on twenty-eight individuals with an average value of 9.04 (SD 6.12, Range: 1.00-25.00).

I considered the possibility of selection bias, and determined since there was no change in outcome variables between groups that bias was not present. Univariate analysis was completed on individuals with a medical record number and calcium value (Table 4). Additionally, univariate analysis was also completed on individuals with a medical record number and hypoxia scale (Table 5). As with calcium value, no

significant deviations were noted between groups. Based on these statistics, further analysis was undertaken without any transformation of data.

Initial Analysis

Calcium

After completing data univariate statistics, initial analysis was completed on three separate individual groups. Individuals with a medical record number and a low calcium value and individuals with a medical record number and an early calcium value were used for further analysis. Finally, initial analysis was completed on individuals with a medical record number and hypoxia scale value.

First, using Pearson's Correlation coefficients, we investigated potential confounding relationships between outcomes and patient demographics (Table 6). Using an alpha significance level of 0.10, the only significant relationships occurred between sex and CDIP Gross Motor Score (p=0.001), sex and DAS Verbal Standard Score (p=0.02), age at cardiac surgery and CBCL Internalizing Problems Score (p=0.01), and finally age at cardiac surgery and DAS Verbal Standard Score (p=0.07). Females were coded as zero, and males as one; as a result, our results complement the higher prevalence of ASD in males as opposed to females. Since these results indicate possible confounding by gender and age at cardiac surgery, they will be forced into modeling to account for bias in final analysis.

The initial analysis, described in Table 7, shows the crude potential relationship between our two predictor variables and outcome variables. For initial analysis we used an alpha significance level of 0.20 to highlight potential relationships for further study.

Correlations were determined for psychological assessments and each of the three predictors: low calcium value, early calcium value, and hypoxia scale. Initial analysis using Pearson's Correlation Coefficient revealed a significant crude correlation between low calcium level and multiple psychological assessment sub-scores. Low calcium value was associated with CSBS Speech total score (r=0.31, p=0.18). Low calcium was also correlated with multiple CDIP section scores, including Social Raw score (r=0.39, p=0.19), Self Help Raw score (r=0.51, p=0.07), Gross Motor score (r=0.46, p=0.11), and Expressive Language score (r=0.41, p=0.16). Using the CBCL assessment, low calcium was correlated with Social score (r=0.75, p=0.09) and Externalizing Problems score (r=-0.63, p=0.09). Finally, low calcium value was correlated with A-TAC Autism sub-score (r= -0.93, 0.02).

The second phase of initial analysis, using Pearson's Correlation Coefficient, also revealed a significant crude correlation between the early calcium level and multiple assessment outcomes (Table 7). Earliest calcium value was associated with CSBS Speech total score (r=0.38, p=0.09), Symbolic total score (r=0.40, p=0.08), and CSBS total score (r=0.37, p=0.11). Earliest calcium was also correlated with multiple CDIP section scores, including Social Raw score (r=0.55, p=0.05), Self Help Raw score (r=0.67, p=0.01), Gross Motor score (r=0.78, p=0.002), Expressive Language score (r=0.57, p=0.06), Language Composition score (r=0.69, p=0.01), Letter score (r=0.47, p=0.10), and Numbers score (r=0.53, p=0.06).

While these initial analysis were beneficial to highlight variables for further analysis, additional variables were also explored regardless of initial correlation results

because of their use in ASD diagnosis. These indices included ADI-R Social Score, ADOS repetitive behavior and social affect total score.

Hypoxia Scale

After completing univariate statistics, initial analysis was completed on all individuals with psychological assessments and a hypoxia scale based on cardiac diagnosis. Since the hypoxia scale is categorical, analysis of variance was used to determine crude relationship between groups (Table 7). While no relationships revealed significant results, this could be be due to uneven distribution of hypoxia scores among individuals with psychological assessments (Figure 2-5), Appendix table 1. Specfically few individuals were located within the no cardiac defect group, therefore limiting analysis. No further analysis was completed on hypoxia data for this study, however future studies could explore potential trends.

While further analysis was not completed to determine the effect of hypoxia on presence of autism symptoms, we calculated correlation between calcium value and hypoxia score. Early calcium value was significantly associated with hypoxia scale ($p=0.05$). Furthermore, using correlation coefficients, the low calcium value was also significantly associated with hypoxia scale ($R^2=0.07$, $p<0.01$).

Regression Analysis

Lowest Calcium Value

Based on literature review, certain variables were forced into model to control for possible confounding. These covariates were the age at assessment, age at calcium

blood draw, and gender. From there, backward selection was used to determine the best fit model. No significant results were found to exist between lowest calcium value and the ADOS assessment, the DAS verbal standard score, or CBCL assessment scores.

The first models explored the CSBS assessment scores. With the outcome variable of total CSBS score (Table 8), the full model with all covariates was first examined ($F=2.86$, $p=0.0605$, adjusted $R^2=0.2813$), however age of CSBS assessment was not significant. Therefore after removing it from the model, the best fit model resulted in only sex and age at calcium blood draw as covariates in the model with lowest calcium level. This model explained 28% of the variability in CSBS total scores ($F=3.48$, $p=0.0407$). Further analysis was completed on individual subsections of the CSBS assessment. The CSBS social total score was modeled with the same variables, lowest calcium age with CSBS assessment age and sex as covariates (Table 9). The model explained 25% of the variability of CSBS Social Score ($F=3.17$, $p=0.0532$). CSBS Speech total scores were best fit by the full model containing all three covariates, and the model explained 32% of the variation in CSBS Speech score ($F=3.22$, $p=0.0427$) (Table 10). Finally, CSBS symbolic total best fit model contained only CSBS assessment age as a covariate (Table 11). This model explained 31% of the variability in CSBS Symbolic total score ($F=5.26$, $p=0.0167$). In each of these final models the sign of the regression coeffcients are positive. Lowest calcium value was also used to predict CBCL Externalizing Problems score. All covariates in this analysis were non-significant in modeling. However lowest calcium value alone was found to have a significant linear relationship to this specific CBCL subsections (Table 12). Lowest calcium predicted 30% of the variability of the externalizing problems subset

(F=3.95, p=0.0942). Finally, significant relationships were found between lowest calcium value and A-TAC Autism scores (Table 13). With only lowest calcium value in the model, the model explained 86% of variation of A-TAC scores (F=18.57, p=0.023). The regression coefficient had a negative sign.

Earliest Calcium Value

Based on literature review, certain variables were forced into model to control for possible confounding. These covariates were the age at assessment, age at calcium blood draw, and gender. From there, backward selection was used to determine the best fit model. No significant results were found to exist between earliest calcium value and CSBS assessment, the ADI-R Social score, the ADOS assessment, CBCL assessment scores, or A-TAC Autism scores.

The first models explored the CDIP assessment scores. Earliest calcium value was used to predict CDIP Social score. All covariates in this analysis were non-significant in modeling. However earliest calcium value alone was found to have a significant linear relationship to this specific CDIP subsection. Earliest calcium predicted 24% of the variability of the Social Score subset (F=4.75, p=0.0512) (Table 14). The second outcome variable of the CDIP assessment was the expressive language section (Table 15). Like social raw sore, all covariates in this analysis were non-significant in modeling. Earliest calcium predicted 27% of the variability of the language composition subset (F=5.47, p=0.0392). The CDIP language composition score, there was statistically significant linear relationship (Table 16). The model only contained the covariate of sex; a significant portion of the variance in language

composition score was explained by earliest calcium and sex value (F=10.54, p=0.0026). Furthermore, CDIP gross motor score was best explained by earliest calcium value and sex (Table 17). This model explained 68% of the variation in CDIP gross motor score (F=13.83, p=0.0013).

DISCUSSION

The current study explored possible associations between peripheral physiologic states and behavioral assessments among patients with 22q11DS. One of the two hypotheses explored within the current study was the effect of hypoxia on risk of autism. One limitation of the current study was that the database did not have enough data on individuals without cardiac defect to fully explore the relationship. There appears to be a complex relationship between hypoxia and brain development, and subsequent risk of psychiatric illness. However, future studies are needed to elucidate this relationship. Interestingly, there appears to be a significant association between hypoxia scale and both low calcium level ($p=0.007$) and early calcium level ($p=0.05$). A future analysis could focus on these physiologic interactions.

The second major hypothesis of the current study looked at the effect of calcium level on risk of ASD symptoms. A strength of the current study is that the calcium value obtained from medical records was drawn from the individual during critical times of neurological development. The average age of calcium blood draw was 2 years for both the lowest and earliest calcium value. Therefore the calcium values used in the current analysis are representative of levels during neurological growth when autism symptoms often begin to appear, and prior to the time that ASD is typically diagnosed. Since calcium is essential to synaptic formation and transmission, it is believed to be a crucial element to neuronal development. One limitation of the study of hypocalcaemia on brain development is the possibility of the individuals undergoing seizures. In children, seizures could go unrecognized, especially early in childhood. There is a possibility that

hypocalcaemia could cause seizures that result in neuronal damage and increased risk of abnormal psychological assessments. In the current study, history of seizures was not involved in analysis. Future studies could incorpate this history and elucidate a possible connection. Recent studies have suggested that individuals with Autism have altered neuronal organization, cortical connectivity, neurotransmitter pathways, and brain growth (Grafodatskaya D, 2010). However studies had not been conducted within a known genetically susceptible population. Calcium level is tightly regulated within the body mainly by parathyroid hormone. Deletions within the chromosomal region of 22q11 can result in abnormal parathyroid development, which in turn can lead to abnormal calcium homeostasis. The normal range for calcium value was 8.0 – 10.7 mg / dL. Among our cohort of patients with 22q11DS, the average lowest calcium value, 8.32, was borderline low value.

In the current study, many associations were found between calcium values and psychological assessment scores. The first part of analysis found that the lowest calcium value was positively correlated with CSBS scores. The lower the calcium level, the lower the CSBS score, which indicates greater concern for developmental delays and communication disabilities. Using backward step regression, low calcium level was significantly associated with CSBS total score ($p=0.02$) indicating that among children with 22q11DS, a lower level of circulating calcium is associated with developmental delays. This model was adjusted for age at CSBS assessment, as well as sex. Using the regression coefficient, we can conclude that for every 1 mg / dL decrease in lowest calcium level, the CSBS total score would decrease by 6.18 points. This significant

decrease in CSBS total score would be indicative of greater concern for communication disabilities, which is one of the presentations of autism.

Lowest calcium was also negatively associated with CBCL Externalizing Problems Score. Using linear regression methods, highercalcium levels were significantly associated with decreases in Externalizing Problems score ($p=0.09$) explaining 30% of the variability. For every 1 increment increase in calcium level there was a 5.25 decrease in CBCL Externalizing Problems score. Thus, among these patients with 22q11DS, children with a higher calcium level had fewer problems with externalizing behaviors (e.g. attention and aggression). Finally, lowest calcium value was also significantly negatively correlated with A-TAC Autism score ($p=0.02$). Using linear regression, lower calcium levels were associated with increases of score, which denotes increasing number of autism symptoms. For every unit decrease in calcium level, there is an increase in A-TAC score by 11 points.

The effect of earliest calcium value on assessments was also analyzed. Among earliest calcium values, there was a positive correlation between value and CDIP assessment scores. The CDIP Social subset is of particular interest for concern for autistic symptoms. Lower calcium value for the earliest value taken was significantly associated with lower CDIP scores ($p=0.05$). For every unit decrease in early calcium level, there was a 3.83 point decrease on CDIP score. Lower CDIP scores, when compared to mean ages, are considered to be indicative of developmental delays. Since this association was shown in particular for social interaction, they may also highlight the present of autistic symptoms. Similar trends were seen for CDIP expressive language

and language composition sections (p=0.03, p=0.07), both of which are also symptoms of autism.

Overall, all of the assessments discussed above follow the same trend. Lower calcium levels, in both the lowest value of calcium on record and the earliest calcium on record, have been shown to correlate with increase in symptoms of autism. One of the major strength of this study is the use of multiple assessments to ascertain Autism symptoms. Furthermore, all results supported a single trend. Among patients with 22q11DS, lack of calcium homeostasis, specifically hypocalcaemia, could lead to disruptions in normal neuronal developments. These disruptions are shown to be related to presence of autism symptoms.

A limitation of the study is that low calcium could be a marker for some other physiological variable that affects the risk for ASD symptoms. Furthermore, severe hypocalcaemia is usually treated in this patient group. Nevertheless, the growing recognition of the ability of developing neurons to sense and respond to fluctuations in extracellular calcium through the calcium-sensing receptor suggests that circulating levels of calcium may play a role in the developmental adversities associated with 22q11DS, and possibly ASD. Future studies could be prospective, monitoring childhood development, and evaluating the effectiveness of calcium supplements and effective calcium control in individuals with 22q11DS. Such studies could be further extended to other perinatal and infant groups at high risk for ASD.

FIGURES

Figure 1: Study Patient Selection

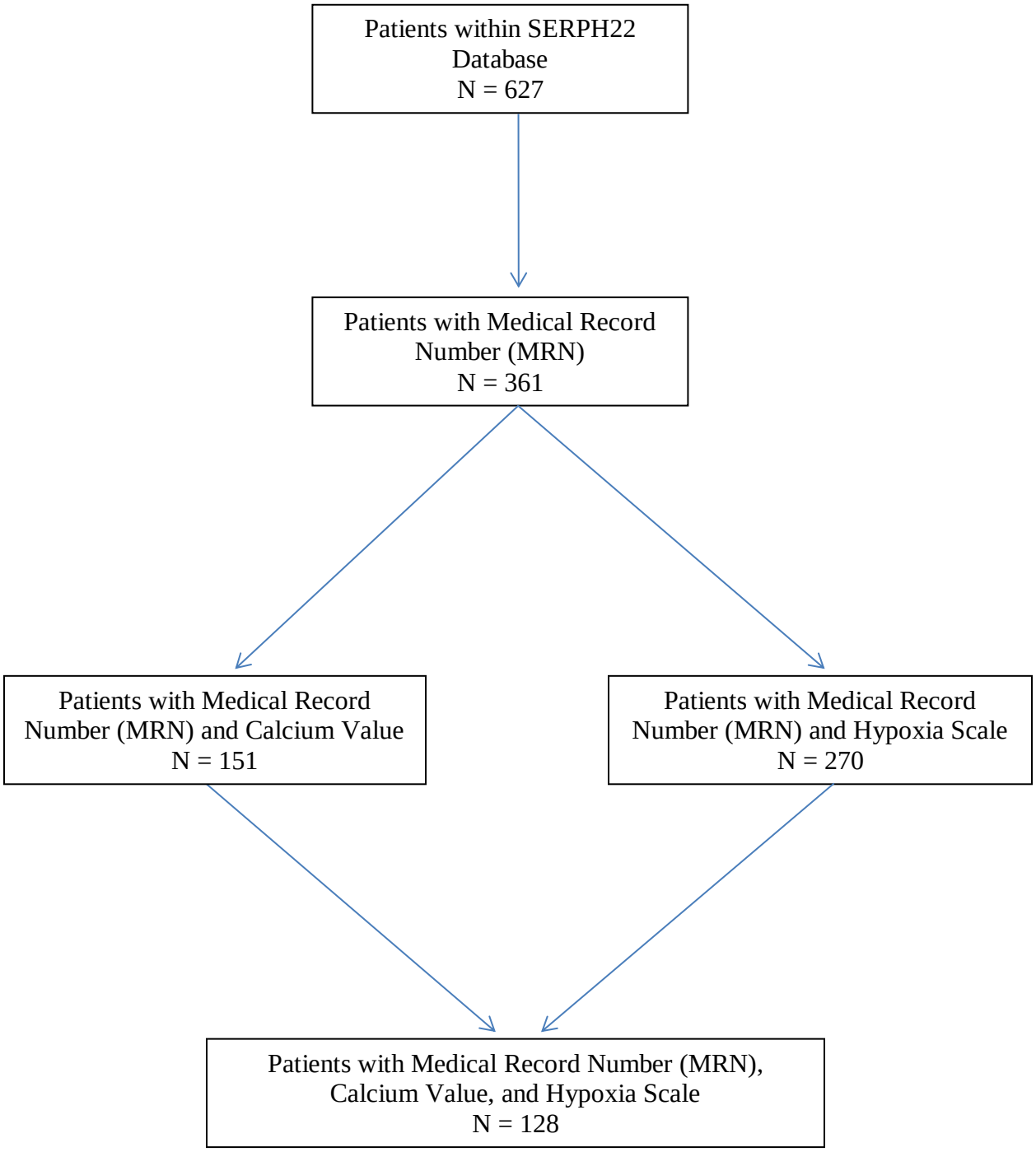


Figure 2: ANOVA Results comparing Hypoxia Scale with CSBS Assessment.

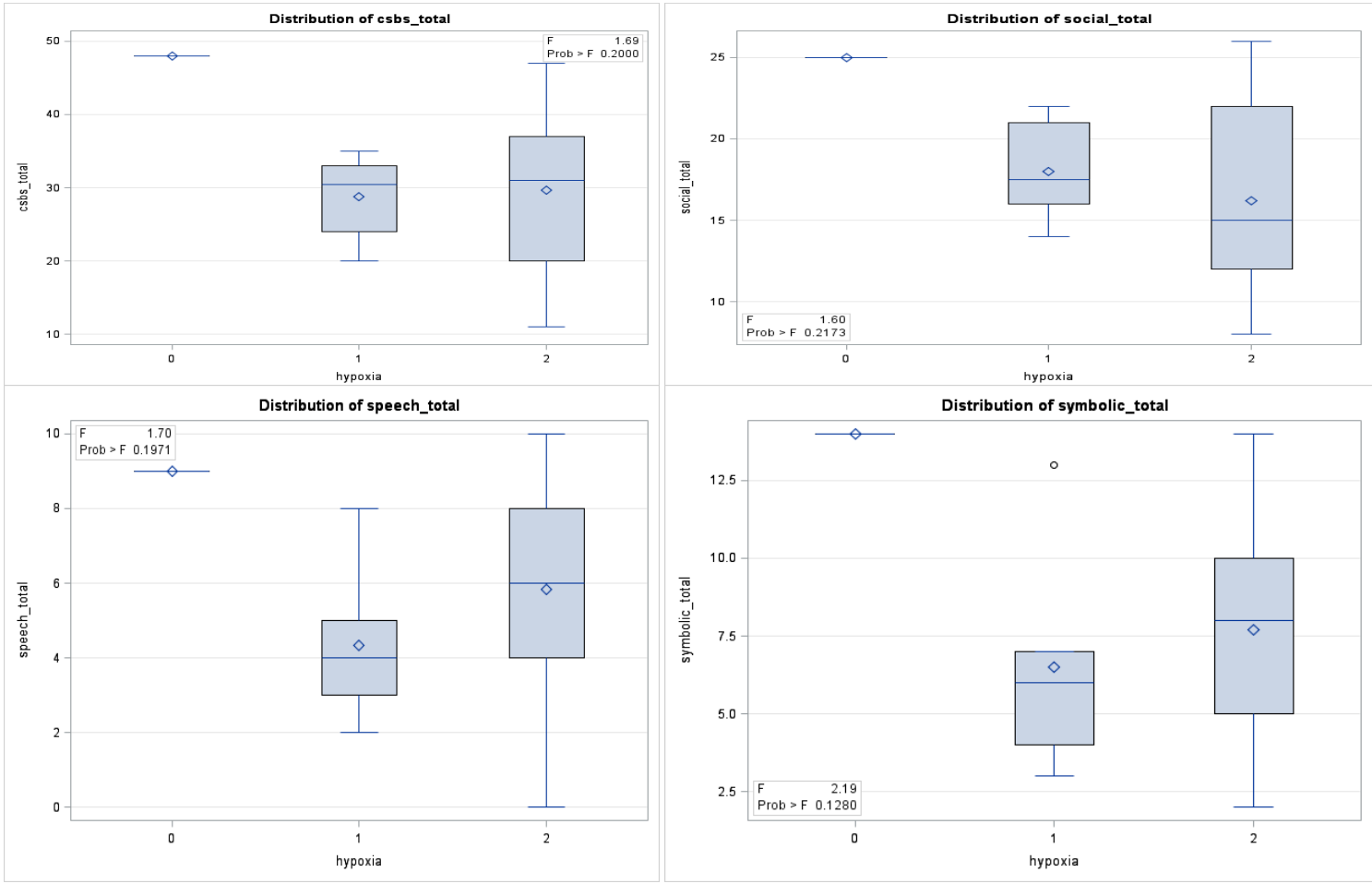


Figure 3: ANOVA Results comparing Hypoxia Scale with CDIP Assessment.

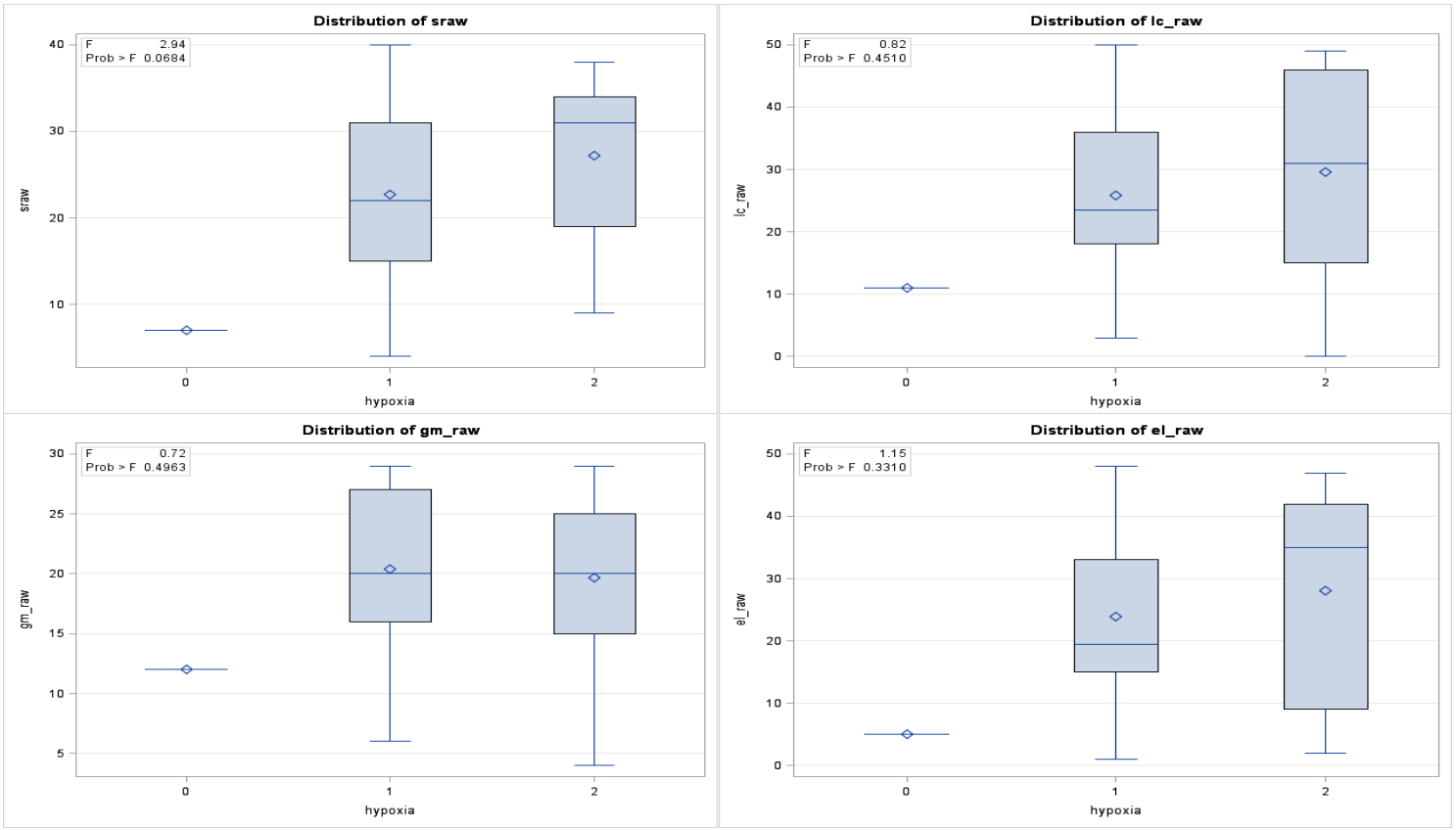


Figure 4: ANOVA Results comparing Hypoxia Scale with ADOS score.

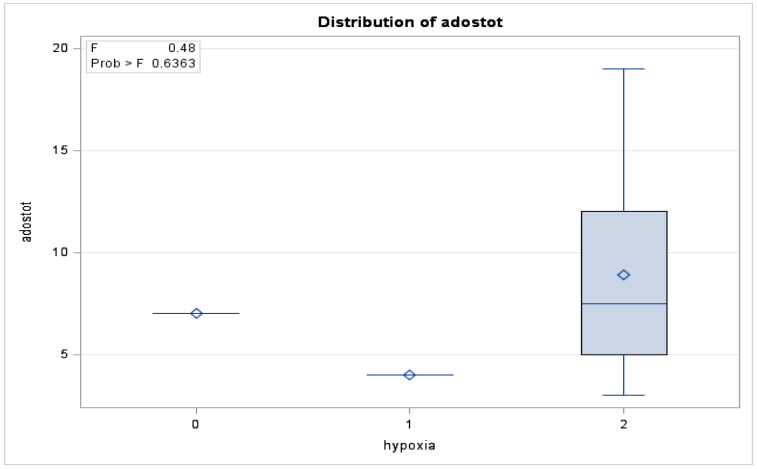
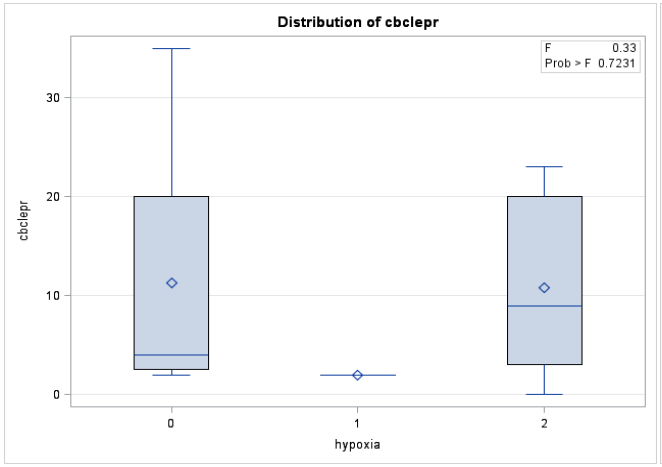
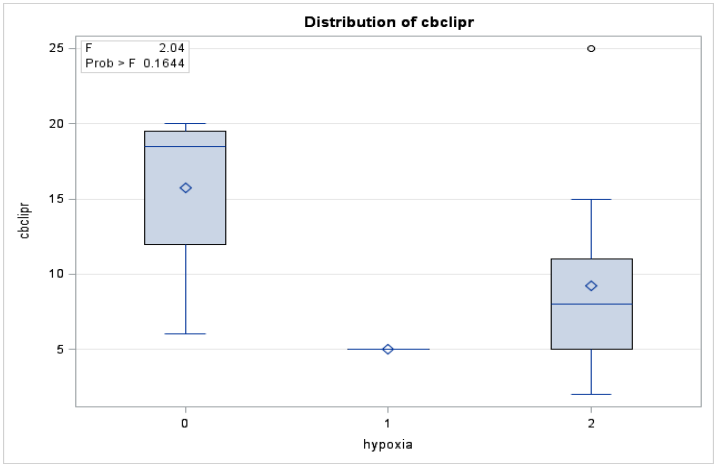


Figure 5: ANOVA Results comparing Hypoxia Scale with CBCL Assessment scores.





TABLES

Table 1: Descriptive Statistics of Patients in Current Study*

Variable	n	Percent
Male	80	53%
Female	68	45%

	n	Mean	SD	Min	Max
Age	361	11.60	7.54	0.3	50.24

Variable	n	Mean	SD	Min	Max	Skewness	Kurtosis
Age at Childhood Visit	6	10.68	2.21	6.22	11.95	-2.34	5.55
Age at CDIP	13	3.88	1.02	2.24	5.70	0.50	0.15
Age at CSBS	20	1.22	0.32	0.78	1.92	0.76	-0.12
Age at Low Calcium	151	2.48	4.56	0.00	19.46	2.24	4.17
Age at Early Calcium	151	2.03	4.29	0.00	19.46	2.53	5.65
Age at Cardiac Surgery	68	0.21	0.44	0.00	3.28	5.52	37.52
Calcium							
Early Calcium	151	8.32	1.07	4.20	11.18	-0.45	1.46
Low Calcium	151	9.08	1.03	5.78	11.18	-0.47	0.46

	n	Percent
Hypoxia		
No Cardiac defect	58	14.57%
Primary Cardiac defects	105	26.38%
Secondary Cardiac defects	235	59.05%

*These patients were those which posessed a medical record number to evaluate cardiac and calcium status.

Table 2: Neuropsychiatric Assements Administered

Child Assessments		**Test**	**Scoring**	**Result**
Autism Diagnostic Interview, Revised	ADI-R	Semi-structured interview given by a clinician to caregivers of children and adults. Contains 93 items assessing three domains: 1) quality of social interaction, 2) communication/ language, and 3) repetitive, restricted and stereotyped interests	Score of 0 is given when behavior of the type specified in the coding is not present; 1 specified behavior present but not severe/ frequent; 2 definite abnormal in specified behavior; 3 "extreme severity" of the specified behavior.	Higher score indicates greater ASD behavior. Cutoff for communication and language domain is >=8 for verbal subjects and >=7 for nonverbal subjects. Cutoff for the social interaction >=10, and the cutoff for restricted and repetitive behaviors is >=3.
Autism Diagnostic Observation Schedule	ADOS	Semi-structured play sessions to evaluate communication, social interaction, and play	Module and scoring algorithm depend on language level/age.	Higher score indicates greater ASD behavior.
Autism, Tics, and ADHD Inventory	A-TAC	Parent telephone interview assessing presence of ASD symptoms during any phase of life. Contains 96 gate items, with 163 corresponding follow-up questions	Presence of symptoms gives a score of 1, no symptoms gives a score of 0. Sum scores for difference diagnoses.	Higher score indicates presence of ASD symptoms. Often used as a screening tool for epidemiologic studies.
Child Behavior Checklist	CBCL	Parent questionnaire to evaluate childhood behavior. 112 items that assess behavior	Scores are summed to assess behavioral problems.	Higher scores suggest more behavioral problems.
Childhood Development Inventory Profile	CDIP	270 statements used to describe behaviors in first year of life.	Developmental skills are compared to means for the appropriate age	Higher scores correspond to better attainment. Scores below the age appropriate mean indicative of developmental delays
Communication and Symbolic Behavior Scales Developmental Profile	CSBS	Checklist for infant-toddlers. 24 questions that have three main aspects: social, speech, and symbolic composites	Scores for each section are totaled for a combined score.	Higher scores indicate better development and less concern for developmental delay.

Table 3: Psychological Assessment Descriptive Statistics for patients in SERPH22 Database.

Assessment	N	Mean	SD	Min	Max	Skewness	Kurtosis
ADOS Repitive Behavior and Social Affect Total Score	13	7.15	3.74	1.00	13.00	0.15	-0.91
ADI-R Social Total	28	9.04	6.12	1.00	25.00	0.85	0.45
A-TAC Autism Score	14	18.64	12.75	0.00	47.00	0.64	0.31
CSBS Total	37	30.54	10.40	11.00	48.00	-0.33	-0.89
CSBS Speech Total	37	5.59	2.66	0.00	10.00	-0.32	-0.59
CSBS Symbolic Total	37	7.92	3.49	2.00	14.00	0.20	-1.08
CSBS Social Total	37	17.05	5.47	8.00	26.00	-0.31	-1.17
CDIP Social Raw Score	36	23.83	9.86	4.00	40.00	-0.15	-1.14
CDIP Expressive Language Score	36	24.25	16.38	1.00	48.00	0.02	-1.53
CDIP Language Composition Score	36	26.47	15.64	0.00	50.00	0.11	-1.37
CDIP Gross Motor Score	36	18.67	7.02	4.00	29.00	-0.26	-0.81
CBCL Social Score	13	5.73	2.20	1.00	9.50	-0.33	1.19
CBCL Externalizing Problems Score	21	11.81	9.35	0.00	35.00	0.74	0.08
CBCL Internalizing Problems Score	21	12.43	6.31	3.00	25.00	0.32	-0.96

Table 4: Psychological Assessment Descriptive Statistics with MRN and Calcium Value.

Assessment	N	Mean	SD	Min	Max	Skewness	Kurtosis
ADOS Social Total	2	5.00	7.07	0.00	10.00		
ADI-R Social Total	9	10.89	8.31	1.00	25.00	0.66	-0.56
A-TAC Autism Score	14	18.64	12.75	0.64	0.31	0.00	47.00
CSBS Total	20	30.60	9.73	11.00	45.00	-0.43	-0.75
CSBS Speech Total	20	5.95	2.42	0.00	9.00	-0.56	0.22
CSBS Symbolic Total	20	7.70	3.40	2.00	13.00	0.10	-1.03
CSBS Social Total	20	16.95	5.24	8.00	26.00	-0.10	-1.06
CDIP Social Raw Score	13	26.54	8.78	9.00	38.00	-0.53	-0.57
CDIP Expressive Language Score	13	26.08	16.10	2.00	47.00	-0.02	-1.45
CDIP Language Composition Score	13	26.69	15.82	0.00	49.00	-0.06	-0.99
CDIP Gross Motor Score	13	18.77	7.61	4.00	29.00	-0.27	-0.54
CBCL Social Score	6	4.75	1.92	1.00	6.50	-1.98	4.52
CBCL Externalizing Problems Score	8	16.25	11.84	0	35	-0.03	-0.81
CBCL Internalizing Problems Score	8	11.25	5.70	3	19	0.09	-1.20

Table 5: Psychological Assessment Descriptive Statistics with MRN and Hypoxia Scale.

Assessment	N	Mean	SD	Min	Max	Skewness	Kurtosis
ADOS Social Total	9	8.00	3.61	3.00	13.00	0.19	-1.34
ADI-R Social Total	18	9.28	7.00	1.00	25.00	0.82	0.07
A-TAC Autism Score	14	18.64	12.75	0.64	0.31	0.00	47.00
CSBS Total	34	30.44	10.44	11.00	48.00	-0.31	-0.83
CSBS Speech Total	34	5.71	2.74	0.00	10.00	-0.43	-0.60
CSBS Symbolic Total	34	7.76	3.48	2.00	14.00	0.25	-1.00
CSBS Social Total	34	16.97	5.44	8.00	26.00	-0.28	-1.10
CDIP Social Raw Score	33	24.18	9.71	4.00	40.00	-0.23	-0.97
CDIP Expressive Language Score	33	25.06	16.40	1.00	48.00	-0.05	-1.50
CDIP Language Composition Score	33	27.15	15.63	0.00	50.00	0.07	-1.35
CDIP Gross Motor Score	33	19.18	6.93	4.00	29.00	-0.34	-0.67
CBCL Social Score	9	5.61	2.68	1.00	9.50	-0.13	-0.08
CBCL Externalizing Problems Score	15	12.2	10.29	0.00	35.00	0.78	-0.23
CBCL Internalizing Problems Score	15	11.6	6.66	3.00	25.00	0.58	-0.77

Table 6: Correlation Analysis between Outcomes and Potential Confounders.

		Gender	Age at Low Calcium	Age at Early Calcium	Age at Cardiac Surgery
ADI-R Social	Correlation	0.02976	0.11853	0.11796	-0.10755
	P- value	*0.8214*	*0.7613*	*0.7625*	*0.7394*
ADOS Total	Correlation	0.01338	-0.51065	-0.51215	-0.26274
	P- value	*0.9472*	*0.3006*	*0.2989*	*0.5296*
A-TAC Autism Total	Correlation	0.29849	0.73082	0.70542	-0.08481
	P- value	*0.2999*	*0.1607*	*0.1832*	*0.8565*
CSBS Total	Correlation	-0.00727	0.16479	0.25179	0.16054
	P- value	*0.964*	*0.4875*	*0.2842*	*0.4334*
CSBS Social Score	Correlation	-0.10893	0.14472	0.2735	0.12624
	P- value	*0.4978*	*0.5427*	*0.2433*	*0.5389*
CSBS Speech Score	Correlation	0.0434	-0.11201	-0.04065	0.23804
	P- value	*0.7876*	*0.6382*	*0.8649*	*0.2416*
CSBS Symbolic Score	Correlation	0.10509	0.32765	0.32743	0.10161
	P- value	*0.5132*	*0.1585*	*0.1588*	*0.6214*
CDIP Social Score	Correlation	0.30283	0.08925	-0.1674	-0.23743
	P- value	*0.0575*	*0.7719*	*0.5846*	*0.3*
CDIP Expressive Language Score	Correlation	0.19849	0.10933	-0.14327	-0.29784
	P- value	*0.2195*	*0.7222*	*0.6406*	*0.1897*
CDIP Language Composition Score	Correlation	0.25208	0.24525	-0.00958	-0.23776
	P- value	*0.1166*	*0.4193*	*0.9752*	*0.2993*
CDIP Gross Motor Score	Correlation	0.41534	0.18364	-0.06965	-0.33844
	P- value	*0.0077*	*0.5481*	*0.8211*	*0.1334*
CBCL Externalizing Problems Score	Correlation	0.11002	0.14984	0.14897	-0.4909
	P- value	*0.4991*	*0.7232*	*0.7248*	*0.1051*
CBCL Internalizing Problems Score	Correlation	-0.12736	0.35883	0.35838	0.72931
	P- value	*0.4336*	*0.3827*	*0.3834*	*0.0071*
CBCL Social Score	Correlation	-0.15025	0.53989	0.54393	-0.12062
	P- value	*0.4544*	*0.2688*	*0.2646*	*0.776*

Table 7: Crude Correlation Analysis between Outcome and Predictors.

		Low Calcium	Early Calcium	Hypoxia Scale
ADI-R Social	Correlation	-0.34733	-0.56438	0.19805
	P- value	*0.3597*	*0.145*	*0.11*
ADOS Total	Correlation	-0.21893	0.42684	0.09558
	P- value	*0.6768*	*0.3986*	*0.6363*
A-TAC Autism Total	Correlation	-0.92787	-0.63428	0.0306
	P- value	*0.023*	*0.2504*	*0.8831*
CSBS Total	Correlation	0.19415	0.37207	0.09033
	P- value	*0.4121*	*0.1062*	*0.2*
CSBS Social Score	Correlation	0.07159	0.25287	0.08587
	P- value	*0.7642*	*0.2821*	*0.2173*
CSBS Speech Score	Correlation	0.31042	0.38037	0.09112
	P- value	*0.1828*	*0.0978*	*0.1971*
CSBS Symbolic Score	Correlation	0.22415	0.40366	0.1139
	P- value	*0.3421*	*0.0776*	*0.128*
CDIP Social Score	Correlation	0.51113	0.54936	0.16379
	P- value	*0.0742*	*0.0518*	*0.0684*
CDIP Expressive Language Score	Correlation	0.41331	0.57643	0.07106
	P- value	*0.1604*	*0.0392*	*0.331*
CDIP Language Composition Score	Correlation	0.33607	0.68655	0.05171
	P- value	*0.2616*	*0.0095*	*0.451*
CDIP Gross Motor Score	Correlation	0.45792	0.77632	0.04564
	P- value	*0.1156*	*0.0018*	*0.4963*
CBCL Externalizing Problems Score	Correlation	-0.6299	-0.42063	0.0423
	P- value	*0.0942*	*0.2994*	*0.7231*
CBCL Internalizing Problems Score	Correlation	-0.17109	-0.22583	0.21392
	P- value	*0.6854*	*0.5907*	*0.1644*
CBCL Social Score	Correlation	0.74925	-0.32209	0.1911
	P- value	*0.0864*	*0.5336*	*0.385*

Table 8: Low Calcium and CSBS Total Score.

Backward Regression

		Overall Model				Coefficient		
	Variables	*R^2*	*Adjusted R^2*	*F*	*P-Value*	*Beta*	*SE*	*Partial P-value*
1	Low Calcium					6.76626	2.48342	0.0157
	Age at Low Calcium					-3.62082	3.62574	0.334
	Age at CSBS Assessment					21.31538	8.71902	0.0273
	Sex					-12.0014	5.44093	0.0434
		0.4326	0.2813	2.86	0.0605*			
2	Low Calcium					6.17961	2.41258	0.0209
	CSBS age					15.45371	6.44397	0.029
	Sex					-11.4038	5.40726	0.0511
		0.395	0.2815	3.48	0.0407*			

*Significant at α=0.10

Table 9: Low Calcium and CSBS Social Total Score.

Backward Regression

		Overall Model				Coefficient		
	Variables	*R^2*	*Adjusted R^2*	*F*	*P-Value*	*Beta*	*SE*	*Partial P-value*
1	Low Calcium					2.98623	1.39073	0.0485
	Age at Low Calcium					-1.17425	2.03145	0.5718
	Age at CSBS Assessment					7.81689	4.88271	0.1302
	Sex					-8.16691	3.04695	0.0171
		0.3863	0.2227	2.36	0.1001*			
2	Low Calcium					2.79597	1.3228	0.0506
	CSBS age					5.91592	3.53318	0.1135
	Sex					-7.97311	2.96477	0.0161
		0.3726	0.255	3.17	0.0532*			

*Significant at α=0.10

Table 10: Low Calcium and CSBS Speech Total Score.

Backward Regression

		Overall Model				Coefficient		
	Variables	*R^2*	*Adjusted R^2*	*F*	*P-Value*	*Beta*	*SE*	*Partial P-value*
1	Low Calcium					1.83898	0.60093	0.0079
	Age at Low Calcium					-1.9987	0.87778	0.0379
	Age at CSBS Assessment					5.97417	2.10979	0.0126
	Sex					-2.32469	1.31657	0.0978
		0.462	0.3185	3.22	0.0427*			

*Significant at α=0.10

Table 11: Low Calcium and CSBS Symbolic Total Score.

Backward Regression

		Overall Model				Coefficient		
	Variables	*R^2*	*Adjusted R^2*	*F*	*P-Value*	*Beta*	*SE*	*Partial P-value*
1	Low Calcium					1.94108	0.88758	0.045
	Age at Low Calcium					-0.44787	1.29649	0.7346
	Age at CSBS Assessment					7.52432	3.11619	0.029
	Sex					-1.5098	1.9446	0.4496
		0.4086	0.2509	2.59	0.0791*			
2	Low Calcium					1.86851	0.83829	0.0405
	CSBS age					6.79928	2.23906	0.0079
	Sex					-1.43589	1.87884	0.4558
		0.4039	0.2922	3.61	0.0364*			
3	Low Calcium					1.53972	0.71062	0.0448
	Age at CSBS Assessment					6.6618	2.20435	0.0077
		0.3822	0.3095	5.26	0.0167*			

*Significant at α=0.10

Table 12: Low Calcium and CBCL Externalizing Problems Score.

Backward Regression

		Overall Model				Coefficient		
	Variables	*R^2*	*Adjusted R^2*	*F*	*P-Value*	*Beta*	*SE*	*Partial P-value*
1	Low Ca					-6.18505	11.77592	0.6921
	Age at Low Ca					-0.24595	7.00735	0.9777
	Child Visit age					42802	12.81847	0.9788
	Sex					-10.0242	11.41534	0.5413
		0.7719	**	0.85	0.6619			
2	Low Ca					-7.39201	3.08361	0.0746
	Age at Low Ca					0.68773	0.67564	0.3663
	Sex					-12.2643	9.13586	0.2506
		0.5996	0.2993	2	0.2568			
3	Low Ca					-6.68553	3.01533	0.1238
	Sex					-8.15748	8.22624	0.3669
		0.4959	0.2943	2.46	0.1804			
4	Low Ca					-5.25232	2.65914	0.0942
		0.3968	0.2962	3.95	0.0942*			

*Significant at α=0.10
** Model did not fit.

Table 13: Low Calcium and A-TAC Autism Score

Backward Regression

		Overall Model				Coefficient		
	Variables	*R^2*	*Adjusted R^2*	*F*	*P-Value*	*Beta*	*SE*	*Partial P-value*
1	Low Calcium					-9.80399	3.64608	0.1149
	Age at Low Calcium					1.05616	1.2155	0.4765
		0.899	0.7981	8.91	0.101			
2	Low Ca					-11.778	2.73292	0.023
		0.8146	0.8609	18.57	0.023*			

*Significant at α=0.10

Table 14: Early Calcium and CDIP Social Score

Backward Regression

		Overall Model				Coefficient		
	Variables	*R^2*	*Adjusted R^2*	*F*	*P-Value*	*Beta*	*SE*	*Partial P-value*
1	Early Calcium					1.36975	1.84914	0.48
	Age at Early Calcium					0.1818	0.91342	0.8472
	CDIP age					-0.97263	1.94232	0.63
	Sex					12.23977	4.89553	0.0369
		0.6408	0.4611	3.57	0.0593*			
2	Early Calcium					1.50271	1.62967	0.3805
	CDIP age					-0.93082	1.825	0.6223
	Sex					11.77737	4.07276	0.0178
		0.639	0.5186	5.31	0.0221*			
3	Early Calcium					1.45067	1.56514	0.3758
	Sex					11.26638	3.79878	0.0141
		0.6285	0.5542	8.46	0.0071*			
4	Early Calcium					3.83039	1.75661	0.0518
		0.3018	0.2383	4.75	0.0518*			

*Significant at α=0.10

Table 15: Early Calcium and CDIP Expressive Language Score.

Backward Regression

		Overall Model				Coefficient		
	Variables	*R^2*	*Adjusted R^2*	*F*	*P-Value*	*Beta*	*SE*	*Partial P-value*
1	Early Calcium					2.36643	3.09669	0.4667
	Age at Early Calcium					0.57259	1.52966	0.7179
	CDIP age					-0.35808	3.25273	0.9151
	Sex					23.34511	8019838	0.0216
		0.7004	0.5505	4.67	0.0306*			
2	Early Calcium					2.35996	2.92127	0.44
	Age at Early Calcium					0.55438	1.43481	0.7082
	Sex					23.10452	7.45543	0.0127
		0.6999	0.5999	7	0.01*			
3	Early Calcium					2.77253	2.6009	0.3115
	Sex					21.76446	6.31266	0.0062
		0.6949	0.6339	11.39	0.0026*			
4	Early Calcium					7.36969	3.14997	0.0392
		0.3323	0.2716	5.47	0.0392*			

*Significant at α=0.10

Table 16: Early Calcium and CDIP Language Composition Score.

Backward Regression

		Overall Model				Coefficient		
	Variables	*R^2*	*Adjusted R^2*	*F*	*P-Value*	*Beta*	*SE*	*Partial P-value*
1	Early Calcium					4.5106	2.97498	0.1679
	Age at Early Calcium					1.1151	1.46955	0.4697
	CDIP age					-2.25776	3.1249	0.4906
	Sex					20.08969	7.87617	0.0341
		0.7137	0.5705	4.98	0.0259*			
2	Early Calcium					4.46984	2.89439	0.1569
	Age at Early Calcium					1.00027	1.4216	0.4995
	Sex					18.57269	7.38682	0.0331
		0.695	0.5933	6.84	0.0107*			
3	Early Calcium					5.21425	2.62521	0.0751
	Sex					16.15485	6.37166	0.0296
		0.6782	0.6139	10.54	0.0035*			

*Significant at α=0.10

Table 17: Early Calcium and CDIP Gross Motor Score.

Backward Regression

		Overall Model				Coefficient		
	Variables	*R^2*	*Adjusted R^2*	*F*	*P-Value*	*Beta*	*SE*	*Partial P-value*
1	Early Calcium					3.39883	1.32793	0.0337
	Age at Early Calcium					0.05519	0.65596	0.935
	CDIP age					-1.08237	1.39485	0.4601
	Sex					6.92741	3.51565	0.0843
		0.7531	0.6297	6.1	0.0149*			
2	Early Calcium					3.4392	1.16795	0.0164
	CDIP age					-1.06968	1.30794	0.4346
	Sex					6.78704	2.91887	0.0451
		0.7529	0.6705	9.14	0.0043*			
3	Early Calcium					3.3794	1.1462	0.0146
	Sex					6.19983	2.78194	0.05
		0.7345	0.6814	13.83	0.0013*			

*Significant at α=0.10

APPENDIX

EMORY UNIVERSITY | Institutional Review Board

TO: Bradley Pearce
Principal Investigator
Epidemiology

DATE: December 8, 2011

RE: **Continuing Review Expedited Approval**
CR1_IRB00045086
IRB00045086
Pathophysiological mechanisms of autism risk in patients with 22q11 deletion syndrome

Thank you for submitting a renewal application for this protocol. The Emory IRB reviewed it by the expedited process on 12/8/2011, per 45 CFR 46.110. This reapproval is effective from **12/8/2011** through **12/7/2012** . Thereafter, continuation of human subjects research activities requires the submission of another renewal application, which must be reviewed and approved by the IRB prior to the expiration date noted above.

Any reportable events (e.g., unanticipated problems involving risk to subjects or others, noncompliance, breaches of confidentiality, HIPAA violations, protocol deviations) must be reported to the IRB according to our Policies & Procedures at www.irb.emory.edu, immediately, promptly, or periodically. Be sure to check the reporting guidance and contact us if you have questions. Terms and conditions of sponsors, if any, also apply to reporting.

Before implementing any change to this protocol (including but not limited to sample size, informed consent, study design, you must submit an amendment request and secure IRB approval.

In future correspondence about this matter, please refer to the IRB file ID, name of the Principal Investigator, and study title. Thank you.

Sincerely,

Andrea Goosen, MPH, CIP
Research Protocol Analyst
This letter has been digitally signed

CC:

Coleman	Karlene	Nursing - Main
Crawford	Mackenzie	Public Health
Cubells	Joseph	H.Genetics
Fernandez-Carriba	Samuel	Psychiatry - Main
Kobrynski	Lisa	Allergy
Oster	Matthew	RTP
Ousley	Opal	Psychiatry - Main
Patel	Sheena	Public Health
Sarrett	Jennifer	Graduate ILA

Appendix Table 1. Hypoxia Scale Frequency by Childhood Psychological Assessment

Assessment	Overall N	Individuals with Hypoxia Scale 0	Individuals with Hypoxia Scale 1	Individuals with Hypoxia Scale 2
ADOS	12	1	1	10
ADI-R	23	4	4	15
CSBS	37	1	6	36
CDIP	34	1	14	19
CBCL	12	1	1	10

Appendix Table 2. Complete Initial Correlations of Calcium values with all subsections of Childhood Psychological Assessments.

	Low Calcium Level			*Early Calcium Level*		
Childhood Assessment	n	Corr Coeff	p-value	n	Corr Coeff	p-value
CSBS Total Social Score	20	0.07159	0.7642	20	0.25287	0.2821
CSBS Speech Total Score	20	0.31042	0.1828	20	0.38067	0.0978
CSBS Symbolic Total Score	20	0.22415	0.3421	20	0.40366	0.0776
CSBS Overall Score	20	0.19415	0.4121	20	0.37207	0.1062
CDIP Social Raw	13	0.39063	0.1869	13	0.54936	0.0518
CDIP Self Help Raw	13	0.51113	0.0742	13	0.66861	0.0125
CDIP Gross Motor Raw	13	0.45792	0.1156	13	0.77632	0.0018
CDIP Fine Motor Raw	13	0.28531	0.3447	13	0.52731	0.0641
CDIP Expressive Language Raw	13	0.41331	0.1604	13	0.57643	0.0392
CDIP Language Composition Raw	13	0.33607	0.2616	13	0.68655	0.0095
CDIP Letters Raw	13	0.25582	0.3989	13	0.47123	0.1041
CDIP Numbers Raw	13	0.18723	0.5402	13	0.52696	0.0643
ADOS Communication Total	2	n/a	n/a	2	n/a	n/a
ADOS Social Total	2	n/a	n/a	2	n/a	n/a
ADOS Social Affect Total	8	-0.47757	0.2314	8	-0.1281	0.7624
ADOS Restricted and Repetitive Behavior Total	8	0.39731	0.3297	8	0.46513	0.2455
ADOS Social Affect + Rest. And Rep. Behavior Total	6	-0.21893	0.6768	6	0.42684	0.3986
ADI-R Social Total (cutoff 10)	9	-0.34733	0.3597	9	-0.23529	0.5422
ADI-R Communication Total (cutoff 8)	8	-0.22608	0.5903	8	-0.56438	0.145
ADI-R NonVerbal Comm Total (cutoff 7 if non-verbal)	1	n/a	n/a	1	n/a	n/a
ADI-R RRB (cutoff 3)	9	-0.43308	0.2443	9	0.23349	0.5454
ADI-R Development Score (cutoff 1)	9	-0.13654	0.7261	9	0.25179	0.5134
DAS Verbal Similarities Raw Score	5	0.43517	0.464	5	0.39035	0.5159
DAS Word Definitions Raw Score	5	-0.29732	0.6271	5	-0.3132	0.6078
DAS Verbal Comprehension Raw Score	1	n/a	n/a	1	n/a	n/a
DAS Naming Vocabulary Raw Score	1	n/a	n/a	1	n/a	n/a

DAS Memory for Digits Raw Score	6	0.52676	0.2829	6	-0.561	0.2468
DAS Verbal Overall Standard Score	6	0.55184	0.2563	6	0.09075	0.8643
Abberrant Behavior Checklist-Irritability Raw	6	-0.92255	0.0088	6	-0.09194	0.8625
Abberrant Behavior Checklist-Lethargy Raw	6	-0.57053	0.2371	6	-0.81639	0.0475
Abberrant Behavior Checklist-Stereotypy Raw	6	-0.75438	0.0831	6	-0.42632	0.3993
Abberrant Behavior Checklist-Hyperactivity Raw	6	-0.75563	0.0823	6	-0.32659	0.5275
Abberrant Behavior Checklist-Inappropriate Speech Raw	6	-0.57547	0.2321	6	0.23918	0.6481
CBCL Activities Total Raw	6	0.03751	0.9438	6	-0.16078	0.7609
CBCL Social Total score	6	0.74925	0.0864	6	-0.32209	0.5336
CBCL School Total Score	6	-0.40732	0.4228	6	0.64348	0.168
CBCL Total Competene Total Score	6	0.64516	0.1665	6	-0.06166	0.9076
CBCL Anxious Depressed Raw Score	8	-0.20102	0.6331	8	0.03932	0.9264
CBCL Withdrawn Depressed Raw Score	8	-0.21865	0.6029	8	-0.59307	0.1212
CBCL Somatic Complaits Raw Score	8	0.0567	0.8939	8	0.16232	0.7009
CBCL Social Problems Raw Score	8	-0.3441	0.4039	8	-0.60509	0.112
CBCL Thought Problems Raw Score	8	-0.56462	0.1448	8	0.32407	0.4336
CBCL Attention Problems Raw Score	8	-0.22454	0.5929	8	-0.08007	0.8505
CBCL Rule-Breaking Behavior Raw Score	8	-0.39383	0.3344	8	-0.47578	0.2334
CBCL Aggressive Behavior Raw Score	8	-0.7297	0.0399	8	-0.36547	0.3733
CBCL Internalizing Problems Total score	8	-0.17109	0.6854	8	-0.22583	0.5907
CBCL Externalizing Problems Total score	8	-0.6299	0.0942	8	-0.42063	0.2994
CBCL Total Problems Total score	8	-0.58702	0.126	8	-0.2705	0.517

CBCL Affective Problems Score (DSM scale)	6	-0.56007	0.2477	6	-0.08473	0.8732
CBCL Anxiety Problems Score (DSM)	6	-0.23444	0.6548	6	0.72059	0.1062
CBCL Somatic Problems (DSM)	6	0.1625	0.7584	6	-0.31452	0.5438
CBCL Attention Deficit Hyperactivity Problems (DSM)	6	-0.50574	0.3061	6	-0.33187	0.5205
CBCL Oppositional Defiant Problems Score (DSM)	6	-0.87475	0.0225	6	-0.10056	0.8497
CBCL Conduct Problems Raw Score (DSM)	6	-0.53974	0.269	6	-0.32028	0.536
CBCL Sluggish Cognitive Temp Raw Score	6	0.09543	0.8573	6	0.12808	0.8089
CBCL Obbsessive Compulsive Problems Raw Score	6	-0.32916	0.5241	6	0.55316	0.2549
CBCL Post-traumatic Stress Problem Raw Score	6	-0.09934	0.8515	6	0.05442	0.9185
A-TAC Autism Score	5	-0.92787	0.023	5	-0.63428	0.2504

SAS coding

I. Initial Analysis

```
*************************************************
                  Data Statistics
*************************************************;
proc univariate data=muldoon.thesis;
      var mrn sex race ic_22qdx
      abcs1r abcs2r abcs3r abcs4r abcs5r
      adicom adicomvv adidev adirrb adisoc
      ados_comtot ados_soctot adosrrb adossocaff adostot
      cbclabr cbclactr cbcladr cbclafpr cbclahpr cbclatpr cbclaxpr
      cbclcpr cbclepr cbclipr cbclocdr cbclodpr cbclptsr cbclrbbr
      cbclschr cbclscr cbclsctr cbclsmpr cbclsocr cbclspr cbcltcr
      cbcltotpr cbcltpr cbclwdrcdipage el_raw fm_raw gm_raw lc_raw
      letter_raw numb_raw sh_raw sraw
      childvisitage childvisit2age
      csbs_total csbsage
      social_total speech_total symbolic_total
      dasmdr dasnvr dasvcr dasvsr dasvss daswdr
      lowca agelowca earlyca ageearlyca
      hypoxia surgeryage;
run;

proc univariate data=muldoon.thesis;
      var mrn sex race ic_22qdx
      abcs1r abcs2r abcs3r abcs4r abcs5r
      adicom adicomvv adidev adirrb adisoc
      ados_comtot ados_soctot adosrrb adossocaff adostot
      cbclabr cbclactr cbcladr cbclafpr cbclahpr cbclatpr cbclaxpr
      cbclcpr cbclepr cbclipr cbclocdr cbclodpr cbclptsr cbclrbbr
      cbclschr cbclscr cbclsctr cbclsmpr cbclsocr cbclspr cbcltcr
      cbcltotpr cbcltpr cbclwdrcdipage el_raw fm_raw gm_raw lc_raw
      letter_raw numb_raw sh_raw sraw
      childvisitage childvisit2age
      csbs_total csbsage
      social_total speech_total symbolic_total
      dasmdr dasnvr dasvcr dasvsr dasvss daswdr
      lowca agelowca earlyca ageearlyca
      hypoxia surgeryage;
      where mrn ne .;
run;

proc univariate data=muldoon.thesis;
      var mrn sex race ic_22qdx
      abcs1r abcs2r abcs3r abcs4r abcs5r
      adicom adicomvv adidev adirrb adisoc
      ados_comtot ados_soctot adosrrb adossocaff adostot
      cbclabr cbclactr cbcladr cbclafpr cbclahpr cbclatpr cbclaxpr
      cbclcpr cbclepr cbclipr cbclocdr cbclodpr cbclptsr cbclrbbr
      cbclschr cbclscr cbclsctr cbclsmpr cbclsocr cbclspr cbcltcr
      cbcltotpr cbcltpr cbclwdrcdipage el_raw fm_raw gm_raw lc_raw
      letter_raw numb_raw sh_raw sraw
      childvisitage childvisit2age
      csbs_total csbsage
      social_total speech_total symbolic_total
```

```
      dasmdr dasnvr dasvcr dasvsr dasvss daswdr
      lowca agelowca earlyca ageearlyca
      hypoxia surgeryage;
      where mrn ne . and lowca ne .;
run;

proc univariate data=muldoon.thesis;
      var mrn sex race ic_22qdx
      abcs1r abcs2r abcs3r abcs4r abcs5r
      adicom adicomvv adidev adirrb adisoc
      ados_comtot ados_soctot adosrrb adossocaff adostot
      cbclabr cbclactr cbcladr cbclafpr cbclahpr cbclatpr cbclaxpr
      cbclcpr cbclepr cbclipr cbclocdr cbclodpr cbclptsr cbclrbbr
      cbclschr cbclscr cbclsctr cbclsmpr cbclsocr cbclspr cbcltcr
      cbcltotpr cbcltpr cbclwdrcdipage el_raw fm_raw gm_raw lc_raw
      letter_raw numb_raw sh_raw sraw
      childvisitage childvisit2age
      csbs_total csbsage
      social_total speech_total symbolic_total
      dasmdr dasnvr dasvcr dasvsr dasvss daswdr
      lowca agelowca earlyca ageearlyca
      hypoxia surgeryage;
      where mrn ne . and earlyca ne .;
run;

proc univariate data=muldoon.thesis;
      var mrn sex race ic_22qdx
      abcs1r abcs2r abcs3r abcs4r abcs5r
      adicom adicomvv adidev adirrb adisoc
      ados_comtot ados_soctot adosrrb adossocaff adostot
      cbclabr cbclactr cbcladr cbclafpr cbclahpr cbclatpr cbclaxpr
      cbclcpr cbclepr cbclipr cbclocdr cbclodpr cbclptsr cbclrbbr
      cbclschr cbclscr cbclsctr cbclsmpr cbclsocr cbclspr cbcltcr
      cbcltotpr cbcltpr cbclwdrcdipage el_raw fm_raw gm_raw lc_raw
      letter_raw numb_raw sh_raw sraw
      childvisitage childvisit2age
      csbs_total csbsage
      social_total speech_total symbolic_total
      dasmdr dasnvr dasvcr dasvsr dasvss daswdr
      lowca agelowca earlyca ageearlyca
      hypoxia surgeryage;
      where mrn ne . and hypoxia ne .;
run;


proc freq data=muldoon.thesis;
      table sex ic_22qdx race
      hypoxia;
run;

proc freq data=muldoon.thesis;
      table hypoxia;
      where hypoxia ne .;
run;


************************************************
```

```
   Correlation Outcomes / Potential confounders
************************************************;

proc corr data=muldoon.thesis;
      var sex;
      with adisoc adostot
      csbs_total social_total speech_total symbolic_total
      sraw el_raw lc_raw gm_raw
      cbclepr cbclipr cbclsocr
      dasvss;
run;

proc corr data=muldoon.thesis;
      var ageearlyca;
      with adisoc adostot
      csbs_total social_total speech_total symbolic_total
      sraw el_raw lc_raw gm_raw
      cbclepr cbclipr cbclsocr
      dasvss;
run;

proc corr data=muldoon.thesis;
      var agelowca;
      with adisoc adostot
      csbs_total social_total speech_total symbolic_total
      sraw el_raw lc_raw gm_raw
      cbclepr cbclipr cbclsocr
      dasvss;
run;

proc corr data=muldoon.thesis;
      var surgeryage;
      with adisoc adostot
      csbs_total social_total speech_total symbolic_total
      sraw el_raw lc_raw gm_raw
      cbclepr cbclipr cbclsocr
      dasvss;
run;


****************************************
   Examine correlations with low calcium
****************************************;

proc corr data=muldoon.thesis;
      var lowca;
      with abcs1r abcs2r abcs3r abcs4r abcs5r;
run;

proc corr data=muldoon.thesis;
      var lowca;
      with adicom adicomvv adidev adirrb adisoc;
run;

proc corr data=muldoon.thesis;
      var lowca;
      with ados_comtot ados_soctot adossocaff adosrrb adostot;
```

```
run;

proc corr data=muldoon.thesis;
      var lowca;
      with cbclabr cbclactr cbcladr cbclafpr cbclahpr cbclatpr cbclaxpr
      cbclcpr cbclepr cbclipr cbclocdr cbclodpr cbclptsr cbclrbbr
      cbclschr cbclscr  cbclsctr cbclsmpr cbclsocr cbclspr cbcltcr
      cbcltotpr cbcltpr cbclwdr;
run;

proc corr data=muldoon.thesis;
      var lowca;
      with csbs_total csbsage
      social_total speech_total symbolic_total;
run;

proc corr data=muldoon.thesis;
      var lowca;
      with cdipage el_raw fm_raw gm_raw lc_raw letter_raw numb_raw
      sh_raw sraw;
run;

proc corr data=muldoon.thesis;
      var lowca;
      with dasmdr dasnvr dasvcr dasvsr dasvss daswdr;
run;

proc corr data=muldoon.thesis;
      var lowca;
      with hypoxia;
run;

proc corr data=muldoon.thesis;
      var lowca;
      with hypoxia;
      where hypoxia eq 0;
run;

proc corr data=muldoon.thesis;
      var lowca;
      with hypoxia;
      where hypoxia eq 1;
run;

proc corr data=muldoon.thesis;
      var lowca;
      with hypoxia;
      where hypoxia eq 2;
run;

proc corr data=muldoon.thesis;
      var lowca;
      with sex;
run;

proc corr data=muldoon.thesis;
      var lowca;
```

```
      with agelowca;
run;



*****************************************
      Examine corr with early calcium
*****************************************;
proc corr data=muldoon.thesis;
      var earlyca;
      with abcs1r abcs2r abcs3r abcs4r abcs5r;
run;

proc corr data=muldoon.thesis;
      var earlyca;
      with adicom adicomvv adidev adirrb adisoc;
run;

proc corr data=muldoon.thesis;
      var earlyca;
      with ados_comtot ados_soctot adossocaff adosrrb adostot;
run;

proc corr data=muldoon.thesis;
      var earlyca;
      with cbclabr cbclactr cbcladr cbclafpr cbclahpr cbclatpr cbclaxpr
      cbclcpr cbclepr cbclipr cbclocdr cbclodpr cbclptsr cbclrbbr
      cbclschr cbclscr  cbclsctr cbclsmpr cbclsocr cbclspr cbcltcr
      cbcltotpr cbcltpr cbclwdr;
run;

proc corr data=muldoon.thesis;
      var earlyca;
      with csbs_total csbsage
      social_total speech_total symbolic_total;
run;

proc corr data=muldoon.thesis;
      var earlyca;
      with cdipage el_raw fm_raw gm_raw lc_raw letter_raw numb_raw
      sh_raw sraw;
run;

proc corr data=muldoon.thesis;
      var earlyca;
      with dasmdr dasnvr dasvcr dasvsr dasvss daswdr;
run;

proc corr data=muldoon.thesis;
      var earlyca;
      with hypoxia;
run;

proc corr data=muldoon.thesis;
      var earlyca;
      with hypoxia;
      where hypoxia eq 0;
```

```
run;

proc corr data=muldoon.thesis;
      var earlyca;
      with hypoxia;
      where hypoxia eq 1;
run;

proc corr data=muldoon.thesis;
      var earlyca;
      with hypoxia;
      where hypoxia eq 2;
run;

proc corr data=muldoon.thesis;
      var earlyca;
      with sex;
run;

proc corr data=muldoon.thesis;
      var earlyca;
      with ageearlyca;
run;

*****************************************
      Examine corr with hypoxia_scale
*****************************************;
proc corr data=muldoon.thesis;
      var hypoxia;
      with abcs1r abcs2r abcs3r abcs4r abcs5r;
run;


proc corr data=muldoon.thesis;
      var hypoxia;
      with adicom adicomvv adidev adirrb adisoc;
run;

proc corr data=muldoon.thesis;
      var hypoxia;
      with ados_comtot ados_soctot adossocaff adosrrb adostot;
run;

proc corr data=muldoon.thesis;
      var hypoxia;
      with cbclabr cbclactr cbcladr cbclafpr cbclahpr cbclatpr cbclaxpr
      cbclcpr cbclepr cbclipr cbclocdr cbclodpr cbclptsr cbclrbbr
      cbclschr cbclscr cbclsctr cbclsmpr cbclsocr cbclspr cbcltcr
      cbcltotpr cbcltpr cbclwdr;
run;

proc corr data=muldoon.thesis;
      var hypoxia;
      with csbs_total csbsage
      social_total speech_total symbolic_total;
run;
```

```
proc corr data=muldoon.thesis;
      var hypoxia;
      with cdipage el_raw fm_raw gm_raw lc_raw letter_raw numb_raw
      sh_raw sraw;
run;

proc corr data=muldoon.thesis;
      var hypoxia;
      with dasmdr dasnvr dasvcr dasvsr dasvss daswdr;
run;

proc corr data=muldoon.thesis;
      var hypoxia;
      with sex;
run;

proc corr data=muldoon.thesis;
      var hypoxia;
      with surgeryage;
run;

*****************************************
hypoxia frequency tables to determine n
*****************************************;

proc freq data=muldoon.thesis;
      tables hypoxia;
      where csbs_total ne .;
run;

proc freq data=muldoon.thesis;
      tables hypoxia;
      where sraw ne .;
run;

proc freq data=muldoon.thesis;
      tables hypoxia;
      where adostot ne .;
run;

proc freq data=muldoon.thesis;
      tables hypoxia;
      where adisoc ne .;
run;

proc freq data=muldoon.thesis;
      tables hypoxia;
      where dasvss ne .;
run;

proc freq data=muldoon.thesis;
      tables hypoxia;
      where cbclsocr ne .;
run;


***********************************************
```

```
      Export data- put in age, re-import
*********************************************;
proc contents data=muldoon.ages;
run;

proc print data=muldoon.ages;
      where cdipage gt 10;
run; *1 patient had cdip age of 38, need to remove; *ages database will
be sued for cdip analysis

*RERUN UNIVARTE FOR CDIP AGE AFTER REMOVING 1 error subject (KLOB228);
*entire SERPH22 database;
proc univariate data=muldoon.age;
      var cdipage;
run;
*now ages with mrn;
proc univariate data=muldoon.age;
      var cdipage;
      where mrn ne .;
run;
*now ages with mrn and low calcium;
proc univariate data=muldoon.age;
      var cdipage;
      where mrn ne . and lowca ne .;
run;
*now ages with mrn and earlyl calcium;
proc univariate data=muldoon.age;
      var cdipage;
      where mrn ne . and earlyca ne .;
run;
*now ages with mrn and hypoxia;
proc univariate data=muldoon.age;
      var cdipage;
      where mrn ne . and hypoxia ne .;
run;
*now ages with all three values;
proc univariate data=muldoon.age;
      var cdipage;
      where mrn ne . and hypoxia ne . and lowca ne .;
run;
```

II. Hypoxia Data Analysis

```
*****************************************
      ANOVA analysis for Hypoxia
*****************************************;
ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
      model adisoc = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
```

```
      model cbclsocr = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;


ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
      model lowca = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
      model earlyca = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
      model csbs_total = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
      model social_total = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
      model speech_total = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
      model symbolic_total = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
```

```
      model sraw = hypoxia*sex;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
      model el_raw = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
      model lc_raw = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
      model gm_raw = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
      model adostot = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
      model dasvss = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
      model cbclepr = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

ods graphics on;
proc glm data=thesis.age;
      class hypoxia;
      model cbclipr = hypoxia;
```

```
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;

   III.  Calcium Data Analysis

*****************************************
      develop model for Low Calcium
*****************************************;
proc reg data=thesis.thesis;
model csbs_total= lowca / vif tol;
run;

proc reg data=thesis.thesis;
model csbs_total= lowca agelowca / vif tol;
run;

proc reg data=thesis.thesis;
model csbs_total= lowca agelowca csbsage /vif tol;
run;

proc reg data=thesis.thesis;
model csbs_total= lowca agelowca csbsage sex /vif tol;
run;
*backward elimination for csbs_total - first remove agelowca (only one
not significant);
proc reg data=thesis.thesis;
model csbs total= lowca csbsage sex /vif tol;
run; *all significant;

proc reg data=thesis.thesis;
model social_total= lowca /vif tol;
run;

proc reg data=thesis.thesis;
model social_total= lowca agelowca /vif tol;
run;

proc reg data=thesis.thesis;
model social_total= lowca agelowca csbsage /vif tol;
run;

proc reg data=thesis.thesis;
model social_total= lowca agelowca csbsage sex /vif tol;
run;
*backward elimination for social_total - first remove agelowca (only
one not significant);
proc reg data=thesis.thesis;
model social total= lowca csbsage sex /vif tol;
run; *csbs age not significant at alpha =0.1 so remove next;

proc reg data=thesis.thesis;
model social total= lowca sex /vif tol;
run; *low age now not significant at alpha =0.1 so remain with original
full model;
```

```
proc reg data=thesis.thesis;
model speech_total= lowca /vif tol;
run;

proc reg data=thesis.thesis;
model speech_total= lowca agelowca /vif tol;
run;

proc reg data=thesis.thesis;
model speech_total= lowca agelowca csbsage /vif tol;
run;

proc reg data=thesis.thesis;
model speech_total= lowca agelowca csbsage sex /vif tol;
run;
*no need for backward elimination for specch_total - all items
significant;

proc reg data=thesis.thesis;
model symbolic_total= lowca /vif tol;
run;

proc reg data=thesis.thesis;
model symbolic_total= lowca agelowca /vif tol;
run;

proc reg data=thesis.thesis;
model symbolic_total= lowca agelowca csbsage /vif tol;
run;

proc reg data=thesis.thesis;
model symbolic_total= lowca agelowca csbsage sex /vif tol;
run;
*backward elimination for symbolic_total - first remove agelowca (most
not significant);
proc reg data=thesis.thesis;
model symbolic total= lowca csbsage sex /vif tol;
run; *sex now not significant, so remove next;

proc reg data=thesis.thesis;
model symbolic total= lowca csbsage /vif tol;
run; *everythings is now significant;

proc reg data=thesis.age; *use age data set because it has corrected
cdipage;
model sraw= lowca /vif tol;
run;

proc reg data=thesis.age;
model sraw= lowca agelowca /vif tol;
run;

proc reg data=thesis.age;
model sraw= lowca agelowca cdipage /vif tol;
run;

proc reg data=thesis.age;
```

```
model sraw= lowca agelowca cdipage sex /vif tol;
run;
*no backward elimination for sraw - not enough data;

proc reg data=thesis.age;
model el_raw= lowca /vif tol;
run;

proc reg data=thesis.age;
model el_raw= lowca agelowca /vif tol;
run;

proc reg data=thesis.age;
model el_raw= lowca agelowca cdipage /vif tol;
run;

proc reg data=thesis.age;
model el_raw= lowca agelowca cdipage sex /vif tol;
run;
*backward elimination for el_raw - first remove cdipage (most not
significant);
proc reg data=thesis.age;
model el_raw= lowca agelowca sex /vif tol;
run; *age at lowca and lowca both nonsignificant so remove age at low
ca next;

proc reg data=thesis.thesis;
model el_raw= lowca sex /vif tol;
run; *low ca still not significant stop here;

proc reg data=thesis.age;
model lc_raw= lowca /vif tol;
run;

proc reg data=thesis.age;
model lc_raw= lowca agelowca /vif tol;
run;

proc reg data=thesis.age;
model lc_raw= lowca agelowca cdipage /vif tol;
run;

proc reg data=thesis.age;
model lc_raw= lowca agelowca cdipage sex /vif tol;
run;
*backward elimination for lc_raw - first remove cdipage (most not
significant besides lowca);
proc reg data=thesis.age;
model lc raw= lowca agelowca sex /vif tol;
run; *all still nonsignif so take out sex;

proc reg data=thesis.age;
model lc raw= lowca agelowca /vif tol;
run; *stop here;

proc reg data=thesis.age;
model gm_raw= lowca /vif tol;
```

```
run;

proc reg data=thesis.age;
model gm_raw= lowca agelowca /vif tol;
run;

proc reg data=thesis.age;
model gm_raw= lowca agelowca cdipage /vif tol;
run;

proc reg data=thesis.age;
model gm_raw= lowca agelowca cdipage sex /vif tol;
run;
*backward elimination for gm_raw - first remove cdipage (most not
significant besides lowca and agelowca);
proc reg data=thesis.age;
model gm raw= lowca agelowca sex /vif tol;
run; *lowca and agelowca still non-significant so take out agelowca;
proc reg data=thesis.age;
model gm raw= lowca sex /vif tol;
run; *lowca still not significant so stop here;

proc reg data=thesis.thesis;
model adostot= lowca / vif tol;
run;

proc reg data=thesis.thesis;
model adostot= lowca agelowca / vif tol;
run;

proc reg data=thesis.thesis;
model adostot= lowca agelowca childvisitage / vif tol;
run;

proc reg data=thesis.thesis;
model adostot= lowca agelowca childvisitage sex / vif tol;
run;
*backward elimination for adostot - first remove sex (most not
significant besides all variables);
proc reg data=thesis.thesis;
model adostot= lowca agelowca childvisitage / vif tol;
run; *nothing significant, remove childvisitage next;
proc reg data=thesis.thesis;
model adostot= lowca agelowca / vif tol;
run; *nothing significant, lowca would be next so stop here;

proc reg data=thesis.thesis;
model dasvss= lowca / vif tol;
run;

proc reg data=thesis.thesis;
model dasvss= lowca agelowca / vif tol;
run;

proc reg data=thesis.thesis;
model dasvss= lowca agelowca childvisitage / vif tol;
run;
```

```
proc reg data=thesis.thesis;
model dasvss= lowca agelowca childvisitage sex / vif tol;
run;
*backward elimination for dasvss - nothing significant so take out sex
which is most non;
proc reg data=thesis.thesis;
model dasvss= lowca agelowca childvisitage / vif tol;
run; *everything now signifcant so stop here;

proc reg data=thesis.thesis;
model cbclepr= lowca / vif tol;
run;

proc reg data=thesis.thesis;
model cbclepr= lowca agelowca / vif tol;
run;

proc reg data=thesis.thesis;
model cbclepr= lowca agelowca childvisitage / vif tol;
run;

proc reg data=thesis.thesis;
model cbclepr= lowca agelowca childvisitage sex / vif tol;
run;
*backward elimination for cbclepr - childvisit age most nonsignificant
of all (all variables non) so remove;
proc reg data=thesis.thesis;
model cbclepr= lowca agelowca sex / vif tol;
run; *agelowca and sex not significant so remove age next;
proc reg data=thesis.thesis;
model cbclepr= lowca sex / vif tol;
run; *sex not significant so stop;

proc reg data=thesis.thesis;
model cbclipr= lowca / vif tol;
run;

proc reg data=thesis.thesis;
model cbclipr= lowca agelowca / vif tol;
run;

proc reg data=thesis.thesis;
model cbclipr= lowca agelowca childvisitage / vif tol;
run;

proc reg data=thesis.thesis;
model cbclipr= lowca agelowca childvisitage sex / vif tol;
run;
*backward elimination for cbclipr - lowage most nonsignificant of all
besides lowca
(all variables non) so remove;
proc reg data=thesis.thesis;
model cbclipr= lowca childvisitage sex / vif tol;
run; *childvisit age next to be removed, everything still nonsig;
proc reg data=thesis.thesis;
model cbclipr= lowca sex / vif tol;
```

```
run; *childvisit age next to be removed, everything still nonsig so
stop;

proc reg data=muldoon.second;
model adisoc = lowca / vif tol;
run;

proc reg data=muldoon.second;
model adisoc = lowca agelowca / vif tol;
run;

proc reg data=muldoon.second;
model adisoc = lowca agelowca childvisitage /vif tol;
run;

proc reg data=muldoon.second;
model adisoc = lowca agelowca childvisitage sex /vif tol;
run;

proc reg data=muldoon.second;
model adisoc = lowca childvisitage sex /vif tol;
run;

proc reg data=muldoon.second;
model adisoc = lowca childvisitage /vif tol;
run;

proc reg data=muldoon.second;
model cbclsocr = lowca / vif tol;
run;

proc reg data=muldoon.second;
model cbclsocr = lowca agelowca / vif tol;
run;

proc reg data=muldoon.second;
model cbclsocr = lowca agelowca childvisitage /vif tol;
run;

proc reg data=muldoon.second;
model cbclsocr = lowca agelowca childvisitage sex /vif tol;
run;

proc reg data=muldoon.second;
model cbclsocr = lowca childvisitage sex /vif tol;
run;

proc reg data=muldoon.second;
model cbclsocr = lowca childvisitage /vif tol;
run;


*****************************************
      develop model for Early Calcium
*****************************************;
proc reg data=thesis.thesis;
model csbs_total= earlyca / vif tol;
```

```
run;

proc reg data=thesis.thesis;
model csbs_total= earlyca ageearlyca / vif tol;
run;

proc reg data=thesis.thesis;
model csbs_total= earlyca ageearlyca csbsage /vif tol;
run;

proc reg data=thesis.thesis;
model csbs_total= earlyca ageearlyca csbsage sex /vif tol;
run;
*backward elimination for csbs_total - early ca age most nonsignificant
so remove;
proc reg data=thesis.thesis;
model csbs total= earlyca csbsage sex /vif tol;
run; *sex now most nonsig so remove;
proc reg data=thesis.thesis;
model csbs total= earlyca csbsage /vif tol;
run; *csbsage now most nonsig so remove stop here;

proc reg data=thesis.thesis;
model social_total= earlyca /vif tol;
run;

proc reg data=thesis.thesis;
model social_total= earlyca ageearlyca /vif tol;
run;

proc reg data=thesis.thesis;
model social_total= earlyca ageearlyca csbsage /vif tol;
run;

proc reg data=thesis.thesis;
model social_total= earlyca ageearlyca csbsage sex /vif tol;
run;
*backward elimination for social_total - csbs age most nonsignificant
so remove;
proc reg data=thesis.thesis;
model social total= earlyca ageearlyca sex /vif tol;
run; *earlyca most non signif but remove second which was earlyca age;
proc reg data=thesis.thesis;
model social total= earlyca sex /vif tol;
run; *stop here;

proc reg data=thesis.thesis;
model speech_total= earlyca /vif tol;
run;

proc reg data=thesis.thesis;
model speech_total= earlyca ageearlyca /vif tol;
run;

proc reg data=thesis.thesis;
model speech_total= earlyca ageearlyca csbsage /vif tol;
run;
```

```
proc reg data=thesis.thesis;
model speech_total= earlyca ageearlyca csbsage sex /vif tol;
run;
*backward elimination for speech_total - sex most nonsignificant so
remove;
proc reg data=thesis.thesis;
model speech total= earlyca ageearlyca csbsage /vif tol;
run; *csbs age now nonsignif so remove;
proc reg data=thesis.thesis;
model speech total= earlyca ageearlyca /vif tol;
run; *early ca age now nonsignif so stop here;

proc reg data=thesis.thesis;
model symbolic_total= earlyca /vif tol;
run;

proc reg data=thesis.thesis;
model symbolic_total= earlyca ageearlyca /vif tol;
run;

proc reg data=thesis.thesis;
model symbolic_total= earlyca ageearlyca csbsage /vif tol;
run;

proc reg data=thesis.thesis;
model symbolic_total= earlyca ageearlyca csbsage sex /vif tol;
run;
*backward elimination for symbolic_total - early ca age most
nonsignificant so remove;
proc reg data=thesis.thesis;
model symbolic total= earlyca csbsage sex /vif tol;
run; *sex most non signif so remove;
proc reg data=thesis.thesis;
model symbolic total= earlyca csbsage /vif tol;
run; *stop here;

proc reg data=thesis.age;
model sraw= earlyca /vif tol;
run;

proc reg data=thesis.age;
model sraw= earlyca ageearlyca /vif tol;
run;

proc reg data=thesis.age;
model sraw= earlyca ageearlyca cdipage /vif tol;
run;

proc reg data=thesis.age;
model sraw= earlyca ageearlyca cdipage sex /vif tol;
run;
*backward elimination - remove ageearly first;
proc reg data=thesis.age;
model sraw= earlyca cdipage sex /vif tol;
run;
```

```
proc reg data=thesis.age;
model sraw= earlyca sex /vif tol;
run;

proc reg data=thesis.age;
model el_raw= earlyca /vif tol;
run;

proc reg data=thesis.age;
model el_raw= earlyca ageearlyca /vif tol;
run;

proc reg data=thesis.age;
model el_raw= earlyca ageearlyca cdipage /vif tol;
run;

proc reg data=thesis.age;
model el_raw= earlyca ageearlyca cdipage sex /vif tol;
run;
*backward elimination for el_raw - cdip age most nonsignif so remove;
proc reg data=thesis.age;
model el raw= earlyca ageearlyca sex /vif tol;
run; *early ca age most nonsignif now so remove;
proc reg data=thesis.age;
model el raw= earlyca sex /vif tol;
run; *stop here;

proc reg data=thesis.age;
model lc_raw= earlyca /vif tol;
run;

proc reg data=thesis.age;
model lc_raw= earlyca ageearlyca /vif tol;
run;

proc reg data=thesis.age;
model lc_raw= earlyca ageearlyca cdipage /vif tol;
run;

proc reg data=thesis.age;
model lc_raw= earlyca ageearlyca cdipage sex /vif tol;
run;
*backward elimination for lc_raw - cdip age most nonsignif so remove;
proc reg data=thesis.age;
model lc raw= earlyca ageearlyca sex /vif tol;
run; *early ca age next;
proc reg data=thesis.age;
model lc_raw= earlyca sex /vif tol;
run; *stop here;

proc reg data=thesis.age;
model gm_raw= earlyca /vif tol;
run;

proc reg data=thesis.age;
model gm_raw= earlyca ageearlyca /vif tol;
run;
```

```
proc reg data=thesis.age;
model gm_raw= earlyca ageearlyca cdipage /vif tol;
run;

proc reg data=thesis.age;
model gm_raw= earlyca ageearlyca cdipage sex /vif tol;
run;
*backward elimination for gm_raw - early ca age most nonsignif so
remove;
proc reg data=thesis.age;
model gm raw= earlyca cdipage sex /vif tol;
run; *cdip age next;
proc reg data=thesis.age;
model gm raw= earlyca sex /vif tol;
run; *stop here;

proc reg data=thesis.thesis;
model adostot= earlyca / vif tol;
run;

proc reg data=thesis.thesis;
model adostot= earlyca ageearlyca / vif tol;
run;

proc reg data=thesis.thesis;
model adostot= earlyca ageearlyca childvisitage / vif tol;
run;

proc reg data=thesis.thesis;
model adostot= earlyca ageearlyca childvisitage sex / vif tol;
run;
*backward elimination for adostot - child visit age most nonsignif so
remove;
proc reg data=thesis.thesis;
model adostot= earlyca ageearlyca sex / vif tol;
run; *early ca age next;
proc reg data=thesis.thesis;
model adostot= earlyca sex / vif tol;
run; *nothing significant;

proc reg data=thesis.thesis;
model dasvss= earlyca / vif tol;
run;

proc reg data=thesis.thesis;
model dasvss= earlyca ageearlyca / vif tol;
run;

proc reg data=thesis.thesis;
model dasvss= earlyca ageearlyca childvisitage / vif tol;
run;

proc reg data=thesis.thesis;
model dasvss= earlyca ageearlyca childvisitage sex / vif tol;
run;
```

```
*backward elimination for dasvss - all significicant so remove nothing;
however VIF all extremely high indicative of collinearity;

proc reg data=thesis.thesis;
model cbclepr= earlyca / vif tol;
run;

proc reg data=thesis.thesis;
model cbclepr= earlyca ageearlyca / vif tol;
run;

proc reg data=thesis.thesis;
model cbclepr= earlyca ageearlyca childvisitage / vif tol;
run;

proc reg data=thesis.thesis;
model cbclepr= earlyca ageearlyca childvisitage sex / vif tol;
run;
*backward elimination for cbclepr - child visit age most nonsignif so
remove;
proc reg data=thesis.thesis;
model cbclepr= earlyca ageearlyca sex / vif tol;
run; *sex next;
proc reg data=thesis.thesis;
model cbclepr= earlyca ageearlyca / vif tol;
run; *stop here;

proc reg data=thesis.thesis;
model cbclipr= earlyca / vif tol;
run;

proc reg data=thesis.thesis;
model cbclipr= earlyca ageearlyca / vif tol;
run;

proc reg data=thesis.thesis;
model cbclipr= earlyca ageearlyca childvisitage / vif tol;
run;

proc reg data=thesis.thesis;
model cbclipr= earlyca ageearlyca childvisitage sex / vif tol;
run;
*backward elimination for cbclipr - child visit age most nonsignif so
remove;
proc reg data=thesis.thesis;
model cbclipr= earlyca ageearlyca sex / vif tol;
run; *early ca age next;
proc reg data=thesis.thesis;
model cbclipr= earlyca sex / vif tol;
run; *stop here;

proc reg data=muldoon.second;
      model adisoc = earlyca / vif tol;
run;

proc reg data=muldoon.second;
```

```
      model adisoc = earlyca ageearlyca / vif tol;
run;

proc reg data=muldoon.second;
model adisoc = earlyca ageearlyca childvisitage /vif tol;
run;

proc reg data=muldoon.second;
model adisoc = earlyca ageearlyca childvisitage sex /vif tol;
run;

proc reg data=muldoon.second;
model adisoc = earlyca ageearlyca sex /vif tol;
run;

proc reg data=muldoon.second;
model adisoc = earlyca sex /vif tol;
run;

proc reg data=muldoon.second;
model cbclsocr = earlyca / vif tol;
run;

proc reg data=muldoon.second;
model cbclsocr = earlyca ageearlyca / vif tol;
run;

proc reg data=muldoon.second;
model cbclsocr = earlyca ageearlyca childvisitage /vif tol;
run;

proc reg data=muldoon.second;
model cbclsocr = earlyca ageearlyca childvisitage sex /vif tol;
run;

proc reg data=muldoon.second;
model cbclsocr = earlyca ageearlyca childvisitage /vif tol;
run;

proc reg data=muldoon.second;
model cbclsocr = earlyca childvisitage /vif tol;
run;
```

IV. Follow-up A-TAC analysis

```
proc univariate data=muldoon.atacset;
      var atac;
run;

proc univariate data=muldoon.atacset;
      var atac;
      where mrn ne .;
run;

proc univariate data=muldoon.atacset;
      var atac;
```

```
      where lowca ne .;
run;

proc univariate data=muldoon.atacset;
      var atac;
      where hypoxia ne .;
run;

*** Potential confounders data;
proc corr data=muldoon.atacset;
      var atac;
      with sex agelowca ageearlyca surgeryage;
run;


*** initial correlations;
proc corr data=muldoon.atacset;
      var atac;
      with hypoxia lowca earlyca;
run;

*** build models;
proc reg data=muldoon.atacset;
      model atac = lowca;
run;

proc reg data=muldoon.atacset;
      model atac = lowca agelowca;
run;

proc reg data=muldoon.atacset;
      model atac = earlyca;
run;

proc reg data=muldoon.atacset;
      model atac = earlyca ageearlyca;
run;



ods graphics on;
proc glm data=muldoon.atacset;
      class hypoxia;
      model atac = hypoxia;
      lsmeans hypoxia / adjust = tukey cl;
run;
ods graphics off;
```

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