Study Title:	Genomic Basis of Neurodevelopmental and Brain Outcomes in Congenital Heart Disease
Short Title:	CHD Brain and Genes
Sponsor:	Pediatric Cardiac Genomics Consortium (PCGC)
Funding:	National Heart, Lung, and Blood Institute (NHLBI), NIH/DHHS; *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), NIH/DHHS
Version and date:	4.0, 08Mar2018

Table of Contents

Table of Contents ii
LIST OF ABBREVIATIONS 1
STATEMENT OF COMPLIANCE 2
PROTOCOL SUMMARY 3
SCHEMATIC OF STUDY DESIGN 4
1 KEY ROLES 4
2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE 5
2.1 Background Information 5
2.2 Rationale 8
2.3 Risk Benefit Analysis 8
3 OBJECTIVES AND PURPOSE 9
4 STUDY DESIGN AND ENDPOINTS 9
4.1 Description of the Study Design 9
4.1.1 Primary Endpoint 10
4.1.2 Secondary Endpoints 10
4.1.3 Exploratory Endpoints 10
5 STUDY ENROLLMENT AND WITHDRAWAL 10
5.1 Subject Inclusion Criteria 10
5.2 Participant Exclusion Criteria 11
5.3 Strategies for Recruitment and Retention 11
5.3.1 Compensation 12
5.4 Subject Withdrawal or Termination 12
5.4.1 Reasons for Withdrawal or Termination 12
5.4.2 Handling of Subject Withdrawals or Termination 13
5.5 Premature Termination or Suspension of Study 13
6 STUDY AGENT 13
7 STUDY PROCEDURES AND SCHEDULE 13
7.1 Study Procedures/Evaluations 13
7.1.1 Study specific procedures 13
7.1.2 Scoring of the Neurodevelopmental and Behavioral Health Assessments 19
7.1.3 Standard of Care Study Procedures 19
7.2 Laboratory Procedures/Evaluations 20
7.2.1 Clinical Laboratory Evaluations 20
7.2.2 Specimen Preparation, Handling, and Storage 20
7.3 Study Schedule 20
7.3.1 Follow-up 20
7.3.2 Final Study Visit/Early Termination Visit 21
7.3.3 Schedule of Events Table 21
7.4 Justification for Sensitive Procedures 22
7.5 Prohibited Medications or Treatments 22
7.6 Rescue Medications, Treatments, and Procedures 22
8 ASSESSMENT OF SAFETY 23
8.1 Specification of Safety Parameters 23
8.1.1 Definition of Adverse Events (AE) 23
8.1.2 Definition of Serious Adverse Events (SAE) 23

8.1.3 Definition of Unanticipated Problems (UP) 23
8.2 Classification of an Adverse Event 23
8.2.1 Severity of Event 23
8.2.2 Relationship to Study 24
8.2.3 Expectedness 24
8.3 Time Period and Frequency for Event Assessment and Follow-Up 24
8.4 Reporting Procedures 25
8.5 Study Halting Rules 26
8.6 Safety Oversight 26
8.7 Known Potential Risks 26
8.8 Known Potential Benefits 29
9 CLINICAL MONITORING 30
10 STATISTICAL CONSIDERATIONS 30
10.1 Statistical Hypotheses 30
10.2 Description of Statistical Methods 30
10.2.1 General Approach 30
10.3 Sample Size 32
10.4 Measures to Minimize Bias 32
10.4.1 Enrollment/ Randomization/ Masking Procedures 32
11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS 33
12 QUALITY ASSURANCE AND QUALITY CONTROL 33
13 ETHICS/PROTECTION OF HUMAN SUBJECTS 34
13.1 Ethical Standard 34
13.2 Institutional Review Board 34
13.3 Informed Consent Process 34
13.3.1 Consent/Assent and Other Informational Documents Provided to Subjects 34
13.3.2 Consent Procedures and Documentation 35
13.4 Subject and Data Confidentiality 36
13.4.1 Research Use of Stored Human Samples, Specimens or Data 38
13.5 Future Use of Stored Specimens 38
14 DATA HANDLING AND RECORD KEEPING 38
14.1 Data Collection and Management Responsibilities 38
14.2 Study Records Retention 40
14.3 Protocol Deviations/Violations 40
14.4 Publication and Data Sharing Policy 41
15 STUDY ADMINISTRATION 41
15.1 Study Leadership 41
16 CONFLICT OF INTEREST POLICY 42
17 APPENDIX A – DESCRIPTION OF TESTS OF NEURODEVELOPMENT, BEHAVIOR, AND QUALITY OF LIFE 43
18 LITERATURE REFERENCES 47

LIST OF ABBREVIATIONS

ACC	Administrative Coordinating Center
ADC	Apparent diffusion coefficient
ADOS	Autism Diagnostic Observation Schedule
AE	Adverse Event
ApoE	Apolipoprotein E
BASC	Behavior Assessment System for Children
BRIEF	The Behavior Rating Inventory of Executive Function
CDI	Children's Depression Index
CDMS	Clinical Data Management System
CHD	Congenital Heart Disease
CNS	Central Nervous System
CNVs	Copy Number Variants
d-TGA	dextro-Transposition of the great arteries
DCC	Data Coordinating Center
D-KEFS	Delis Kaplan Executive Function System
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
MASC	Multidimensional Anxiety Scale for Children
MOO	Manual of Operations
MRI	Magnetic Resonance Imaging
NICHD	*Eunice Kennedy Shriver* National Institute of Child Health and Human Development
ND	Neurodevelopmental
NDD	Neurodevelopmental Disabilities
NS	Noonan Syndrome
NV	Number variant
NHLBI	National Heart, Lung, and Blood Institute
PCGC	Pediatric Cardiac Genomic Consortium
PedsQL	Pediatric Quality of Life Inventory
PI	Principal Investigator
PDI	Psychomotor Development Index
QOLS	Quality of Life Scale
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SRS	Social Responsiveness Scale
TOF	Tetralogy of Fallot
UP	Unanticipated Problem
VMI	Beery Visual Motor Integration Test
WAIS	Wechsler Adult Intelligence Scale
WES	Whole Exome Sequencing
WGS	Whole Genome Sequencing
WIAT	Wechsler Individual Achievement Test
WISC	Wechsler Intelligence Scale for Children
WRAML	Wide Range Assessment of Memory and Learning
WRAT	Wide Range Achievement Test

STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the ICH E6, the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and the NHLBI Terms of Award. The Principal Investigator will endeavor to ensure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

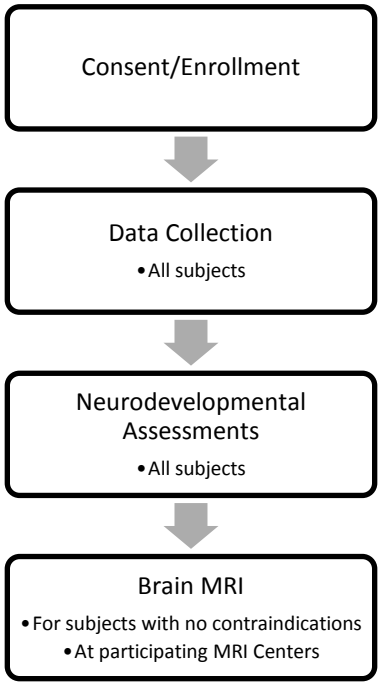
Principal Investigator: _____
Print/Type Name

Signed: _____ Date: _____
Signature

PROTOCOL SUMMARY

Title:	Genomic Basis of Neurodevelopmental and Brain Outcomes in Congenital Heart Disease
Précis:	Approximately 400 CHD patients will participate in the research study which will include 1 or more research visits for neurodevelopmental testing, brain MRI, and collection of medical history including previously collected genetic sequencing results.
Objectives:	1. To compare neurodevelopmental and behavioral health outcomes in individuals with CHD who have deleterious mutations (damaging de novo mutations or stringently defined deleterious missense mutations) on whole exome sequencing (WES) or whole genome sequencing (WGS) vs. those without such variants. 2. To compare abnormalities in brain structure and microstructure on MRI in individuals with CHD who have deleterious mutations on WES or WGS vs. those without any variants
Endpoint	The primary neurodevelopmental outcome will be the math/reading/writing domain assessed by the WRAT-4 composite score.
Population:	CHD patients aged 8 years and older with prior WES or WGS results
Number of Sites enrolling subjects:	8
Study Duration:	5 years
Participant Duration:	6 months for completion of neurodevelopmental assessment and brain MRI, followed by yearly phone follow up for 5 years

SCHEMATIC OF STUDY DESIGN



1 KEY ROLES

Funding Agencies: National Heart, Lung, and Blood Institute (NHLBI); *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD)

Study Sponsor: Pediatric Cardiac Genomic Consortium (PCGC)

Administrative and Data Coordinating Center (ACC/DCC): Cincinnati Children's Hospital Medical Center (CCHMC)

MRI Core: University of California, San Diego

Participating Centers: Boston Children's Hospital
Children's Hospital of Philadelphia
Yale New Haven Children's Hospital
University of Rochester Medical Center
Children's Hospital Los Angeles
University of Utah/Primary Children's Hospital
Icahn School of Medicine at Mt. Sinai
University of California San Francisco/Gladstone Institute
Cohen Children's Medical Center (referring center)
Columbia University Medical Center (referring center)

2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

Neurodevelopmental disabilities (NDD) are the most common, and potentially the most distressing, sequelae of complex congenital heart disease. Remarkable progress in cardiac surgery has improved the survival of patients with critical congenital heart disease. However, the burgeoning population of children and adults with CHD has exposed a high prevalence of neurodevelopmental (ND) impairment in survivors.[1] Specifically, survivors of infant heart surgery have more problems with reasoning, learning, executive function, inattention and impulsive behavior, language skills, and social skills compared with peers without CHD.[2-5] Lower abilities in these domains may lead to poor school performance, strained interpersonal relationships, and behavior problems. Children with CHD are more likely to require remedial services, including tutoring and special education, as well as physical, occupational, and speech therapy.[1] As these children reach adulthood, ND disabilities can limit educational achievements, employability, insurability, and quality of life.[6,7] Importantly, studies have identified few modifiable risk factors for adverse ND outcomes.[7-9] Moreover, known risk factors only explain approximately 30% of observed variation in ND outcome after cardiac surgery in infancy,[8] suggesting that genetic and epigenetic factors may play an important role. Critical barriers to elucidating these factors have been the genetic heterogeneity of CHD, the expense and technical challenges of genomic analysis, and the time and resources required to ascertain a sufficient number of cases for statistical analysis.

Known genetic associations between CHD and neurodevelopment: Genetic abnormalities, including chromosomal disorders (e.g., Trisomy 21), microdeletions (e.g.,22q11 microdeletion), or mutations (e.g., Noonan syndrome), may cause both congenital heart defects and abnormalities of central nervous system structure and function. Children with genetic syndromes have much worse ND outcome than those without recognizable syndromes.[10] Furthermore, it is suspected that genetic factors may underlie delayed development without other explanation even in some patients without a recognizable constellation of congenital abnormalities.

Specific types of congenital heart defects may be associated with different chromosomal abnormalities with varying influence on molecular pathways that impact central nervous system structure and function. For example, tetralogy of Fallot (TOF) can be associated with mutation or deletion of several different genes (*NKX2.5, JAG1, TBX5, TBX1, and FOXC2*) and with several clinical syndromes, such as 22q11 deletion syndrome or trisomy 21.[11,12] Three genes known thus far to cause TOF (i.e., *TBX5, NKX2.5*, and *JAG1*) have been found in the brain as well as the heart (http://www.ncbi.nlm.nih.gov, UniGene). *JAG1*, mutated or deleted in Alagille syndrome, encodes a ligand for the notch intercellular signaling pathway of tremendous importance in brain development.[13-17,18]

Noonan syndrome (NS) serves as a good single gene model of the intersection between the genetic underpinnings of cardiac and neurocognitive development. A multiple congenital anomaly syndrome characterized by short stature, CHD, distinctive facial features, and musculoskeletal abnormalities, the syndrome is caused by germline mutations of genes in the RAS–MAPK signaling cascade.[19,20] Variable

neurocognitive impairments are observed with effects ranging from absent or mild learning problems to severe intellectual disability.[20,21, 22-25] Experimental animal models have demonstrated that RAS–MAPK pathway proteins may play a key role in the process of memory formation and consolidation[26,27] suggesting a plausible molecular explanation for the observed deficits.

Human genomes harbor copy number variants (CNVs), which are regions of DNA gains or losses. Microdeletions causing congenital heart defects may be associated with specific ND profiles. For example, in adults with 22q11 deletion, specific deficits have been reported in visual–spatial ability, problem solving and planning (executive functions), abstract social thinking, and attentiveness.[28 29,30 31,32] A recently published study demonstrated that pathogenic CNVs among 223 infants with single ventricle physiology were associated with inferior neurocognitive and somatic growth outcomes.[33] Putatively pathogenic CNVs had a prevalence of 13.9%, significantly greater than the 4.4% rate of such CNVs among controls. In this study, pathogenic CNVs seemed to contribute to the cause of single ventricle forms of CHD in ≥10% of cases and, though clinically subtle, adversely affect outcomes in children harboring them. The impact on NDD of genetic abnormalities was similarly shown in a cross-sectional ND outcome study of adolescent subjects aged 10 - 19 years who recently had the Fontan procedure performed at Boston Children's Hospital.[34] Of the previously published loci thought to confer a significant risk for CHD,[35-37] there was an overall 6% (8/132) pathogenic CNV detection rate by chromosomal microarray. Adolescents who underwent the Fontan procedure with vs. without genetic abnormalities were more likely to have received developmental services and to have worse processing speed, full scale IQ, and memory.

Epigenetic analyses, the study of protein changes that affect gene regulation without altering core DNA sequence, are at their beginning stages for the field of neurodevelopment.[38] The best understood types of epigenetic modification include DNA methylation and histone modification but epigenetic changes that affect chromatin structure have more recently been found to alter gene expression of particular cell types or during specific developmental stages. Such chromatin modifications have been shown to importantly influence CNS development.[39] Because the molecular determinants of neuronal regeneration after CNS injury are not completely known, understanding their epigenetic regulation is an exciting frontier in understanding ND outcomes in CHD. Comparison of frequency of deleterious variants in DNA methyltransferase enzymes, histone modifying enzymes, and chromatin modifying genes in CHD patients with and without neurocognitive abnormalities may begin to uncover these important regulators of gene expression in the CNS.

Genetic polymorphisms affecting host susceptibility and resiliency may affect the response of the brain to stresses associated with CHD, including cardiopulmonary bypass and perioperative events.[8] For example, apolipoprotein E (APOE) genotype has been shown to have an important role as a determinant of neurologic recovery after CNS ischemia, intracerebral hemorrhage, and traumatic brain injury. The *APOE å*2 allele has been shown to be an independent risk factor for worse Psychomotor Development Index (PDI) scores in multivariable regression adjusting for preoperative and postoperative covariates in infants undergoing cardiac surgery and was seen across the spectrum of children with and without genetic syndromes.[7] The adverse effect of *APOE å*2 allele is most likely related to decreased neuroresiliency and impaired neuronal repair after central nervous system injury. Interestingly, this

finding underscores that children and adults may differ with respect to the effects of particular genotypes. In contrast to infants, adults carrying the APOE å4 allele who undergo open heart surgery have increased levels of biochemical markers of brain injury[40,41] and a greater rate of postoperative cognitive decline.[42,43]

There are likely additional genetic polymorphisms that impair neuroresiliency and CNS recovery that may explain variations in ND outcome after surgery for CHD. Studies of focal and global ischemia have shown upregulation of immediate early genes, stress response genes, genes that regulate apoptosis, neurotransmission related genes, ion channel genes, genes of the inflammatory process, cytoskeletal proteins, and neurotrophic genes.[44] Proteomic screen of rat pup brains after hypoxic injury confirmed upregulation of MAP-2 (a known regulator of neuronal polarity and dendritic extension) and proteomic analysis found 193 proteins present only in the hypoxic group. [45,46] Rodent models of endogenous brain protection by hypoxic-ischemic preconditioning (a non-lethal hypoxic-ischemic event, followed by a second, more severe hypoxic-ischemic event whereby the preconditioning stimulus activates endogenous protective mechanisms and lead to a better outcome of the second event alone) show evidence of upregulation of three DAVID annotated biological terms: 9 response to organic substance genes, 7 regulation of transcription from RNA polymerase II promoter genes, and 6 MAPK signaling pathway genes.[44, 47] The genes implicated by these animal models are a good place to begin to look for alterations that may explain decreased neuroresiliency after perturbed brain perfusion and resultant neurocognitive delay.

Brain structure and function as assessed by brain MRI. Brain development is abnormal in children with CHD. Magnetic resonance imaging (MRI) studies in CHD fetuses, including studies at Boston Children's Hospital, show smaller gestational age- and weight-adjusted total brain volumes and abnormal brain metabolism, as well as delayed cortical development and folding.[48-52] Indeed, in fetuses with HLHS, compared with fetuses without CHD, Limperopoulos *et al.,* demonstrated progressive third trimester fall-off in cortical gray and white matter volumes and subcortical gray matter, as well as significant delays in cortical gyrification. Post-natal MRI studies have shown that white matter abnormalities are evident in one in five infants before cardiac surgery.[53-55] A lower brain maturity score at birth by MRI is associated with greater brain injury in both the preoperative and postoperative periods.[54] Thus, altered brain development associated with CHD may increase vulnerability to perioperative hemodynamic instability and intraoperative hypoxia-ischemic injury.

Moreover, a growing body of literature has suggested that macrostructural and microstructural changes on brain MRI could underlie cognitive impairment in patients with CHD. The Boston Circulatory Arrest Study cohort demonstrated that, despite scant white matter injury on conventional brain MRI, adolescents with d-TGA repaired in infancy demonstrated significant white matter left parietal fractional anisotropy reduction which correlated with math problem solving skill level.[56] Symptoms of ADHD as well as executive function were related to frontoparietal white matter microstructure. Finally, collaboration between Boston Children's Investigators and University of Pittsburgh on the Boston Circulatory Arrest study of the brain "connectome," demonstrated that adolescents with d-TGA have differences in network properties that mediate neurocognitive differences between the d-TGA and healthy referent subjects.[57] In TOF, a diagnosis with a range of disturbance of fetal cerebral

hemodynamics, patients also experience neurodevelopmental morbidity.[58] This together with the understanding that several CNVs associated with CHD relate to neuron protection and development,[59] suggest that characterizing the effect of such genetic variation on the structure and function of the brain is likely critical for prognostication and for identifying new opportunities for intervention to improve outcomes. Taken together, brain MRI studies in patients who underwent infant heart surgery highlight the challenges of discriminating neurocognitive deficits that results from genetic abnormalities, deficient cerebral substrate delivery in fetal life, and postnatal injury. Brain MRI will help to distinguish among these potential etiologies, for example by demonstrating developmental abnormalities and acquired lesions such as stroke.

In summary, patients with CHD carry a high risk of neurological, developmental, and behavioral morbidities. With a burgeoning population of CHD survivors reaching reproductive age, research on the genetic underpinnings of NDD assumes ever greater importance. Combining cutting-edge genetic technologies with innovative neuroimaging and ND phenotyping tailored to CHD patients, we will interrogate the effect of genetic factors on structure and function in CHD patients. By narrowing the knowledge gap in understanding the as-yet-undescribed genetic or epigenetic determinants of NDD in children with CHD, the proposed study will improve prenatal screening and counseling, prognostication, and treatments for children targeted early as requiring specialized support services.

2.2 RATIONALE

Neurodevelopmental disabilities (NDD) remain the most common sequelae of CHD, causing problems in school function, behavior, employability, and quality of life. Earlier prospective studies suggest that preoperative, intraoperative, and postoperative factors together can explain only one third of the variance in neurodevelopmental (ND) outcomes, suggesting an important role for as-yet-undescribed genetic factors. In this protocol, we will explore the association between genetic variants, neurodevelopmental deficits, and brain MRI endophenotype. Analyses will compare groups with and without deleterious de novo mutations or stringently-defined missense mutations by meta-SVM, frequency matched for type of CHD, age group, and sex. We will exclude subjects with copy number variants (CNVs) deemed to be known clinically pathogenic (e.g. 22q11) from both groups of subjects. Variants will be classified as pathogenic using accepted types of variant evidence (e.g., population data, computational data, functional data, segregation data) as detailed in the American College of Medical Genetics and Genomics “ACMG Standards and Guidelines for the interpretation of sequence variants”.[60]

2.3 RISK BENEFIT ANALYSIS

The risk/benefit ratio is favorable for this study, for the following reasons:

1. The baseline risk is minimal because there are no therapeutic interventions; adverse events are unlikely.
2. We offer an option for subjects to learn, at the end of the study, whether they/their children carry pathogenic genetic variants that are likely to have caused their congenital heart disease and/or affected their development and behavior. If participants choose to receive their genetic results, confirmed in a CLIA-approved laboratory and have a pathogenic mutation, they will have the

opportunity to speak with a geneticist or a genetics counselor about the results. The costs of the additional CLIA testing and genetic counseling will be paid for by this research study and will not be charged to study participants.

3. Although an individual subject may not benefit from participation, the results of this study will make important contributions to understanding the genetic basis of CHD and neurocognitive impairment.
4. Neurodevelopment and genetic abnormalities have never been studied in such a large population of individuals with CHD.
5. The in-person evaluation will provide accurate and rich information about neurocognitive function for use by patients, their families, and schools.

Data generated from this study will be unique in terms of the breadth and depth of the information that can be provided to probands, parents and medical care providers of patients with congenital heart disease.

3 OBJECTIVES AND PURPOSE

1. Aim 1: To compare neurodevelopmental and behavioral health outcomes in individuals with CHD who have deleterious mutations (damaging de novo mutations or stringently defined deleterious missense mutations) on whole exome sequencing (WES) or whole genome sequencing (WGS) vs. those without such variants. Patients with stringently defined pathogenic CNVs or mutations in previously established CHD genes will be excluded from the study.

   We will compare groups with respect to achievement, IQ, learning disability, specific neuropsychological domains (e.g., memory, attention, executive functions, and visual-spatial/motor integration), adaptive function, behavior, social cognition and symptoms of autism spectrum disorder, and quality of life. The primary study outcome for this aim will be the WRAT4 composite score.

2. Aim 2: To compare abnormalities in brain structure and microstructure on MRI in individuals with CHD who have deleterious mutations on WES or WGS vs. those without any deleterious variants. Neither group will have stringently defined pathogenic CNVs or mutations in previously established CHD genes.

   We will compare the groups with respect to measured and derived parameters including, but not limited to, 1) regional volumetric and cortical thickness, 2) regional surface metrics, 3) voxel-based DTI eigenvectors and apparent diffusion coefficient (ADC) values, and resting state principal component analysis.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

This is a multi-center, prospective, observational, non-interventional trial of individuals with CHD. Assessments will include neurodevelopmental test battery and brain MRI imaging.

4.1.1 PRIMARY ENDPOINT

The primary neurodevelopmental outcome will be the math/reading/writing domain assessed by the WRAT-4 composite score.

4.1.2 SECONDARY ENDPOINTS

Secondary neurodevelopmental outcomes using age appropriate instruments will include the following:

- Visual spatial skill (VMI-6)
- Intelligence (WISC-V or WAIS-IV)
- Memory (WRAML-2 story memory subtest)
- Memory (WRAML-2 picture memory subtest)
- Executive function (D-KEFS verbal fluency, trail making subtext, and tower subtest)
- Language (WIAT-III oral language composite score) (listening comprehension and oral expression)
- Social cognition (Reading the mind in the eyes)

Secondary neurodevelopmental outcomes for probands assessed for autism include the following:

- ADOS-2

Brain structure/microstructure outcomes including but not limited to the following:

- Regional volumetric and cortical thickness measures
- Regional surface metrics
- Voxel-based DTI eigenvectors and apparent diffusion coefficient (ADC) values
- Resting state principal components

4.1.3 EXPLORATORY ENDPOINTS

Exploratory brain MRI endpoints may be initiated by the brain MRI imaging group or by individual centers that are accessing the imaging dataset.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 SUBJECT INCLUSION CRITERIA

To be eligible for the study, subjects must meet all of the following criteria:

1. Subjects in whom WES or WGS has already been performed, either during the CHD GENES study or, for new centers (Utah or UCSF/Gladstone), after trios in existing biobanks undergo analysis by WES or WGS during the PCGC2 grant cycle

2. Presence of deleterious mutations (damaging de novo mutations or stringently defined deleterious missense mutations) identified on sequencing (Cases) OR absence of such known deleterious mutations (Controls)
3. Males or females, age ≥8 years
4. Diagnosis of CHD
5. Informed consent obtained

5.2 PARTICIPANT EXCLUSION CRITERIA

To be eligible for this study, subject must not have any of the following criteria:

1. History of cardiac transplant
2. A cardiac surgical procedure within 6 months of enrollment
3. Known genetic syndrome due to a pathogenic variant identified in a gene associated with abnormalities of the brain structure or function, structural heart disease, and potentially other associated features.
4. Presence of CNV known to be clinically pathogenic. Variants will be classified as pathogenic using accepted types of variant evidence (e.g., population data, computational data, functional data, segregation data) as detailed in the American College of Medical Genetics and Genomics "ACMG Standards and Guidelines for the interpretation of sequence variants" (Richards et al, GIM 2015).
5. Overwhelming acquired brain injury, such as a major stroke or severe ischemic injury, that would overshadow the effect of a genetic mutation on outcome in the opinion of the center investigator
6. Lack of ability to communicate in English or Spanish

Exclusion criteria for brain MRI:

1. Contraindication to having brain MRI scan
2. Claustrophobia or inability to lie still while in the MRI scanner for the required time (sedation will not be allowed)
3. Pregnancy

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

Subjects will be recruited from among those in whom WES or WGS was performed in trios gathered by the PCGC Congenital Heart Disease Genetic Network Study (CHD GENES) or from subjects at Utah or UCSF/Gladstone with trios that have undergone WES or WGS during the PCGC grant period. The latter subjects may also be recruited to participate in CHD GENES during the consent process for this study. All cases (as defined in section 5.1) deemed eligible may be recruited. Controls (as defined in section 5.1) will be recruited and will be frequency matched to cases for CHD type, age category, and sex within each site to the greatest extent possible. The matching will include four CHD type categories (i.e. single ventricle with arch obstruction, single ventricle without arch obstruction, bi-ventricle with arch

obstruction, bi-ventricle without arch obstruction), two sex categories, and three age categories (8-12, 13-17, 18+). This results in 24 unique combinations of CHD type, sex, and age category within each of the sites. A list of cases and controls will be generated for each site. To the extent possible, study personnel at the site that will have direct contact with the subjects should remain blinded as to which subjects are cases and controls. The site lists will be presented such that the blind IDs are presented in random order of cases and controls. Sites should approach each of the subjects on their lists, and subjects/families can be approached in any order. The ACC will monitor the balance of cases and controls within each site and across all sites to determine if any modifications in recruitment are necessary due to severe imbalance; if so, the ACC will work with individual sites to restore balance. Of note, some sites may be recruiting controls to match cases at other sites. Because we are using a frequency matching process in this protocol, no specific control is matched to a specific case, but rather we aim to have approximately the same number of controls as cases within each of the 24 strata.

The medical record will be reviewed or the subject's cardiologist will be contacted before initiating contact with subjects or their parent/guardian to be sure that the subject is alive.

Subjects will be contacted by various methods, as permitted by local institutional policy, and may include: approaching the patient or parent/guardian at a routine clinic visit, mailing of letter/study brochure, email, and phone introductions. All materials for recruitment will be IRB approved prior to use in the study. If used, contact by phone will include a standard phone script that will have IRB approval.

Based on current data in the CHD GENES database, and an estimate of the number of subjects with qualifying sequencing results plus the needed controls, we anticipate approximately 300 patients with deleterious variants and a similar number of subjects without such variants will be eligible to participate in the study. If 60% give consent, 180 subjects with exomic mutations and approximately 180 controls, together with some subjects contributed by Utah and UCSF/Gladstone, would participate in the study.

5.3.1 COMPENSATION

Subject to approval by the IRB, subjects returning for neurodevelopmental testing and, in a subset, brain MRI, will receive $100 for the subject and, if MRI is done, the subject (or parent/guardian as applicable) will receive a composite set of MPGR MRI images of the subject's brain if desired. Subject (or parent/guardian as applicable) will also receive the results of their neurodevelopmental testing, and in the subset who undergo brain MRI, results of neuroimaging. Reimbursement for travel expenses incurred due to study participation will be provided to all participants including, but not limited to, parking, mileage, ground transportation, and meals. In addition, for subjects traveling from afar, reimbursement for hotel and transportation (e.g. airfare, train) will be provided to participants in accordance with guidelines set by the ACC.

5.4 SUBJECT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

Subjects may withdraw from participation in the study at any time.

An investigator may terminate participation in the study for the following reasons, including but not limited to:

- Any condition or abnormality that develops such that continued participation in the study would not be in the best interest of the subject
- The subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- The subject is unable and/or unwilling to complete the study visits/procedures

5.4.2 HANDLING OF SUBJECT WITHDRAWALS OR TERMINATION

Data from subjects who withdraw or are terminated from the study will remain in the database unless the subject specifies in writing to the Investigator that they wish for their data to be removed. No other special procedures are required for subject withdrawals or terminations.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for the study suspension or termination, will be provided by the suspending or terminating party to the investigator, funding agency, and sponsor. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

The study may resume once concerns about the study that led to suspension (e.g. safety, protocol compliance, data quality) are addressed and satisfy the Sponsor, funding agency, and the IRB.

6 STUDY AGENT

This is an observational study. No study agent is used.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY SPECIFIC PROCEDURES

A subject will be considered enrolled following documentation of informed consent.

Following informed consent (as outlined in section 13.3) the following procedures will be performed as part of the study and should occur within 6 months of the first in-person evaluation:

- Demographic, medical history, concomitant medication and clinical characteristics data collection
- Neurocognitive and Behavioral Health Evaluation
- Brain MRI Imaging, in a subset of enrolled subjects

Data Collection

- Review of Inclusion/Exclusion criteria
- Medical history will be obtained by interview with the subject/parent/legal guardian as well as review of the medical chart. Medical history will include collection of WES or WGS results and other relevant data from the CHD GENES database, where available.
- Measurement of subject height and weight
- Concomitant Medication Review
- Collection of primary care physician name (if subject consents for results to be shared)

Brain MRI Imaging

- Brain MRI imaging will be performed in a subset of subjects, i.e., those enrolled at selected centers who have no contraindications to having brain MRI.
- Brain MRI imaging without contrast or sedation will be performed.
- Institutional policy for pregnancy testing prior to research MRI will be followed.
- Details of the brain MRI imaging parameters will be defined in the study specific Manual of Operations. The following MRI sequences will be performed:
    - Structural MRI
    - Diffusion Tensor MRI
    - Resting State Functional MRI
- Subjects unable or unwilling to have the brain MRI imaging may still be enrolled into the study to collect neurocognitive and behavioral health endpoints.

Neurocognitive and Behavioral Health Evaluation

- Neurodevelopmental assessment of the subject will be performed by a licensed psychologist or supervised psychometrician at each site according to Table 5 below. Multiple areas will be assessed including intellectual functioning, academic (reading and math) functioning, language, memory, attention/executive functioning, visual spatial, fine motor, social functioning, adaptive skills, emotional/behavioral functioning, and quality of life.
- Standardization and Certification: The evaluators (licensed psychologist and/or supervised psychometrician) at each site will complete a certification process to optimize standardization of the testing battery. If the testing is performed by someone other than a licensed psychologist, a licensed psychologist at the site will be expected to provide training and oversight for the individuals performing the evaluation. For all evaluators, a recording of the evaluator performing the test battery on a staff volunteer will be submitted for review by the co-chairs of the neurocognitive and behavioral health testing protocol. The certification videos can be in any format and sent on any medium (jump drive, SD card, DVD) as long as the file can be opened and reviewed. Any concerns related to the performance of testing will be relayed back to the site licensed psychologist and to the individual performing the testing. Upon review of the

administration video and test protocols, a feedback form that includes a pass/fail decision will be provided to the ACC for inclusion in the trial master file. A follow-up video and test protocol submission will be necessary if an evaluator fails the certification process.

7.1.1.1 Developmental Assessments and Testing

Parent, Participant, and Teacher Report Instruments - Questionnaires to be completed by the parent/caregiver are listed in Tables 1 and 2. Questionnaires to be completed by the participant are listed in Table 3. Teacher Reports are listed in Table 4. After consent has been obtained, the parent and teacher response instruments (per the tables below) may be mailed to the parents prior to the scheduled ND testing of their child along with a cover letter explaining the process for completing the instruments. Parent/caregiver will be instructed to pick the teacher whom they feel knows their child best to complete the teacher instruments. Consent for teacher completion of the instruments will be implied based on the teacher completing the instrument. Parent/caregiver instruments may be completed either before or during the child's evaluation as per the tables below. If completed before, completion must be within 3 months of the in-person evaluation and within 6 months of the MRI. (See details of consent described in section 13.3.2 for consent procedure prior to completing forms at home.)

The parent/caregiver questionnaires will require approximately 1-2 hours to complete. Teacher questionnaires will require approximately 40 minutes to complete.

Table 1 – Questionnaires Generally Completed by the Parent/Caregiver Prior to Study Visit*		
Measure	**Age Range**	**Time to Administer**
Social Responsiveness Scale, 2nd Edition (SRS-2)	≥ 8 yrs	15-20 min

*****May be mailed to parent/caregiver and completed prior to study visit. If mailed, participants will be phone consented following procedures in section 13.3.2. The purpose of SRS completion prior to the site visit is to facilitate the scheduling of the ADOS during the in-person visit. However, the SRS can also be completed at the time of the visit.

The version of the SRS-2 that will be completed will depend on the age and independent functioning of the participants as follows: For participants <18 years of age, parent/caregiver will complete the SRS-2 School-age form. Parent/caregiver (or legally appointed representative, if applicable) of all participants ≥ 18 years old will be asked to complete the SRS-2 Adult (Relative/Other) Form. For participants ≥ 18 years old, the coordinator will obtain the name/address of the designated parent/caregiver from the study subject and mail the questionnaire, with a self-addressed return envelope.

Table 2 - Questionnaires Generally Completed by Parent/Caregiver During Study Visit*		
Measure	**Age Range**	**Time to Administer**
BASC-3 Parent Report	< 22 yrs	10-20 min
BRIEF-2 Parent Report	< 18 yrs	20 min
Children's Depression Inventory, 2nd Edition (CDI-2)	< 18 yrs	5 min
Conners-3 Parent Report	< 18 yrs	20 min
MASC-2 Parent Report	< 18 yrs	5 min
Peds QL Parent Report (generic and cardiac modules)	< 18 yrs	10 min
Vineland-3 Caregiver Rating Form	≥ 8 yrs	20-60 min

*****May be mailed to parent/caregiver and completed prior to study visit. If mailed, participants will be phone consented following procedures in section 13.3.2. Also, if the parent/caregiver is primarily Spanish-speaking and/or indicates they are unable to read English fluently, the Spanish version of the questionnaires will be administered when available.

The version of the BASC-3 that will be completed will depend on the age and independent functioning of the participants as follows: For participants ages 8-11 years of age, parent/caregiver will complete the BASC-3 Child Form. For participants ages 12 to < 22 years (i.e., through 21 years, 11 months), parent/caregiver or the LAR will complete the Adolescent form. The coordinator will determine which form to send based on the age of the participant.

The Vineland-3 Caregiver Rating Form is a survey form which must be filled out by the parent/caregiver for participants ages 8 and up.

Table 3 - Questionnaires to be Administered to Participants During Study Visit		
Measure	**Age Range**	**Time to Administer**
Beck Anxiety Inventory	≥ 18 yrs	5 – 10 min
Beck Depression Inventory, 2nd Edition (BDI-2)	≥18 yrs	5 min
CAARS Self-Report (CAARS-S)	≥ 18 yrs	20 min
Peds QL (generic and cardiac modules)*	< 18 yrs	10 min
QOLS*	≥ 18 yrs	5 min

*****May be mailed and completed prior to study visit. If mailed, participants will be phone consented following procedures in section 13.3.2. If participant is primarily Spanish-speaking and/or indicates s/he is unable to read English fluently, the Spanish version of the questionnaires will be administered when available.

Table 4 - Questionnaires to be Mailed to Teachers		
Measure	**Age Range**	**Time to Administer**
Conners-3	< 18 yrs	20 min
BRIEF-2	< 18 yrs	20 min

Direct testing battery - The direct testing evaluation will require approximately 3.5 hours of in-person testing without the autism tests, and approximately 4.5 hours with autism testing. Breaks will be provided for snacks/lunch and in response to the level of fatigue displayed, as appropriate for the

subject. Whenever possible, the in-person battery will begin in the morning as the first activity for the subject. Following the visit, parents/guardians will be sent a summary of their child's test results, if requested, that will be scored based on age-based norms after all ND and MRI testing has been completed for the subject. In-person tests will be administered in the order specified in Table 5 whenever possible. Additional information regarding the testing is provided in the CHD Brain and Genes Neurodevelopmental (ND) Testing MOO.

Table 5 – Battery of ND Tests to be Administered in Sequential Order During Study Visit

Order	Measure	Age Range	Time to Administer*
1	VMI-6	≥ 8 yrs	5 min
2	WISC-V - Core Subtests: • Similarities • Vocabulary • Block Design • Matrix Reasoning • Figure Weights • Digit Span • Coding • Symbol Search	< 16 yrs	60 min
3	WAIS-IV - Core Subtests: • Block Design • Similarities • Digit Span • Matrix Reasoning • Vocabulary • Arithmetic • Symbol Search • Visual Puzzles • Information • Coding	≥ 16 yrs	60-90 min
4	WRAML-2 Story Memory & Picture Memory Immediate Recall Tasks	≥8 yrs	20 min
5	WRAT-4 Word Reading	≥8 yrs	35-45 min (total for all subtests)
6	WRAML-2 Story Memory & Delayed Recognition Recall Tasks	≥8 yrs	20 min
7	WRAT-4 Remaining Subtests • Sentence Comprehension • Spelling • Math Computation	≥8 yrs	35-45 min (total for all subtests)
8	D-KEFS (Selected Subtests) • Verbal Fluency • Trail Making • Tower Subtests	≥ 8 yrs	20 min
9	WIAT-III • Listening Comprehension • Oral Expression Subtests Only	≥ 8 yrs	 14 min 19 min

10	Reading the Mind in the Eyes Task • Child Version • Adult Version	 < 18 yrs ≥ 18 yrs	 15 – 18 min 15 – 18 min
11	Questionnaire Measures for Older Participants: • BDI-2 • BAI • CAARS	 ≥ 18 yrs ≥ 18 yrs ≥ 18 yrs	 5 min 5 min 20 min
12	ADOS-2**	≥ 8 yrs	40-60 min + scoring time

*'Time to Administer' in Tables 1-5 above are approximations.

**The ADOS-2 will be administered if the SRS-2 Total Score ≥ 60 or if the coordinator, in his or her best judgment and after consulting with the research site's psychologist, believes that there is a strong likelihood of an Autism Spectrum Disorder despite the absence of an elevation ≥ 60 on the SRS-2. This determination will be made in consideration of the current DSM-V diagnostic criteria for Autism Spectrum Disorder. That is, if the SRS-2 is < 60 but it is apparent from discussion with the parent/caregiver that there appears to be a strong likelihood of persistent deficits in social communication and social interactions in addition to the indication of restricted, repetitive patterns of behavior, interests, or activities. If such a determination is made, it will be discussed with the site PI for final confirmation.

Counseling procedures – If the subject/parent exhibits undue stress or signs of suicidal ideation during the neurodevelopmental testing, counseling will be provided.

7.1.1.2 Methods for MRI Testing.

Subjects with certain types of metal implants, such as surgical clips or pacemakers, will not have an MRI scan due to the use of powerful magnets to make images. During the scanning session, each patient will wear protective earphones, which will reduce the noise heard from the scanner. Audio-video entertainment with headphones may be provided to the subjects during the MRI sessions except during the resting state fMRI acquisition when the entertainment system will be deactivated. If a subject indicates that they are experiencing discomfort from lying in the confined space of the scanner and does not wish to continue, the scanning will be stopped immediately. No sedation will be used for brain MRIs that are performed for research purposes only. Prior to conducting the study scan, participants may undergo acclimation procedures, as described in the CHD Brain and Genes MRI MOO.

MRI scan staff will use all available tools at their disposal to make the scanning experience comfortable and the scans acceptable. Examples of comfort measures that may be used by centers include: form-fitting pillows, a magnet compatible sound system, protective earphones, a parent in attendance, and a two-way intercom. An attendant must remain at the bore and remain at the scanner until the proband is helped out of the scan room.

Subjects who cannot undergo this test without sedation will not be included in the MRI portion of this protocol. Subjects who decline to undergo MRI may still be enrolled in the study and will complete the neurodevelopmental assessments and data collection aspects of the protocol only.

The scanners used to acquire the brain MRIs will be calibrated and harmonized across sites during study start-up. The specific calibration and image acquisition process will be contained within the CHD Brain and Genes MRI MOO that each site will receive prior to enrolling their first subject. Training for site MRI staff will be described in the CHD Brain and Genes MRI MOO.

MRI variables

Specific sequences will allow derivation of a variety of brain MRI measures. The most important major categories of MRI outcomes are derived from the following core MRI sequences:

1. Structural MRI
2. Diffusion Tensor MRI
3. Resting State Functional MRI

Measured and derived parameters to be recorded include, but may not be limited to, the following:

1. Volume and image intensity (i.e. T1-weighted, T2-weighted) measures for subcortical volumes.
2. Surface-based morphometry measures (e.g. thickness, area) and image intensity measures (i.e. T1-weighted, T2-weighted) for cortical surface parcellations
3. Diffusion derived measures (e.g. fractional anisotropy (FA) and mean diffusivity (MD)) for white matter tracts, subcortical regions, and cortical surface parcellations
4. Resting state network connectivity measures

The specific methods and sequences for each of these domains will be specified by the PCGC Imaging Working Group (IWG) that will contain representative(s) from the MRI Core Lab and will be included in the MRI MOO.

7.1.2 SCORING OF THE NEURODEVELOPMENTAL AND BEHAVIORAL HEALTH ASSESSMENTS

Scoring: Assessments will be scored by staff at the clinical site per the CHD Brain and Genes ND Testing MOO. Scores will then be entered into the study database.

7.1.3 STANDARD OF CARE STUDY PROCEDURES

Based on current AHA/AAP guidelines for neurodevelopmental testing in children with congenital heart disease, it is possible that subjects will have recently undergone a clinical ND assessment. Repetition of the same ND test administered twice within 6 months can affect the score of the second test. For this reason, scores from tests that overlap with our research battery that were administered for clinical testing will be used as the scores for that test for this protocol, if the research evaluation takes place within 6 months of the clinical testing. If more than 6 months has elapsed between clinical and research testing, the entire research battery will be administered. If a subject has previously completed some or

all of the research battery of tests within the past 6 months, the subject will be scheduled for evaluation after 6 months have passed allowing the entire battery of assessments to be given to the subject in the order specified in this protocol. If it is not possible to delay the evaluation for the subject, the subject will be administered only those research ND tests that were not done as clinical testing. For those subjects that have had any of the above named ND tests performed clinically within the past 6 months, the subject will be asked to sign a medical release form to allow the study staff to obtain the clinical neurodevelopmental testing results from the clinical testing site (if at institution other than the research site) to be used as part of the research data. If the subject does not wish to sign the release for results, then the subject will be rescheduled to return after 6 months has passed, if possible. If not possible to reschedule the subject, the subject will be administered only those tests that have not been taken by the subject within the past 6 months and the remainder of the tests will have missing data for that subject.

7.2 LABORATORY PROCEDURES/EVALUATIONS

7.2.1 CLINICAL LABORATORY EVALUATIONS

Female subjects of childbearing potential in whom brain MRI is performed may require a pregnancy test if deemed applicable per institutional policy.

7.2.2 SPECIMEN PREPARATION, HANDLING, AND STORAGE

No specimens will be collected.

7.3 STUDY SCHEDULE

Following consent, subjects may have one or more study visits to complete study assessments as outlined in section 7.1.1, however, all in-person assessments must be completed within a maximum of six months.

If needed, MRI and neurodevelopmental testing can occur over two days, with a maximal interval between testing of 6 months, assuming that there are no intervening operations or catheterizations. If neurodevelopmental testing and brain MRI are performed on the same day, ND testing should be completed prior to obtaining the MRI.

7.3.1 FOLLOW-UP

Yearly follow up as described in section 13.4 (item m) to obtain updated medical information may be ascertained.

In the case of possible suicidal ideation and intent, follow up will be completed as described in section 7.4. Additionally, patients will be provided with the option of receiving results from the ND testing. Results will be provided in a written report provided to the patient/parent/LAR. If additional

conversation is needed this will be communicated to the patient either during the visit or via phone call. If the study physician feels referral is needed, the referral will be made and any further follow up will be per clinical care and not considered part of the research study.

It is our hope to continue to follow the subjects who are in this study for years to come. When subjects agree to join this study, the Investigator or designated research staff may continue to contact them once each year over the next five years by a brief telephone call or letter. We will ask about how they/their child is doing, if this information is not available in the medical record, and we will describe any further follow-up studies. Subjects and/or their parent/guardian will **not** be committed to entering any other studies.

7.3.2 FINAL STUDY VISIT/EARLY TERMINATION VISIT

There is no final study visit or early termination visit planned for the study. Subject enrollment will be considered complete once all study procedures are completed.

7.3.3 SCHEDULE OF EVENTS TABLE

Procedures	**Screening**[a]	**Study Procedures**[a]
Informed Consent	X	
Review of Inclusion/Exclusion Criteria	X	
Measure subject height/weight		X
Review of Medical History		X
Collect Concomitant medications/medication history/prior WES results		X
Subject ND test battery[b]		X
Brain MRI[c]		X
Parent/Teacher ND test battery		X[d]
Yearly follow up phone call for up to 5 years (see section 13.4. m)		X

[a]All screening and study procedures *may* occur on the same day. However, if needed, the ND testing and MRI may be scheduled over 2 or more visits with a maximal interval between testing of 6 months.

[b]If ND testing and brain MRI are performed on the same day, ND testing should be completed prior to performing the Brain MRI.

[c]In females of childbearing age, pregnancy testing may be performed per institutional procedure before brain MRI. Participants positive for pregnancy will not undergo the brain MRI.

[d] Parent/Teacher questionnaires may be mailed to the parent up to 3 months prior to the in-person ND testing, or may be done at the time of the ND testing.

If ND testing and/or brain MRI images are deemed incomplete, repeat testing may occur at the discretion of the PI, if feasible, and if the participant agrees to the repeat testing. If repeat testing occurs, a process note will be placed in the study subject binder documenting the discussion and the participant/parent/LAR agreement to repeat testing.

7.4 JUSTIFICATION FOR SENSITIVE PROCEDURES

As part of the assessment procedure, both suicidal ideation and intent may become evident. If subjects exhibit suicidal thoughts or intentions, this will be carefully discussed both with the subject (e.g., adolescent or adult) and, for those <18 years of age, with parent(s)/LAR. Suicidal intent, plans, and means will be evaluated by a licensed clinician. For subjects at low risk (those without active intent, plan or means and with adequate social support), parents and/or the subject, if an adult, will be directed on how to monitor risk and the frequency of contact with the treatment team will be increased. Subjects judged to be at moderate or higher risk will be referred for further evaluation and intervention. Referrals for emergency evaluation would be made to the participating institution's Psychiatric Emergency Service or to hospitals closer to their homes, if appropriate. The on-call and emergency service behavioral health providers will be notified of the study's existence.

If a subject exhibits a significant depression or appears to require psychiatric hospitalization, s/he will have access to referral for treatment. If during the assessment, the subject has a suicide plan or attempt or the severity of the subject's depression requires hospitalization, the psychiatric clinician at the participating center will facilitate hospitalization. If the subject requires additional care but does not require hospitalization, the research team will refer the subject to appropriate clinical follow up.

When findings of clinical relevance are found that could potentially affect other family members, those patients will be referred to an expert in that area who will provide counseling for other family members.

In summary, the risk level, interventions, and follow up will be determined by a licensed clinician at the time of discovery and subsequent interview. Clinical interventions for a subject's mental health will become part of his/her medical record, in accordance with standard medical practice.

7.5 PROHIBITED MEDICATIONS OR TREATMENTS

There are no prohibited medications or treatments that would prevent a subject from taking part in this study

7.6 RESCUE MEDICATIONS, TREATMENTS, AND PROCEDURES

No anticipated need for rescue medication, rescue treatment, or rescue procedure of any kind.

8 ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

Adverse events are not expected during the conduct of the study. However, if any adverse events are documented, they will be reported to the ACC, OSMB, NHLBI and NICHD.

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

For recording and reporting purposes of this study, adverse events (AEs) will include any untoward event, deemed by the PI to be at least possibly related to study procedures, that occurs during or within 24 hours of any study related evaluation including neurodevelopmental assessments or brain MRI. Inability to lie in the MRI scanner due to discomfort from the closed space or inability to lie still will NOT be considered an AE.

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An AE is considered 'serious' if, in the view of either the investigator or the sponsor, it results in any of the following outcomes: death, life-threatening, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that do not meet one of the above criteria may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

OHRP considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets ***all*** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Related or possibly related to the participation in the research; and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) that was previously known or recognized.

This study will use the OHRP definition of UP.

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

The Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 MedDRA 12.1 (http://ctep.cancer.gov) provides a grading system that is used to categorize the severity of adverse

events, as follows:

Grade 1	Mild	Transient, requires no special treatment or intervention, does not interfere with daily activities
Grade 2	Moderate	Alleviated with simple treatments, may limit daily activities
Grade 3	Severe	Requires therapeutic intervention and interrupts daily activities
Grade 4	Life-threatening or disabling	
Grade 5	Death	

8.2.2 RELATIONSHIP TO STUDY

Relationship to study procedures/evaluations will be determined by the Investigator as follows:

- **Not Related:** The event is clearly related to other factors, such as the subject's clinical state, or non-study drugs or interventions.
- **Unlikely to be Related**: An event whose temporal relationship to drug administration makes a causal relationship improbable (e.g. the event did not occur within a reasonable time after the study procedure) and in which other causes or underlying disease provides a plausible explanation.
- **Possibly Related:** The event follows a compatible temporal sequence from the time of study evaluation, but could have been produced by other factors such as the subject's clinical state or non-study drugs or interventions.
- **Probably Related:** The event follows a reasonable temporal sequence from the time of study evaluation, and cannot be reasonably explained by other factors such as the subject's clinical state, or non-study drugs or interventions.
- **Definitely Related:** There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The event occurs in a plausible time relationship to the study procedure and cannot be explained by concurrent disease or other cause.

8.2.3 EXPECTEDNESS

Expectedness will be determined by the Investigator or sponsor as follows:

- **Unexpected:** An unexpected AE or adverse reaction is one for which the nature or severity is not consistent with information in the protocol, or consent form. An AE or adverse reaction also may be categorized as unexpected if the event has not previously been observed at the same specificity and/or severity.
- **Expected:** An event is considered expected if it is known to be associated with the particular evaluation

8.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

For AEs with a causal relationship to the study conduct, follow-up by the Investigator is required until the event or its sequelae resolve or stabilize at a level acceptable to the Investigator.

8.4 REPORTING PROCEDURES

Fatal or life-threatening SAEs are to be reported to the ACC within 24-hours of first knowledge of the event. Those that are unexpected and considered possibly, probably, or definitely related to the study will be reported by the ACC to the OSMB Chair, the medical monitor, the NHLBI and NICHD, and all study Investigators as soon as possible, but no later than 7 calendar days after first knowledge of the event, followed by a complete report within 15 calendar days. All other fatal or life threatening events that are unrelated to the study will be reported semiannually to the OSMB and the NHLBI and NICHD.

All other SAEs (i.e., non-fatal or not life-threatening) that are unexpected and considered possibly, probably, or definitely related to the study will be reported to the ACC within 24-hours of learning of the event. The ACC will report the event to the NHLBI, NICHD, OSMB and all study Investigators within 15 calendar days after first knowledge of the event.

All other AEs not meeting the criteria for expedited reporting will be reported to the ACC within 7 calendar days of first knowledge of the event. The ACC will report these AEs quarterly to NHLBI and NICHD.

Reporting of Adverse Events

Seriousness	Reporting Timeframe to ACC
Fatal or life threatening	Within 24-hours of learning of the event
Serious, but not fatal or life threatening, and pregnancy	Within 24-hours of learning of the event
All other	Within 7 calendar days of learning of the event

Reporting Adverse Events to Institutional Review Boards for NIH-Supported Multi-Center Clinical Trials: The site Investigator or designee is responsible for reporting all serious adverse events to the local IRB in accordance with local policies and procedures. In the case of sites using the central IRB, the site will report the event to the Central IRB via the ACC. An OSMB Summary Report of Adverse Events will be prepared within 30 days of each meeting and distributed by ACC staff to each Principal Investigator.

8.5 STUDY HALTING RULES

As no component of the proposed study is a clinical trial or intervention study, we do not anticipate early stopping of the study due to patient safety concerns.

8.6 SAFETY OVERSIGHT

Data and Safety Monitoring Plan: Interim monitoring by the PCGC's Observational Study Monitoring Board (OSMB), appointed by the NHLBI and NICHD, will occur. The board is made up of subject matter experts the NHLBI and NICHD have invited to participate. None of the members have a connection with the study sites or the study itself. The board includes specialists in ethics, pediatrics, statistics, and neurodevelopment. The board will review patient screening, enrollment, data completeness and quality, and protocol violations every 6-12 months or as needed. Study data will be presented to the board in a number of lists, figures and tables which will be prepared and provided by the ACC. The OSMB will follow NHLBI guidelines for conduct and report recommendations which will be submitted to the central IRB (and any local IRB if applicable) at the conclusion of each formal meeting.

8.7 KNOWN POTENTIAL RISKS

Risks from having an MRI scan: MRI does not involve exposure to X-rays. The MRI uses powerful magnets to make images. Therefore, persons with metal implants, such as surgical clips or pacemakers, should not have an MRI. Although there are no known long-term harmful effects from having an MRI scan, it is possible that there are effects that are not presently known. The MRI scanner uses radio frequency waves that can on rare occasions cause mild warming sensations similar to a warm day at the beach. The MRI scanner also uses switching magnetic fields that make loud banging noises. During the

scanning session, the patient will wear protective earphones, which will both reduce the noise heard from the scanner and permit listening to relaxing music. It is possible that the switching of the magnetic fields in the scanner could cause mild nerve and muscle stimulation in the arms and legs. However, this is very rare and the switching rates are kept well below the levels at which such effects have been known to occur. Finally, some people find it uncomfortable to lie in the confined space of the scanner. If a subject develops such discomfort, the scan will be stopped immediately.

Investigators will follow their institutional standard of care to make the MRI scan comfortable and to minimize motion for better scan quality. The following comfort measures may be used: Form-fitting pillows to immobilize the head comfortably within the head coil; A magnet-compatible sound system, including audio headphones worn by the subject, to permit easy 2-way communication; An attendant to remain in proximity to the subject throughout the scan; A movie to watch during the scanning session if available to help reduce head motion, and to improve comfort and satisfaction on the part of the subjects. MRI scans will not be performed in subjects who cannot undergo this test without sedation. If a subject declines to undergo the MRI scan, he or she can still be in the study.

Risks from neurodevelopmental testing: The interviews and questionnaires that are part of the testing cover sensitive and personal material and might cause subjects to think about issues involving themselves, their children, and family that have not been explored before. Such self-examination, although usually positive, could be negative for some individuals in that it might lead to consideration of bad feelings. While we do not expect significant distress to occur frequently, we recognize the possible risk. At each participating center, a clinical psychologist or psychiatrist will be available by page for consultation regarding such feelings. If there is any indication of risk for harm to self or suicidality during any phase of the study, we will conduct a suicide risk assessment and a behavioral health clinician will advise the subject and/or parent/guardian on the safest course of action.

Additionally, there is some inconvenience and burden of completing questionnaires and some subjects/parents/guardians may feel uncomfortable answering questions.

Risk of Breach of Confidentiality: To help to protect the privacy of subjects participating in this protocol, we have a Certificate of Confidentiality, as described in section 13.4 of this protocol.

Risk of Data Storage: There is a chance that participation in a repository may cause psychological stress or long-term anxiety. For this study, all data entered into a repository will be de-identified.

Risk of Genetic Testing: Subjects will be recruited from among those in whom WES or WGS was performed in trios gathered by the PCGC Congenital Heart Disease Genetic Network Study (CHD GENES) or from subjects at Utah or UCSF/Gladstone with trios that have undergone WES or WGS during the PCGC grant period. The latter subjects may also be recruited to participate in CHD GENES during the consent process for this study. Genetic testing itself is thus not a component of this study.

Under the leadership of the PCGC's "Disclosure of Results" Committee, we will include an option in the CHD Brain and Genes consent form asking subjects and their families whether they would like us to inform them and/or their doctor about whether they/their children were found to carry CHD or neurodevelopment-related genetic variants considered to be pathogenic at the end of the study. We will state that to find out whether the research tests result might be relevant to them/their child's care, it must first be confirmed in a CLIA-approved laboratory using new blood or saliva samples. We will offer participants the ability to consult with a genetic counselor or geneticist before having the clinical test or after the results are known, and will help participants and their physicians in this process. For participants who elect to be informed of their genetic variants, the cost for obtaining a new sample for testing, the testing of the sample in the CLIA lab, and the genetic counseling will be paid for as part of the research study and will not be charged to the patient.

There are several reasons for disclosing results at the end of the study to those study participants who wish to be informed about pathogenic CHD or neurodevelopmental variants. The clinical importance of many of the variants is unknown; indeed, our study results will provide insights on the association of these mutations with adverse outcomes. In addition, the final "grouping" of subjects into mutation positive or negative groups could change over the course of the study in light of new knowledge, including results of whole genome sequencing. The ultimate classification of subjects into the two groups will not take place until the final year of the study. Although subjects with known pathogenic mutations in previously established CHD genes will have been excluded from this protocol at study onset, we expect that we will have discovered new pathogenic mutations by the end of the study period. A further advantage of disclosure of results at the end of the study is that knowledge of the presence of a mutation could influence the results of subjective tests, like quality of life measures, and even responses to questions about cognitive function and behavioral measures.

All disclosure of genetic results at the end of the CHD Brain and Genes Study will be guided by the PCGC's Disclosure of Results Committee, chaired by Drs. Amy Roberts and Wendy Chung. In the first cycle of the PCGC (PCGC1), subjects/families signed an informed consent that stated that genetic results would not be disclosed to them.

There is some chance that analysis of the relationship of genetic findings to neurodevelopmental outcomes could cause psychological distress. Some people involved in genetic studies feel anxious about the possibility of carrying (or their child carrying) an altered gene that places them at risk or that might be passed on to their children.

Risk of Pregnancy Testing: Female study subjects who are of child-bearing potential and who are participating in the MRI portion of the study will be assessed (per institutional policy) for pregnancy prior to undergoing the MRI. Sites will follow institutional policy for pregnancy testing. Institutional policy for determination of pregnancy prior to research MRI will be filed in the study binder at the site. If the subject tests positive for pregnancy, she will be withdrawn from the MRI portion of the study. For minor subjects, sites will follow institutional policy and state law (as applicable) regarding reporting pregnancy results to the parent/guardian. Reporting of pregnancy results (or not reporting) to parent/guardian will be clearly noted in the site specific assent and parental permission forms. Even in

instances where the site does not report pregnancy test results to the parent/guardian (per applicable site policy/state law) the parent or guardian may suspect that the child is pregnant if the subject is unable to take part in the MRI despite best efforts to maintain confidentiality.

Time: One minimal risk of participation is the total time burden of completing the tests, questionnaires, and interviews. Estimated timings for the evaluations include the following: brain MRI 1.5 hours, neuropsychology testing 4 hours, and breaks for lunch and between neuropsychology tests totally 1.5 hours. Thus, it is estimated that the entire evaluation will take 7 hours for those subjects who have both neuropsychology tests and brain MRI. To ease the time burden, if needed, MRI and neurodevelopmental testing can occur over two days, with a maximal interval between testing of 6 months, assuming that there are no intervening operations or catheterizations.

8.8 KNOWN POTENTIAL BENEFITS

The possible benefits of participation are as follows:

- Subjects and their parent/guardian who return for neurodevelopmental evaluation will learn about those aspects of the proband's neurodevelopmental status that are assessed by the battery of tests. Findings will be summarized in a formal written report , with the opportunity for subjects to discuss findings with the center Investigator(s). If the subject/parent/guardian provides consent, this information will also be shared with the primary caregiver and/or cardiologist.

- If there are areas in which a subject is functioning poorly or if neurological problems are discovered, these can be identified and recommendations for further evaluation or intervention provided, as appropriate.

- We offer an option for subjects to learn, at the end of the study, whether they/their children carry pathogenic genetic variants that are likely to have caused their congenital heart disease and/or affected their development and behavior. Although subjects with pathogenic mutations in previously known CHD genes will have been excluded from this protocol at study onset, we expect that we will have discovered new pathogenic mutations by the end of the study period. All disclosure of genetic results at the end of the CHD Brain and Genes Study will be guided by the PCGC's Disclosure of Results Committee, chaired by renowned geneticists Drs. Amy Roberts and Wendy Chung.

- To ascertain whether the research tests result might be relevant to subjects, they must first be confirmed in a CLIA-approved laboratory using new blood and/or saliva samples. If participants choose to receive their genetic results and have a pathogenic mutation, they will have the opportunity to speak with geneticists or genetics counselors about the results. The costs of the additional blood test and genetic counseling will be paid for by this research study and will not be charged to study participants.

- In most cases, knowing the results of DNA analysis done as part of PCGC1 will not provide direct benefit to individual subjects or families. The benefits are those to society as a whole in the improvement of knowledge of the genetic causes of CHD and of NDD, in the development of new diagnostic tests and ultimately in the improvement of treatment and prognosis.

- An indirect benefit may also come from the awareness that the results of this study may serve to help improve the care of patients with similar neurodevelopmental challenges in the future. CHD patients and their families may derive a sense of altruism, accomplishment, and contribution to furthering understanding of neurodevelopmental outcomes in individuals with congenital heart disease through their participation

The research examining the association of DNA results, neurodevelopmental performance, and brain MRI may result in inventions or discoveries that could create new tests and medicines that have commercial value. Although subjects and/or their parent/guardian will not receive compensation now or in the future for use of their data, income that may be derived from future research or sales of the grouped data may be used to support biomedical research.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirements. Risk-based monitoring will be utilized for this trial. Details of clinical site monitoring are documented in the Clinical Monitoring Plan (CMP).

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESES

We hypothesize that patients with deleterious mutations will have more neurodevelopmental abnormalities than CHD patients without these mutations. In exploratory analyses with limited statistical power, we expect to see the greatest developmental deficits in subjects with deleterious mutations in loss of function genes that are expressed in both the heart and brain. We also hypothesize that there will be worse outcomes in patients with independent deleterious mutations in heart and brain, as well as in those with either deleterious mutations or unfavorable polymorphisms in neuroresilience genes.

10.2 DESCRIPTION OF STATISTICAL METHODS

10.2.1 GENERAL APPROACH

Patients will be classified into one of two groups: 1) patients with deleterious mutations and 2) patients without deleterious mutations. Although cases and controls will be chosen based upon available information at the study onset, the final classification will be performed at the conclusion of the study

and prior to data base lock and will be based on the current state of the science at that time by the genetic protocol team.

Demographic and clinical characteristics will be summarized for each of the two groups using mean, standard deviation, median, and interquartile range for continuous variables and frequency and percent for categorical variables. Statistical comparisons between the two groups will be performed using the two-independent sample t-test or the Wilcoxon rank sum test for continuous variables and the chi-square test or Fisher's Exact test for categorical variables. All tests will be performed at the two-sided 5% level of significance.

Neurodevelopmental Outcomes:

The primary neurodevelopmental study outcome is the WRAT-4 composite score, which has a mean of 100 and SD 15. Secondary outcomes include composite scores from each of the following 5 domains: Visual Spatial Skill, Intelligence, Memory, Executive Function, Language, Tests for Autism, Social Cognition, parent and teacher reported instruments.

The primary analysis for all endpoints (primary and secondary) will compare patients with and without known deleterious mutations and will include the matching factors of site, CHD type, sex, and age category using a generalized analysis of variance model. Because socioeconomic status (SES) has a proven effect on neurodevelopmental performance in virtually all published studies, we will also adjust for this variable in all models. An appropriate link and distribution function will be used depending on the distribution of the outcome variable (e.g. identity link and normal distribution for normal data, logit link and binomial distribution for dichotomous data). If assumptions of the model are violated, appropriate transformations will be performed (e.g. log, square root, rank) in order to satisfy the assumptions. Models supporting the primary analysis will include variables that may be associated with adverse neurodevelopment as covariates, (e.g., maternal education, history of prematurity or early term birth, number of open heart surgeries, number of cardiac catheterizations, number of serious adverse events, etc.). We will perform separate analyses for each potential covariate that include the variable, along with the matching factors and SES, and consider those variables associated with endpoint at a p-value<0.1 as covariates in stepwise generalized linear models. Collinearity will be assessed. The primary analyses and all secondary analyses will be conducted at the two-sided 5% level of significance. No multiple testing adjustments will be used for the secondary outcomes, although it is recognized that results of these tests should be interpreted with caution. Exploratory analyses of effect modification by neuro-resiliency polymorphisms may be performed by including interaction terms in the models.

MRI Outcomes:

MRI outcomes will include measures based on voxel-level analyses (e.g. grey matter volumes, regional cortical thickness, etc.) and outcomes that are summarized at the patient level (e.g. mean global FA). For unadjusted voxel-level analyses, two-sample t-tests (with appropriate transformations, if needed to satisfy the assumptions of the analyses) or Wilcoxon Ranks sum tests will be conducted to identify voxels that are significantly different between the two groups. P-value adjustments for multiple testing will be done to control the false discovery rate at 5% for voxel-level analyses for each MRI measure.

Using clinical and demographic variables that are differentially distributed between the two groups (as identified by p<0.10 in univariate analyses) as potential covariates, adjusted analysis at the voxel level will be conducted using stepwise generalized linear modeling with appropriate distribution and link functions.

Unadjusted comparisons of MRI outcomes measured at the patient level for the two groups will be made using the two-sample t-test or Wilcoxon Rank Sum test. Additional analyses for patient level MRI outcomes will include adjustment for matching factors (site, sex, CHD type and age category) and other demographic and clinical characteristics that are differentially distributed between groups (as identified by p<0.10 in univariate analyses) using stepwise regression models. Collinearity will be assessed and transformations used, if required, to satisfy the assumptions of the analyses. All tests will be conducted at the two-sided, 5% level of significance and no adjustment will be made for multiple testing for the MRI outcomes measured at the patient level.

Alternative approaches for analyzing the MRI and genetic data to determine if there is a significant association between genetic variants and brain MRI endophenotype in patients with CHD will be based on the state of the art statistical approaches that are available when the data base for this protocol is closed. The statistical analysis of MRI and genetic data is a rapidly evolving field and it is expected that more sensitive statistical approaches will be developed during the course of this study.

10.3 SAMPLE SIZE

Mean total composite achievement score will be compared for subjects with and without deleterious mutations using a two-sided, two-sample test conducted at the 0.05 level of significance. The standard deviation of achievement score is assumed to be 15 in each group. With 176 subjects with genetic mutations and 175 without mutations, we have 80% power to detect a difference in means of 4.5, or 0.3 standard deviations. If the true effect size is 0.5 standard deviations, samples of 64 subjects in each group would provide 80% power to detect a mean difference of 7.5.

Similarly, the following table provides the samples size required to attain 80% power to detect the specified effect size (i.e. standardized difference between means) between the two groups for any continuous neurodevelopmental or MRI patient level outcome at the two-sided 5% level of significance.

Effect Size	Sample Size for each group for 80% Power using Two-sample T-test
0.3	176
0.4	100
0.5	64

10.4 MEASURES TO MINIMIZE BIAS

10.4.1 ENROLLMENT/ RANDOMIZATION/ MASKING PROCEDURES

The study does not involve randomization, blinding, or masking.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate study research records, in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in a NIH affiliated study, each site will permit authorized representatives of the study sponsor, the NIH, and regulatory agencies to examine (and when permitted by applicable law, to copy) clinical research records for the purpose of quality assurance reviews, audits, and evaluation of the study safety, progress, and data validity.

Source data are all information, original records of clinical findings, results, observations, or other activities conducted for the reconstruction and evaluation of the study. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aids or evaluation checklists, recorded audio tapes of counseling sessions, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete. It is acceptable in some instances to use CRFs, test instruments, or questionnaires as source documents. It is not acceptable for the CRF to be the only record of a subject's participation in the study.

12 QUALITY ASSURANCE AND QUALITY CONTROL

The ACC has primary responsibility for QC/QA activities of the study data. The ACC also requires that the sites complete certain QC activities, most of which are monitored by the ACC.

The key QC/QA activities include but are not limited to:

- Development of a study Manual of Operations;
- Clearly formatted and carefully constructed Data Collection Forms with clear, up-to-date manuals of instruction;
- Sign-Off Procedures for all study forms;
- Central protocol training and certification of all Center data collection staff with the use of standardized checklists;
- Central Clinical Data Management System (e.g. Rave) training and certification of Center staff responsible for entering data;
- Database management and cleaning
- On-going monitoring of all protocols/data collection activities;
- Completion of reliability and/or pilot studies for key measurements as appropriate;

- Inclusion of repeat measurements, as feasible, in the course of the study; and
- Monitoring visits
- Assurance of site compliance with the study, research regulations and Good Clinical Practice
- Management of the Master Study File
- Management of events when non-compliance is identified
- Training and onboarding of new staff as needed
- Collaboration with OSMB, NHLBI, and NICHD

Certification of quality of neurodevelopmental testers and brain MRIs will be carried out by the neurodevelopmental committee co-chairs and MRI Core Lab, respectively.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 ETHICAL STANDARD

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and ICH E6.

13.2 INSTITUTIONAL REVIEW BOARD

All participating sites will submit to a single IRB for the study and will not begin any study procedures until the site has approval from the single IRB. The single IRB will be chosen by the PCGC Steering Committee. The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the IRB for review and approval prior to enrollment and study procedures. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. After initial study approval, any changes to the original approved consent form will be IRB approved and a determination will be made regarding whether previously consented subjects need to be re-consented.

13.3 INFORMED CONSENT PROCESS

13.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO SUBJECTS

Consenting documents that will be used for this study include, but are not limited to: Adult consent form, parental permission form, assent form, consent process note, translation forms. The ACC will provide each site with English consenting templates to be used. Sites may modify the templates to meet institutional requirements, but will be required to obtain ACC approval for any changes prior to IRB submission. All consent forms (and any subsequent updates) will be IRB approved prior to use in the

study. Delegation for site staff to perform consenting procedures will be documented on the Delegation of Authority Log and will be signed by the PI.

13.3.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent will be obtained from all subjects taking part in the study prior to collection of study data or study procedures. The Principal Investigator or his/her designated study staff will explain the study and all study expectations in detail using language that is understandable to the subject. The subject/parent/LAR will be given time to have all questions answered. For minor subjects taking part, parental permission will be obtained from parent or legal guardian. Assent will be obtained from children aged 11-17. Phone consent may be obtained from subjects and will follow institutional/IRB phone consenting procedures. If using a phone consenting procedure, the staff will ensure that the subject receives a copy of the consent form prior to the phone discussion. Following the phone consent discussion, participants/parent/LAR will be instructed to email or fax the signed consent form to the study center. In instances where the parent/participant/LAR does not have the ability to email or fax the consent to the study center, the consent may be mailed back to the study center via postal mail. Upon receipt at the study center, the staff person who performed the phone consent will then sign and date the consent form. If used, phone consent will be documented in the patient record. In all cases (in person and phone consent), a copy of the signed consent will be provided to the subject/parent/LAR and the original maintained in the subject research record. For all centers, an informed consent process note will be completed to describe the consenting process and to ensure that all elements of informed consent have been followed.

Completion of parent (for parent of child or LAR) or participant instruments at home: Documented consent via in-person consent or phone consent (as described above) will be required prior to parent and/or participant completion of at home instruments. Consent must be received and signed by site staff prior to sending parent and/or participant instruments home for completion.

Completion of parent instruments at home for adult participants: For parents completing the parent forms for adult participants (who are not designated as LAR), we request for a waiver of documentation of consent from the IRB for the parent of the adult participant. We will provide an information letter to the parent briefly explaining the study and the required elements of consent, but will not request the information letter to be signed. The information letter will be IRB approved prior to use.

Ability to consent: The Site Investigator will determine the capacity of the subject to provide consent/assent. In instances where the Investigator deems the subject does not have capacity to consent/assent, the parent/LAR will be asked to consent for the subject. Even if the subject does not have the capacity to consent/assent, he or she should be informed about the study to the extent compatible with his/her understanding in a manner that is developmentally appropriate.

Translations: For subjects that are unable to fluently read English, a translated consent (Spanish) will be provided and an interpreter available for the consenting process and study procedures. Translation of consent forms will be documented with a certificate of translation. Short form consents may also be used. Subjects who do not fluently read English or Spanish will not be enrolled.

Age of majority: If a proband turns 18 years of age (or younger if the state law for legal age of consenting is less than 18) while participating in the study, re-consent will be obtained from the legal proband. For subjects who turn legal age to consent while in the 5 year follow up, verbal phone consent will be obtained. Once all data is collected, and yearly follow up is complete, re-consent will not be requested for analysis of the existing limited dataset.

13.4 SUBJECT AND DATA CONFIDENTIALITY

Measures to protect confidentiality will be similar to those used in the PCGC CHD GENES study. Investigators will take all reasonable measures to protect the confidentiality of subjects, including the following:

a) The results of tests performed for research purposes will not be placed in the medical record with the exception of the brain MRI images and report. Each participating site will follow local site policy/standard practice with regard to placing the research MRI in the medical record. If a site is required per local policy/standard practice to include the MRI images and report in the medical record, the consent form will state this.
b) If non-paternity information is present in the prior WES/WGS results, it will be kept in the strictest confidence and will not be divulged to research subjects or their parent/guardian.
c) Each subject is assigned a subject identification number (SID). All interview and clinical research data are stripped of identifiers other than dates and labeled with the study number. The enrollment log with subject identifiers will be maintained at each site in a secured, locked location available only to the study staff.
d) The study will follow good clinical practices at all times. Databases will be secured as previously discussed.
e) All participating laboratories and analysis facilities will follow good clinical practices maintaining data integrity and subject confidentiality.
f) The risk of breach of subject confidentiality will be minimized by storage of all study materials in a locked file cabinet in a location separate from the laboratory data. The informed consent form states that study data will be made available to the Administrative Coordinating Center (ACC) and NHLBI and NICHD to ensure study safety and quality control.
g) The subject's name and any other identifying information will not appear in any presentation or publication resulting from this study.
h) Results of previous testing on biological specimens may be shared with study subjects under conditions that will be determined by the Committee on Disclosure of Results (Co-Chairs Chung and Roberts). If subjects prefer or institutions mandate that the results should not be shared, then they cannot purposely or mistakenly be included in the medical record, which could jeopardize that person's insurability and employability. At the end of the study, the results of the genetic testing may be published for all the subjects as a group. There is a reasonable possibility that no genetic findings will result

from this research effort. If genetic findings are detected, it may be years before any utility of these findings are realized.

i) In the future, information from DNA analyses and clinical studies or medical records will be placed into an NIH-sponsored central data repository such as the National Center for Biotechnology Information (NCBI) repository. When the results of the genetic tests and other study data are placed in a federal data repository, any information that could identify a subject will be removed and the information will be labeled with a new number that is different from the subject study identification number and cannot be linked back to an individual subject. The purpose of a central data repository is to make the study data available for future, yet to be identified research. The NCBI or a similar repository makes data accessible through the Internet. The repository has two databases, open access and controlled access. The open access database is available to anyone and includes DNA sequence traces that are not linked to medical or personal information. The controlled access database includes de-identified medical information and more detailed analyses of de-identified samples that are made available to researchers with IRB/EC approval to conduct human genetic studies and who have received approval from an NIH Data Access Committee.

j) If an incidental finding of clinical significance is found on a study clinical test such as a brain MRI (to be interpreted by a board-certified radiologist within each center), the PI or other qualified member of the research team will take full responsibility for disclosing the findings to the patients/parents, communicating with their primary care physicians with permission, and making appropriate referrals to a neurologist or other specialist as indicated and in accordance with Good Clinical Practice and IRB policies. The subject may choose to seek a second opinion and/or appropriate clinical care. This might change the subject's insurability and employability as it relates to the clinical finding only.

k) The study has a Certificate of Confidentiality from the National Institutes of Health (NIH). With this Certificate, the researchers of this study cannot be forced to disclose information that may identify a subject, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The Certificate cannot be used to resist a request for information from the United States government when it is used for evaluating federally funded study projects or for information that must be disclosed to meet the requirements of the Food and Drug Administration (FDA). A Certificate of Confidentiality does not prevent a subject or his/her parent/guardian from voluntarily releasing information about the subject’s involvement in this research. If an insurer, employer, or other person obtains a subject’s written consent to receive research information, then the researchers will not use the Certificate to withhold that information.

l) Prior to beginning the neurodevelopmental evaluation, subjects will be told that the information they provide will be held in confidence and not revealed to school officials, employers or other authorities without their permission, and that names will not be associated with data in the research database. For minor subjects, parents will be

informed of evaluation findings unless otherwise dictated by local/state regulations. In all instances, if the evaluator judges that the he or she is at risk of suicide or of hurting another, this concern will be shared with the accompanying parent or guardian. Possible referrals will be discussed with the parent/guardian. Similarly, parents will be told that the PI is required by law (as applicable) to report any evidence that suggests child abuse. As part of the debriefing, both the child and parent will be asked if they would like additional care or services. If so, the research team will provide referrals (e.g., behavioral health provider, substance abuse program, etc.). If a patient's responses suggest engagement in risk-taking behaviors, appropriate resources will be discussed and information provided (e.g., for adolescents who admit to involvement in drinking and driving, information about the Designated Drivers Campaign and the Contract for Life). An experienced psychiatric clinician at each participating institution will always be available to help should the testing or questionnaires stimulate any distress in either the subject and/or parents.

m) Subjects will be done with all tests and questionnaires after they/their child completes neurodevelopmental testing and, for some subjects, a brain MRI. However, we may wish to follow the study subjects for years to come. When subjects agree to join this study, the nurse or doctor may continue to contact them once each year over the next five years by a brief IRB approved script/telephone call or letter requesting follow-up medical information. We will ask about how they/their child is doing, if this information is not available in the medical record, and we will describe any further follow-up studies. Subjects and their parent/guardian are **not committed** to entering any other studies or providing this long-term information.

13.4.1 RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS OR DATA

Data and specimens from the PCGC Data Hub will be used to identify patients with de novo mutations and to rule out such mutations in control patients. Neurodevelopment and MRI data from this protocol will be linked to genetic data derived from the CHD GENES protocol. Specimens will not be obtained as part of this protocol.

13.5 FUTURE USE OF STORED SPECIMENS

No specimens will be obtained as part of this trial.

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

The ACC will be responsible for the creation, distribution and training materials related to all CRFs that are not the standardized instruments listed in 7.1.1. The ACC will provide the sites with CRF Completion Guidelines and Data Entry Guidelines specific to each CRF to ensure consistent and accurate collection and entry of data.

All data collected at the site (including AEs, concomitant medications, and expected adverse reactions data) will be entered by trained trial site staff into the Medidata Rave CDMS, a 21 CFR Part 11-compliant data capture system provided and managed by the ACC. The CDMS includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Integrated into the data entry system are real time validations, including both inter- and intra-instrument data checks. Inconsistent or questionable values are flagged during entry, and a query is automatically generated to the data entry client. These queries provide the information necessary to investigate any data entry errors or resolved questions regarding out-of-range or questionable values. Second-level query tracking allows monitors and data managers real time access to unresolved queries as well as the date and time of query generation and resolution. In some instances, with prior approval from the study Steering Committee, some data to be used for additional sub-analyses may be entered and stored in a study specific REDCap or other appropriate database. ACC staff are responsible for the tracking of data entry at each site to ensure timely submissions and query resolution so data are available as close to real-time as possible for all reporting and analysis done during the execution of the protocol.

Data will come from source documents that may include paper CRFs and information from the medical record. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the subject's official electronic study record. Data are entered by subject study identification number; names will not be linked with subject data in the database. Study sites will maintain records in secure areas linking the subject name with the identification number assigned for the study. Study sites will have full access to their own data and be able to view these data remotely. Study staff will not be able to view subject data associated with other sites.

All data changes are written to an audit trail. The audit trail identifies the data item by table, column and key field. The entry includes the user, date and time, as well as the old value and new value. Both subject related data as well as trial configuration data are written to the audit trail. Data are saved at regular intervals during data entry to prevent loss of information in the event of a disruption of the Internet connection.

Several levels of security are employed to ensure privacy and integrity of the study data, including the following:

- Study access requires use of assigned user names and passwords.
- Individual roles and access levels are assigned by the study data manager.
- Passwords are changed regularly.
- Web-based entry uses secure socket layer (SSL) data encryption.
- Data will not be stored on laptop computers.

Once the protocol is completed and all data queries are resolved, the ACC will lock the database following the ACC's Standard Operating Procedure (SOP) for Database Lock. Data will be provided to study statisticians for analyses in the format specified in the SAP. In the event the database will need to be unlocked for corrections, the Database Lock SOP provides the processes and procedures to be followed.

Data collected from the Brain MRI will be sent to the MRI Core for processing and analysis. The ACC will receive final results. Methods and location for MRI data storage will be specified in the Manual of Operations.

14.2 STUDY RECORDS RETENTION

In compliance with Protection of Human Subjects regulations, (45CFR Part 46), records related to the conduct of this trial, including but not limited to source documentation, case report forms, informed consent forms, essential study documentation, and documentation of IRB activities, will be retained by the Investigator for a period of 3 years following the official close of the study. Such records may be preserved in hardcopy, electronic or other media form and must be accessible for inspection and copying by authorized representatives of HHS, NIH, the study sponsor and/or their representatives at reasonable times and in a reasonable manner. At the conclusion of the 3 years institute storage, the NHBLI must be consulted for final storage or destruction decisions.

14.3 PROTOCOL DEVIATIONS/VIOLATIONS

A protocol deviation is any noncompliance with the protocol, GCP, or MOO requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. Deviations should be recorded by the site staff, forwarded to the ACC per the MOO and maintained with the study records. Deviations can affect the integrity of the study data and in some cases the protection of human subjects. As a result of a deviation, an immediate corrective action should be developed by the site and implemented promptly. The ACC, NHLBI/NICHD, or OSMB may request a site develop a corrective action plan to address recurring deviations. It is the responsibility of the site to use continuous vigilance to identify and report deviations to the ACC. Protocol deviations that place a subject at increased safety risk may be considered protocol violations and should be reported to the ACC within 24 hours of the investigators awareness of the violation. Protocol deviations and violations will be reported to the site IRB per institutional/IRB policy.

14.4 PUBLICATION AND DATA SHARING POLICY

In the current PCGC funding period, we have a data sharing agreement with Cincinnati Children's Hospital Medical Center's Administrative Coordinating Center.

Data generated by this project will be shared in conformance with the requirements described in the NIH Final Statement on the Sharing of Research Data. Our plan assumes that final research data are recorded factual material commonly accepted in the scientific community as necessary to document, support, and validate research findings. This does not mean summary statistics or tables; rather, it means the data will be a computerized dataset. The final research data will include the computerized dataset upon which accepted publications were based. The final dataset may also include both raw data and derived variables, which would be described in the documentation associated with the dataset. Through the PCGC's ACC, data products from this study will be made available without cost to researchers and analysts. User registration will be required in order to access or download files. As part of the registration process, users must agree to the conditions of use governing access to the public release data, including restrictions against attempting to identify study subjects, destruction of the data after analyses are completed, reporting responsibilities, restrictions on redistribution of the data to third parties, and proper acknowledgement of the data resource. Registered users will receive user support, as well as information related to errors in the data, future releases, workshops (if held), and publication lists. The information provided to users will not be used for commercial purposes, and will not be redistributed to third parties. We are aware of the need to protect human subject information and Protected Health Information in accordance with HIPPA standards and in other situations where data sharing may not be appropriate or allowed. Recognizing that the value of data often depends on their timeliness, data sharing will occur in a timely fashion. Every effort will be made to release and share the data no later than following the acceptance for publication of the main findings from the final dataset. We will take steps to protect the proprietary information of our institutions and those of any collaborators or other participating third parties. Recognizing that the value of data often depends on their timeliness, every effort will be made to release and share data upon the acceptance for publication of the main findings from any dataset.

15 STUDY ADMINISTRATION

15.1 STUDY LEADERSHIP

The National Heart, Lung, and Blood Institute-supported Pediatric Cardiac Genomics Consortium (PCGC) currently consists of 5 main (Core) centers located in Boston, New Haven, New York, Salt Lake City and San Francisco/Gladstone. Auxiliary centers are located primarily in the United States, with a few being international. All of the centers will work collaboratively to recruit a sufficient number of subjects to achieve the scientific goals of the Consortium.

The consortium is supported by an administrative and data coordinating center, Children’s Hospital Medical Center (CHMC) in Cincinnati, as well as a Steering Committee.

The Consortium will also develop resources that will benefit the wider congenital heart disease research community. The de-identified clinical and genetic data (for PCGC genetic studies) will be deposited in publicly accessible databases for use by outside investigators in accordance with NHLBI data sharing policies and an approved plan developed by the PCGC Steering Committee.

16 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of the trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in this trial. The study leadership in conjunction with the NIH has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest. It is the responsibility of the Investigator to disclose all perceived conflicts of Interest to the study leadership.

17 APPENDIX A – DESCRIPTION OF TESTS OF NEURODEVELOPMENT, BEHAVIOR, AND QUALITY OF LIFE

ADOS-2: The Autism Diagnostic Observation Schedule- Second Edition (ADOS-2)[68] is a test which allows for accurate assessment and diagnosis of autism spectrum disorders across age, developmental level, and language skills. The test modules used will be 1-4 Cutoff scores are provided. There is no mean or standard deviation. Comparison scores can be used as a continuous variable (1- 10) since you cannot compare raw scores across modules.

Cut off scores are as follows.

- Module 1: few to no words autism cut off is 16. Some words autism cutoff score is 12
- Module 2: Younger than 5 years, autism cut off score is 10. Aged 5 and older autism cut-off score is 9
- Module 3: cutoff is 9
- Module 4: Autism cutoff score for Communication + Social Interaction is 10.

BASC-3: The Behavior Assessment System for Children-Third Edition (BASC-3)[73] is a parent, teacher, and self-report questionnaire. The BASC-3 measures emotional and behavioral functioning. It gives a multidimensional measure of a child's behavior both at home and at school. The BASC-3 is usually reported in T-scores, with a mean of 50 and a standard deviation of 10.

BRIEF-2: The Behavior Rating Inventory of Executive Function: Second Edition (BRIEF-2)[72] is a parent administered questionnaire which measures executive skills functioning. The BRIEF looks at General Executive Functioning, Emotional Regulation, Behavioral Regulation, and Cognitive Regulation. The BRIEF includes a parent report form, teacher report form, and for adults, a self-report form. The BRIEF is usually reported in T-Scores, with a mean of 50 and a standard deviation of 10.

Beck Anxiety Inventory (BAI): The Beck Anxiety Inventory[75] is a self-report form which assesses for anxiety in adults. The Beck is measured via cut off score. There is no mean and standard deviation. The following are cut-off scores.

Score Range:

0-9	Minimal
10-16	Mild
17-29	Moderate
30-63	Severe

Beck Depression Inventory, 2nd Edition (BDI-II): The BDI-II is a self-report measure of depression in adults. The BDI-II consists of 21 items that measure the intensity of depression within the past two week timeframe. The Response options for each item are a list of four statements that are presented in increasing order of severity. Scores on the BDI-II are tallied and compared to cut-off scores that indicate the level of severity of depression.

Children's Depression Index (CDI): The Children's Depression Index (CDI)[76] is a self and parent report form which assess depressive symptoms in children. It assesses for both emotional problems and functional problems related to depressive symptomology. It is normed for children ages 8-19. The CDI-2 is usually reported in T-scores, with a mean of 50 and a standard deviation of 10.

Connors, 3rd Edition (Connors-3): The Connors-3 is a multi-informant questionnaire measure of Attention-Deficit/Hyperactivity Disorder (ADHD) and its most common related conditions in children and adolescents, ages 6-18 years old. The Connors-3 yields content scales measuring inattention, hyperactivity/impulsivity, learning problems/executive functioning, , DSM-V symptoms scales, validity scales, indices, anxiety and depression screener items, critical items, and items measuring impairment and strengths/skills. There are parent, teacher, and self-report forms of the Connors-3. Scores are presented as T-scores, which have a mean of 50 and a standard deviation of 10.

Connors' Adult ADHD Rating Scales (CAARS): The CAARS is a questionnaire measure of the presence and severity of Attention Deficit Hyperactivity Disorder in adults. The CAARS yields DSM subscales measuring inattentive symptoms, hyperactive-impulsive symptoms, and total ADHD symptoms. Additionally, factors are available that include Inattention/Memory Problems, Hyperactivity/Restlessness, Impulsivity/Emotional Lability, and Problems with Self-Concept. Scores on the CAARS are presented as T-scores with a mean of 50 and a standard deviation of 10.

D-KEFS (Selected Subtests): The Delis Kaplan Executive Function System (DKEFS)[65] measures overall executive functioning. The specific subtests that will be tested are as follows – the Verbal Fluency Subtest, Trail Making Subtest, and the Tower Subtest. Verbal Fluency assesses letter fluency, category fluency, and category switching. Trail Making measures processing speed and simple cognitive flexibility. The Tower Subtest measures planning and inhibition of impulsive behavior.[61] The mean is 100 and the standard deviation is 15.

The Pediatric Quality of Life Inventory: The Pediatric Quality of Life Inventory (PedsQL)[77-80] is a Parent report form which assesses overall quality of life in children with chronic illness. The 23-item PedsQL 4.0 Generic Core Scales encompassing Physical Functioning, Emotional Functioning, Social Functioning, and School/Work Functioning is administered to measure quality of life. Emotional, Social, and School/Work combine to form a Psychosocial Summary score. The PedsQL scales are comprised of parallel child self-report (ages 5-18 years) and parent proxy-report formats (ages 2-18 years). In addition, the PedsQL™ 4.0 Generic Core Scales Young Adult Version has essentially identical items, the only notable difference being the inclusion of the word 'work' on some of the School Functioning items. This consistency facilitates the evaluation of differences in health-related quality of life across and between age groups, as well as the tracking of health-related quality of life longitudinally. The PedsQL has demonstrated reliability, validity, sensitivity and responsiveness for child self-report and parent proxy report and has been shown to be related to other key constructs in pediatric healthcare such as access to needed care, healthcare barriers, and quality of primary care. The disease-specific PedsQL Cardiac Module has 6 scales related to symptoms, treatment barriers, perceived physical appearance, treatment anxiety, cognitive problems, and communication. Formatting and scoring are the same as the PedsQL generic core scales. The validity and reliability of the PedsQL Cardiac Module for parent-proxy report (age 2-18 years) and for self-report (age 8 -

18 years) has been demonstrated. The PedsQL Generic Core and Cardiac Module scales can be completed by parents/adults in 5 minutes and by children in 5 to 10 minutes

Multidimensional Anxiety Scale for Children – Second Edition (MASC-2): The Multidimensional Anxiety Scale for Children (MASC-2)[74] is a parent questionnaire. It measures children's level of anxiety in multiple areas including, overall anxiety level, separation anxiety, Generalized Anxiety, Obsessions and Compulsions, Social Anxiety, and Physical manifestations of anxiety. It is normed for children ages 8-19. The MASC-2 is usually reported in T-scores, with a mean of 50 and a standard deviation of 10.

Quality of Life Scale (QOLS): The QOLS is a 16-item self-report form that assesses overall quality of life on a scale of 16-112 (higher scores indicate better quality of life).[81,82] The QOLS has high test-retest reliability, strong content validity, and good convergent and divergent construct validity. Scores have been validated in populations of healthy individuals (mean=90) and a number of disease specific groups.[81] The total score is our main endpoint. The Linear Analog Scale is a self-report tool for assessing global QOL.[83] The scale is a vertical, 10-centimeter line with graded increments from 0-100 (higher scores indicating better QOL).[84] Linear analog scales have shown good validity and reliability in previous studies of adults with CHD.[84]

Reading the Mind in the Eyes Task[69]: This task measures general social intelligence and the ability to read and understand facial expression. Both the child and adult version will be administered. The mean for adults (≥ age 18 years) is 26.2 (SD 3.6).

Social Responsiveness Scale (SRS-2): The Social Responsiveness Scale- Second Edition (SRS-2)[71] is a parent administered questionnaire. It measures interpersonal behavior, communication, and repetitive/stereotypic behavior characteristic of autism spectrum disorders (ASD). The SRS-2 is normed from ages 2.5 years through adulthood. The SRS scale is measured via T-score. A cut off T score of 76 or higher indicates a clinical diagnosis of autism. T scores of 66-75 indicate moderate deficiencies. T scores of 60-65 are mild deficits. A T score of 59 or lower indicates functioning that is age appropriate.

Vineland-III Questionnaire: The Vineland Adaptive Behavior Scales[70] measures the adaptive functioning of subjects, which generally refers to level at which they perform their activities of daily living. There are three indices – Communication, Daily Living, and Socialization. This measure is a parent report form. The mean is 100 and the standard deviation is 15.

VMI-6: The Beery Visual Motor Integration Test – 6th Edition (VMI-6)[66] measures the extent to which individuals can integrate their visual and motor abilities. It is commonly used to identify subjects who are having significant difficulty with visual-motor integration. The test is normed for ages 2- adult. The mean is 100 and the standard deviation is 15.

WAIS IV: The Wechsler Adult Intelligent Scale-Fourth Edition (WAIS IV)[63] is a comprehensive intelligence test for adults ages 16 and up. The WAIS IV takes 48–65 minutes to administer and generates a Full Scale IQ which represents general intellectual ability. It also provides four primary index scores (i.e., Verbal Comprehension Index, Perceptual Reasoning Index, Working Memory Index, and Processing Speed Index) that represent a child's abilities in more discrete cognitive domains. The mean is 100 and the standard deviation is 15.

WIAT-III Oral Language Composite: The Wechsler Individual Achievement Test – Third Edition (WIAT-III)[64,65] Oral Language Component acts as a language screener for children and adults from age 5 to 50. The Listening Comprehension subtest measures receptive language skills, such as the ability to listen and understand details. The Oral Expression subtest is a screening tool for expressive language skills such as naming. The mean is 100 and the standard deviation is 15.

WISC V: The Wechsler Intelligence Scale for Children-Fifth Edition (WISC V)[62] is a comprehensive intelligence test for children between the ages of 6 and 16. The WISC-V takes 48–65 minutes to administer and generates a Full Scale IQ which represents a child's general intellectual ability. It also provides five primary index scores (i.e., Verbal Comprehension Index, Visual Spatial Index, Fluid Reasoning Index, Working Memory Index, and Processing Speed Index) that represent a child's abilities in more discrete cognitive domains. The mean is 100 and the standard deviation is 15.

WRAML-2: The Wide Range Assessment of Memory and Learning: Second Edition (WRAML-2)[67] is a test which measures memory. The Story memory subtest measures the ability to remember verbal information for both immediate and delayed recall. The Picture Memory subtest assesses the ability to remember visual data. The test is normed for subjects ages 5 to 90. The mean is 100 and the standard deviation is 15.

WRAT-4: The Wide Range Achievement Test 4 (WRAT-4) is a norm-referenced test that measures the basic academic skills of word reading, sentence comprehension, spelling, and math computation.[61] It was standardized on a representative national sample of over 3,000 individuals ranging in age from 5 to 94 years. The normative sample was selected according to a stratified national sampling procedure with proportionate allocation controlled for age, gender, ethnicity, geographic region, and parental/obtained education as an index of socioeconomic status. The WRAT-4 takes 35-45 minutes to administer. The composite score of the WRAT4 is the primary outcome variable for the study had has a mean of 100 and the standard deviation of 15.

18 LITERATURE REFERENCES

1. Marino BS, Lipkin PH, Newburger JW, et al. Neurodevelopmental outcomes in children with congenital heart disease: evaluation and management: a scientific statement from the American Heart Association. *Circulation.* 2012;126(9):1143-1172.

2. Bellinger DC, Wypij D, Rivkin MJ, et al. Adolescents with d-transposition of the great arteries corrected with the arterial switch procedure: neuropsychological assessment and structural brain imaging. *Circulation.* 2011;124(12):1361-1369.

3. Gaynor JW, Nord AS, Wernovsky G, et al. Apolipoprotein E genotype modifies the risk of behavior problems after infant cardiac surgery. *Pediatrics.* 2009;124(1):241-250.

4. Creighton DE, Robertson CM, Sauve RS, et al. Neurocognitive, functional, and health outcomes at 5 years of age for children after complex cardiac surgery at 6 weeks of age or younger. *Pediatrics.* 2007;120(3):e478-486.

5. Neufeld RE, Clark BG, Robertson CM, et al. Five-year neurocognitive and health outcomes after the neonatal arterial switch operation. *J. Thorac. Cardiovasc. Surg.* 2008;136(6):1413-1421, 1421 e1411-1421 e1412.

6. Bellinger DC, Newburger JW, Wypij D, Kuban KC, duPlesssis AJ, Rappaport LA. Behaviour at eight years in children with surgically corrected transposition: The Boston Circulatory Arrest Trial. *Cardiol. Young.* 2009;19(1):86-97.

7. Gaynor JW, Gerdes M, Zackai EH, et al. Apolipoprotein E genotype and neurodevelopmental sequelae of infant cardiac surgery. *J.Thorac.Cardiovasc.Surg.* 2003;126(6):1736-1745.

8. Gaynor JW, Wernovsky G, Jarvik GP, et al. Patient characteristics are important determinants of neurodevelopmental outcome at one year of age after neonatal and infant cardiac surgery. *J. Thorac. Cardiovasc. Surg.* 2007;133(5):1344-1353, 1353 e1341-1343.

9. Atallah J, Dinu IA, Joffe AR, et al. Two-year survival and mental and psychomotor outcomes after the Norwood procedure: an analysis of the modified Blalock-Taussig shunt and right ventricle-to-pulmonary artery shunt surgical eras. *Circulation.* 2008;118(14):1410-1418.

10. Goldberg CS, Lu M, Sleeper LA, et al. Factors associated with neurodevelopment for children with single ventricle lesions. *J. Pediatr.* 2014;165(3):490-496 e498.

11. Fokstuen S, Arbenz U, Artan S, et al. 22q11.2 deletions in a series of patients with non-selective congenital heart defects: incidence, type of defects and parental origin. *Clinical Genetics.* 1998;53(1):63-69.

12. Goldmuntz E, Clark BJ, Mitchell LE, et al. Frequency of 22q11 deletions in patients with conotruncal defects. *J. Am. Coll. Cardiol.* 1998;32(2):492-498.

13. Cohen JD, Perlstein WM, Braver TS, et al. Temporal dynamics of brain activation during a working memory task. *Nature.* 1997;386:603-607.

14. Gaiano N, Fishell G. The role of notch in promoting glial and neural stem cell fates. *Annual Review of Neuroscience.* 2002;25:471-490.

15. Whitford KL, Dijkhuizen P, Polleux F, Ghosh A. Molecular control of cortical dendrite development. *Annual Review of Neuroscience.* 2002;25:127-149.

16. Pasini A, Wilkinson DG. Stabilizing the regionalisation of the developing vertebrate central nervous system. *Bioessays.* 2002;24(5):427-438.

17. Brennan K, Gardner P. Notching up another pathway. *Bioessays.* 2002;24(5):405-410.

18. Alagille D, Estrada A, Hadchouel M, Gautier M, Odievre M, Dommergues JP. Syndromic paucity of interlobular bile ducts (Alagille syndrome or arteriohepatic dysplasia): review of 80 cases. *J. Pediatr.* 1987;110(2):195-200.

19. Tartaglia M, Zampino G, Gelb BD. Noonan syndrome: clinical aspects and molecular pathogenesis. *Molecular syndromology.* 2010;1(1):2-26.

20. Zenker M. Clinical manifestations of mutations in RAS and related intracellular signal transduction factors. *Curr. Opin. Pediatr.* 2011;23(4):443-451.

21. Pierpont EI, Pierpont ME, Mendelsohn NJ, Roberts AE, Tworog-Dube E, Seidenberg MS. Genotype differences in cognitive functioning in Noonan syndrome. *Genes, brain, and behavior.* 2009;8(3):275-282.

22. Alfieri P, Cesarini L, Mallardi M, et al. Long term memory profile of disorders associated with dysregulation of the RAS-MAPK signaling cascade. *Behav. Genet.* 2011;41(3):423-429.

23. Alfieri P, Cesarini L, De Rose P, et al. Visual processing in Noonan syndrome: dorsal and ventral stream sensitivity. *Am. J. Med. Genet. A.* 2011;155A(10):2459-2464.

24. Lee DA, Portnoy S, Hill P, Gillberg C, Patton MA. Psychological profile of children with Noonan syndrome. *Dev. Med. Child Neurol.* 2005;47(1):35-38.

25. Wingbermuhle E, Egger JI, Verhoeven WM, van der Burgt I, Kessels RP. Affective functioning and social cognition in Noonan syndrome. *Psychol. Med.* 2012;42(2):419-426.

26. Costa RM, Silva AJ. Molecular and cellular mechanisms underlying the cognitive deficits associated with neurofibromatosis 1. *J. Child Neurol.* 2002;17(8):622-626; discussion 627-629, 646-651.

27. Pagani MR, Oishi K, Gelb BD, Zhong Y. The phosphatase SHP2 regulates the spacing effect for long-term memory induction. *Cell.* 2009;139(1):186-198.

28. Niklasson L, Rasmussen P, Oskarsdottir S, Gillberg C. Chromosome 22q11 deletion syndrome (CATCH 22): neuropsychiatric and neuropsychological aspects. *Dev.Med Child Neurol.* 2002;44(1):44-50.

29. Henry JC, van Amelsvoort T, Morris RG, Owen MJ, Murphy DG, Murphy KC. An investigation of the neuropsychological profile in adults with velo-cardio-facial syndrome (VCFS). *Neuropsychologia.* 2002;40(5):471-478.

30. Goldberg R, Motzkin B, Marion R, Scambler PJ, Shprintzen RJ. Velo-cardio-facial syndrome: a review of 120 patients. *Am J Med Genet.* 1993;45(3):313-319.

31. Kok LL, Solman RT. Velocardiofacial syndrome: learning difficulties and intervention. *J Med Genet.* 1995;32(8):612-618.

32. Swillen A, Vogels A, Devriendt K, Fryns JP. Chromosome 22q11 deletion syndrome: update and review of the clinical features, cognitive-behavioral spectrum, and psychiatric complications. *Am J Med Genet.* 2000;97(2):128-135.

33. Carey AS, Liang L, Edwards J, et al. Effect of copy number variants on outcomes for infants with single ventricle heart defects. *Circ. Cardiovasc. Genet.* 2013;6(5):444-451.

34. Bellinger DC, Watson CG, Rivkin MJ, et al. Neuropsychological Status and Structural Brain Imaging in Adolescents With Single Ventricle Who Underwent the Fontan Procedure. *Journal of the American Heart Association.* 2015;4(12).

35. Soemedi R, Topf A, Wilson IJ, et al. Phenotype-specific effect of chromosome 1q21.1 rearrangements and GJA5 duplications in 2436 congenital heart disease patients and 6760 controls. *Hum. Mol. Genet.* 2012;21(7):1513-1520.

36. Greenway SC, Pereira AC, Lin JC, et al. De novo copy number variants identify new genes and loci in isolated sporadic tetralogy of Fallot. *Nat. Genet.* 2009;41(8):931-935.

37. Fahed AC, Gelb BD, Seidman JG, Seidman CE. Genetics of congenital heart disease: the glass half empty. *Circ. Res.* 2013;112(4):707-720.

38. Lindner R, Puttagunta R, Di Giovanni S. Epigenetic regulation of axon outgrowth and regeneration in CNS injury: the first steps forward. *Neurotherapeutics.* 2013;10(4):771-781.

39. Feng J, Fan G. The role of DNA methylation in the central nervous system and neuropsychiatric disorders. *Int. Rev. Neurobiol.* 2009;89:67-84.

40. Grocott HP, Newman MF, El Moalem H, Bainbridge D, Butler A, Laskowitz DT. Apolipoprotein E genotype differentially influences the proinflammatory and anti-inflammatory response to cardiopulmonary bypass. *J. Thorac. Cardiovasc. Surg.* 2001;122(3):622-623.

41. Drabe N, Zund G, Grunenfelder J, et al. Genetic predisposition in patients undergoing cardiopulmonary bypass surgery is associated with an increase of inflammatory cytokines. *Eur.J Cardiothorac.Surg.* 2001;20(3):609-613.

42. Lelis RG, Krieger JE, Pereira AC, et al. Apolipoprotein E4 genotype increases the risk of postoperative cognitive dysfunction in patients undergoing coronary artery bypass graft surgery. *J Cardiovasc.Surg.(Torino).* 2006;47(4):451-456.

43. Tardiff B, Newman M, Saunders A, et al. Apolipoprotein E allele frequency in patients with cognitive deficits following cardiopulmonary bypass. *Circulation.* 1994;90 (Suppl 1):201.

44. Cox-Limpens KE, Gavilanes AW, Zimmermann LJ, Vles JS. Endogenous brain protection: what the cerebral transcriptome teaches us. *Brain Res.* 2014;1564:85-100.

45. Poulain FE, Sobel A. The microtubule network and neuronal morphogenesis: Dynamic and coordinated orchestration through multiple players. *Mol. Cell. Neurosci.* 2010;43(1):15-32.

46. Yang LJ, Ma DQ, Cui H. Proteomic analysis of immature rat pups brain in response to hypoxia and ischemia challenge. *Int. J. Clin. Exp. Pathol.* 2014;7(8):4645-4660.

47. Kitagawa K. Ischemic tolerance in the brain: endogenous adaptive machinery against ischemic stress. *J. Neurosci. Res.* 2012;90(5):1043-1054.

48. Miller SP, McQuillen PS, Hamrick S, et al. Abnormal brain development in newborns with congenital heart disease.[see comment]. *N. Engl. J. Med.* 2007;357(19):1928-1938.

49. Limperopoulos C, Tworetzky W, McElhinney DB, et al. Brain volume and metabolism in fetuses with congenital heart disease: evaluation with quantitative magnetic resonance imaging and spectroscopy. *Circulation.* 2010;121(1):26-33.

50. Licht DJ, Shera DM, Clancy RR, et al. Brain maturation is delayed in infants with complex congenital heart defects. *J. Thorac. Cardiovasc. Surg.* 2009;137(3):529-536; discussion 536-527.

51. Clouchoux C, Kudelski D, Gholipour A, et al. Quantitative in vivo MRI measurement of cortical development in the fetus. *Brain structure & function.* 2012;217(1):127-139.

52. Ortinau C, Beca J, Lambeth J, et al. Regional alterations in cerebral growth exist preoperatively in infants with congenital heart disease. *J. Thorac. Cardiovasc. Surg.* 2012;143(6):1264-1270.

53. Licht DJ, Wang J, Silvestre DW, et al. Preoperative cerebral blood flow is diminished in neonates with severe congenital heart defects. *J. Thorac. Cardiovasc. Surg.* 2004;128(6):841-849.

54. Andropoulos DB, Hunter JV, Nelson DP, et al. Brain immaturity is associated with brain injury before and after neonatal cardiac surgery with high-flow bypass and cerebral oxygenation monitoring. *J. Thorac. Cardiovasc. Surg.* 2010;139(3):543-556.

55. Beca J, Gunn JK, Coleman L, et al. New white matter brain injury after infant heart surgery is associated with diagnostic group and the use of circulatory arrest. *Circulation.* 2013;127(9):971-979.

56. Rollins CK, Watson CG, Asaro LA, et al. White Matter Microstructure and Cognition in Adolescents with Congenital Heart Disease. *J. Pediatr.* 2014.

57. Panigrahy A, Schmithorst VJ, Wisnowski JL, et al. Relationship of white matter network topology and cognitive outcome in adolescents with d-transposition of the great arteries. *NeuroImage. Clinical.* 2015;7:438-448.

58. Bellinger DC, Rivkin MJ, DeMaso D, et al. Adolescents with tetralogy of Fallot: neuropsychological assessment and structural brain imaging. *Cardiol. Young.* 2015;25(2):338-347.

59. Silversides CK, Lionel AC, Costain G, et al. Rare copy number variations in adults with tetralogy of Fallot implicate novel risk gene pathways. *PLoS genetics.* 2012;8(8):e1002843.

60. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* 2015;17(5):405-424.

61. Wilkinson GSR, G..J. Wide Range Achievement Test 4 Professional Manual. Lutz, FL: Psychological Assessment Resources; 2006.

62. Wechsler D. *Wechlser Intelligence Scale for Children - Fifth Edition.* 5th ed: Psychological Corporation, Inc.; 2014.

63. Wechsler D. Wechsler adult intelligence scale: Fourth edition. San Antonio, TX: Pearson, Inc.; 2008.

64. Wechsler D. Wechsler individual achievement test: Third edition. San Antonio, TX: Pearson, Inc; 2009.

65. Delis D, Kaplan E. *Delis-Kaplan Executive Function System.* San Antonio The Psychological Corporation; 2001.

66. Beery KE. The beery-buktenica developmental test of visual-motor integration. In: Steer RA, ed. San Antonio, TX: Pearson, Inc; 2010.

67. Sheslow DA, W. Wide range assessment of memory and learning: Second edition. Lutz, FL: Wide Range, Inc.; 2003.

68. Lord C, Rutter, M.; DiLavore, P.C.; Risi, S.; Gotham, K.; Bishop, S.L., et al. Autism diagnostic observation schedule: Second edition. Torrance, CA: Western Psychological Services; 2012.

69. Baron-Cohen SW, S; Scahill, V; Lawson, J; Spong, A. Reading the mind in the eyes test. Cambridge, UK: ARC Publications; 1997.

70. Sparrow SS, Cicchetti, D.V.; Saulnier, CA. Vineland adaptive behavior scales: Third edition. Bloomington, MN: Pearson, Inc.; 2016.

71. Constantino JN. Social responsiveness sale: Second edition. Torrance, CA: Western Psychological Services; 2012.

72. Gioia GAI, PK, Guy, S.C.; Kenworthy, L. Behavior rating inventory of executive function: Second edition. Lutz, FL: Psychological Assessment Resources, Inc.; 2015.

73. Reynolds CRK, R.W. Behavior assessment system for children: Third edition. . Bloomington, MN: Pearson, Inc.; 2015.

74. March JS. Multidimensional anxiety scale for children: Third edition. Tonawanda, NY: Multi-Health Systems, Inc.; 2013.

75. Beck AT. Beck anxiety inventory. In: Steer RA, ed. San Antonio, TX: The Psychological Corporation; 1993.

76. Kovacs M. Children's depression inventory: Second edition. North Tonawanda, NY: Multi-Health Systems, Inc.; 2011.

77. Uzark KC, Collins B, Kirkpatrick S, Spicer RL, Varni JW, Lamberti JJ. Measurement of quality of life in children with heart disease. *Circulation.* 2000;100:253.

78. Varni JW. The PedsQL: Measurement model for the pediatric quality of life inventory. *http://www.pedsql.orgpedsql3.html*. 1999.

79. Varni JW, Seid M, Knight TS, Uzark K, Szer IS. The PedsQL 4.0 Generic Core Scales: sensitivity, responsiveness, and impact on clinical decision-making. *J Behav.Med.* 2002;25(2):175-193.

80. *The PedsQL4.0: Reliability and validity of the Pediatric Quality of Life Inventory* [computer program]. Version 4.01999.

81. Burckhardt CS, Anderson KL. The Quality of Life Scale (QOLS): reliability, validity, and utilization. *Health and quality of life outcomes.* 2003;1:60.

82. Burckhardt CS, Anderson KL, Archenholtz B, Hagg O. The Flanagan Quality Of Life Scale: evidence of construct validity. *Health and quality of life outcomes.* 2003;1:59.

83. Moons P, Budts W, De Geest S. Critique on the conceptualisation of quality of life: a review and evaluation of different conceptual approaches. *Int. J. Nurs. Stud.* 2006;43(7):891-901.

84. Moons P, Van Deyk K, De Bleser L, et al. Quality of life and health status in adults with congenital heart disease: a direct comparison with healthy counterparts. *Eur. J. Cardiovasc. Prev. Rehabil.* 2006;13(3):407-413.