PEDIATRIC CARDIAC GENOMICS CONSORTIUM

CONGENITAL HEART DISEASE GENETIC NETWORK STUDY (CHD GENES)

Funded by the
National Heart, Lung, and Blood Institute,
NIH/DHHS

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Summary Table

Title	Congenital Heart Disease GEnetic NEtwork Study (CHD GENES)
Grant Number	UM1-HL098123, UM1-HL098147, U01-HL098153, UM1-HL098162, U01-HL098163, UM1-HL128711, UM1-HL128761, U01-HL131003
Study Objectives	Investigate relationships between genetic factors and phenotypic and clinical outcomes in patients with congenital heart defects (CHD).
Study Design	Multi-center, prospective observational cohort study of those with CHD and those without CHD who have a genotype strongly associated with CHD. Acquisition of both phenotypic data and source DNA from participant, parents, and family of interest.
Primary Aim	Through genomic analyses, genome-wide association studies, whole exome sequencing, and whole genome sequencing discover gene(s) responsible for CHD.
Secondary Aim	• Identification of mutations responsible for CHD in large numbers of participants. • Genotype/Phenotype correlation including long-term clinical follow-up of enrolled participants to determine how genetics influences the clinical outcome in CHD.
Accrual Objective	Accrual of thousands of participants with CHD, parents, and related family of interest and participants without CHD who have a genotype strongly associated with CHD.
Study Duration	10 years (longer, if funding extended)
Inclusion Criteria	• Affected Individuals with CHD or participants without CHD who have a genetic syndrome strongly associated with CHD (such as Trisomy or Chr22q deletion) • Age 0 – 99 years of age • Males and females • No ethnic or race restrictions • Sporadic and familial cases will be screened • Pregnant women who have a fetus with diagnosed CHD
Exclusion Criteria	• Isolated patent foramen ovale (except in confirmed cases of trisomy 21) • Isolated prematurity-associated patent ductus arteriosus • Lack of consent • Isolated pulmonary stenosis secondary to twin-twin transfusion syndrome

INVESTIGATOR SIGNATURE PAGE	
Protocol Number: U01-HL131003 *(Each site is to insert their unique grant number)*	Date: June 5, 2019 Version: 13.0
Site Principal Investigator	*(Please insert name)*
Title: Congenital Heart Disease GEnetic NEtwork Study (CHD GENES)	
Study Sponsor: National Heart, Lung and Blood Institute; Division of Cardiovascular Sciences	

INSTRUCTIONS: The Principal Investigator must print, sign, and date this form indicating he/she is in full agreement with the statement being signed. A copy should be kept in the study records and the original signature page sent to the ACC.

After signature, please return the original of this form by surface mail to:

Office for Clinical and Translational Research
Children's Hospital Medical Center
3333 Burnet Avenue, MLC 7004
Cincinnati, OH 45229

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 21 CFR Parts 45, 50, 56, and 312, and the International Conference on Harmonization (ICH) document "Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance." Further, I will conduct the study in keeping with local, legal, and regulatory requirements.

- **As the Site Principal Investigator, I agree to conduct [**Congenital Heart Disease GEnetic NEtwork Study (CHD GENES)**.**
- **I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the NHLBI.**

_____
Site Principal Investigator Name (Print)

_____ _____
Site Principal Investigator (Signature) **Date**

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A. Background

Congenital heart defects (CHD) are the most common major human birth malformation, affecting ~8 per 1,000 live births [1-4]. CHD are associated with significant morbidity and mortality, and are second only to infectious diseases in contributing to the infant mortality rate [1,2,5-7]. Mortality and morbidity rates in patients with CHD have decreased due to the advent of surgical and medical intervention; however, these individuals often require lifelong care, including repeat interventions, resulting in enormous burdens on families, and a large financial burden on the health care system [8]. Children with arrhythmias and cardiomyopathies similarly require extensive medical care throughout life.

Current understanding of the etiology of pediatric cardiovascular disorders is limited. The etiology of congenital heart disease is multifactorial and consists of a combination of environmental, teratogenic, and genetic causes [9-18]. Furthermore, both genetic and environmental factors have been proposed to act as disease modifiers, accounting for the wide variation in phenotypic expression and clinical outcomes of these disorders [11-18]. To date however, few specific genetic or environmental causative factors have been identified. It is estimated that ~11% of CHD patients have some form of chromosomal abnormality causing cardiac malformation [1]. This includes patients with aneuploidy (such as trisomy 13, 18, and 21, as well as Turner Syndrome) who have an elevated prevalence of CHD as compared to the general population [19,20]. Chromosomal structural abnormalities, such as monosomy 22q11, are a second significant genetic contributor to CHD [9]. More recently, disease-related single-gene defects have been identified in a small number of cases, including mutations in the transcription factors *NKX2.5*, *ZIC3*, *JAG1* and *NOTCH1* [11,21-25]. Despite these recent discoveries, the cause of the majority of cases of CHD remains largely unknown despite strong evidence of a significant genetic component [9].

The analysis of CHD genetics is complicated by the limited correlation between the genetic defect and specific CHD phenotype. In particular:

1) Similar cardiac defects may be caused by more than one genetic alteration (genetic heterogeneity) [26].
2) One gene may cause more than one CHD phenotype (variable expressivity) [26].
3) A person without CHD may carry a disease-causing gene mutation (reduced penetrance).

4) One or more genes may be causal for CHD associated with chromosomal alterations [9, 27].
5) Single gene defects can cause CHD with or without being recognized as a clinical syndrome [23].

Analyses are further complicated by the likelihood that phenotypic variability results from genetic modifiers, the interaction of genes and environment, and/or stochastic effects.

Due to both the genetic heterogeneity of CHD and the investigators' clinical inability to discriminate subtle anatomic variants, it is likely that within each cohort of patients with anatomically similar CHD, causative genes affecting distinct developmental pathways will be found. Clinical outcome may therefore at least in part depend upon which specific biological pathway is affected, and genotype variants may have distinct, clinically relevant physiologic phenotypes.

Although human genetic analyses are increasingly identifying mutations that cause congenital heart disease (CHD), the molecular events triggered by a CHD gene mutation that leads to the malformation remain poorly defined. For example, 60-70% of patients with deletion of chromosome 22q11.2 (DiGeorge syndrome) have CHD, most often tetralogy of Fallot. Similarly, CHD occurs in 40% of Down syndrome patients, most often atrioventricular septal defects that occur in over 40% of Down syndrome patients with CHD. By contrast, CHD occurs in only ~1% in the general population. While mutations such as chromosome 22q11.2 (DiGeorge syndrome) and Trisomy 21 (Down Syndrome) are the unequivocal causes of each syndrome, the reason why some individuals with these predisposing genotypes do not have CHD is unknown. To address this gap in knowledge, and to understand the absence of CHD in some subjects who have normal heart structure and function despite having genotypes that are strongly associated with CHD, we will also enroll subjects without CHD who have a genotype strongly associated with CHD (such as Trisomy, or Chr22q deletion).

Because of low recurrence rates, small sample size, and high phenotypic variability, traditional linkage analysis has met with limited success in defining the genetics of CHD. This study will use state-of-the-art genomic technology coupled with large-scale, multi-center participant recruitment to comprehensively elucidate the genetic causes of CHD. A large number of participants will be necessary to capture the highly variable phenotype and genotype of CHD. Developing a cardiac genetic registry with a DNA repository will aid in rapidly advancing the understanding, diagnosis, and treatment of congenital heart disease and serve as a foundation on

which the investigators plan to build a clinical research program in the molecular genetics of congenital heart disease.

To accomplish these goals, the consortia centers will work collaboratively as part of the National Heart, Lung, and Blood Institute-sponsored Pediatric Cardiac Genomics Consortium (PCGC).

B. Study Aims and Hypothesis

B.1 Study Aims

The Aims of this study are:

- *Gene discovery* of a comprehensive repertoire of genes responsible for CHD through genomic analyses including (but not limited to) copy-number variation, genome-wide association studies, whole exome sequencing, and whole genome sequencing,
- *Identification of mutations* responsible for CHD in large numbers of participants through sequencing of known CHD candidate genes,
- *Genotype/Phenotype correlation* of enrolled participants to determine how genetics influences the clinical outcome in CHD.

To accomplish this, the Consortium will develop and maintain a biorepository of specimens (DNA) and genetic data, along with detailed, phenotypic and clinical outcomes data in order to investigate relationships between genetic factors and phenotypic and clinical outcomes in congenital heart disease. Biospecimens and the derived genetic data will be shared with cardiovascular genetics researchers at participating Centers. Data will also be shared with investigators outside the Consortium consistent with NHLBI data-sharing policies. These biological samples will remain linked to detailed clinical data and will serve as a resource for long-term investigations into the genetic basis and clinical outcome of pediatric cardiovascular disorders beyond the current funding period. Through an increased understanding of the causes and modifiers of congenital heart disease, this initiative's long-term goal is to enhance early detection, treatment and prevention of cardiovascular disease in newborns, children, and adults.

B.2 Hypothesis

Genetic alterations contribute to both the etiology and the outcome of congenital heart disease.

To test this hypothesis, the investigators will recruit large numbers of subjects with sporadic as well as familial pediatric cardiovascular disorders (including adults with congenital heart disease), as well as subjects with normal heart structure and function who have a genotype strongly associated with CHD (such as Trisomy, or Chr22q deletion) from the participating Centers. Each consented participant and parents (and other family members when available) will donate biological specimens for banking and detailed phenotypic and clinical outcome data will be gathered. Analysis of these biospecimens will permit identification of genetic loci responsible for abnormal cardiovascular development. The identification of such genes and their modifiers will enhance understanding of both normal and abnormal cardiovascular development and will provide the foundation for investigations into the treatment and ultimately the prevention of cardiovascular anomalies. Furthermore, linking genetic information to clinical outcomes is expected to yield information that will facilitate risk stratification and improve treatment through individual genetically tailored regimens.

To address the gap in knowledge as to why some individuals with predisposing genotypes (e.g. trisomy, 22q11.2qdel or others) do not have CHD, we will derive induced pluripotent stem cells (iPSCs) from subjects with trisomy 21 (Down Syndrome) or 22q11.2qdel or others with and without CHD that we will differentiate into cardiomyocytes (iPSC-CMs), as part of the related IRB approved study (iPSC) iNDUCED PLURIPOTENT STEM CELLS. We plan to analyze cardiomyocyte gene expression between subjects with the same genotype (e.g., trisomy 21, 22q11.2qdel or others) who are discordant for CHD. We predict that the expression of genes not encoded in these mutated regions will be different. We expect that the identity of these modifiers and the pathways in which these participate may improve our understanding of the molecular mechanisms for normal and aberrant heart development.

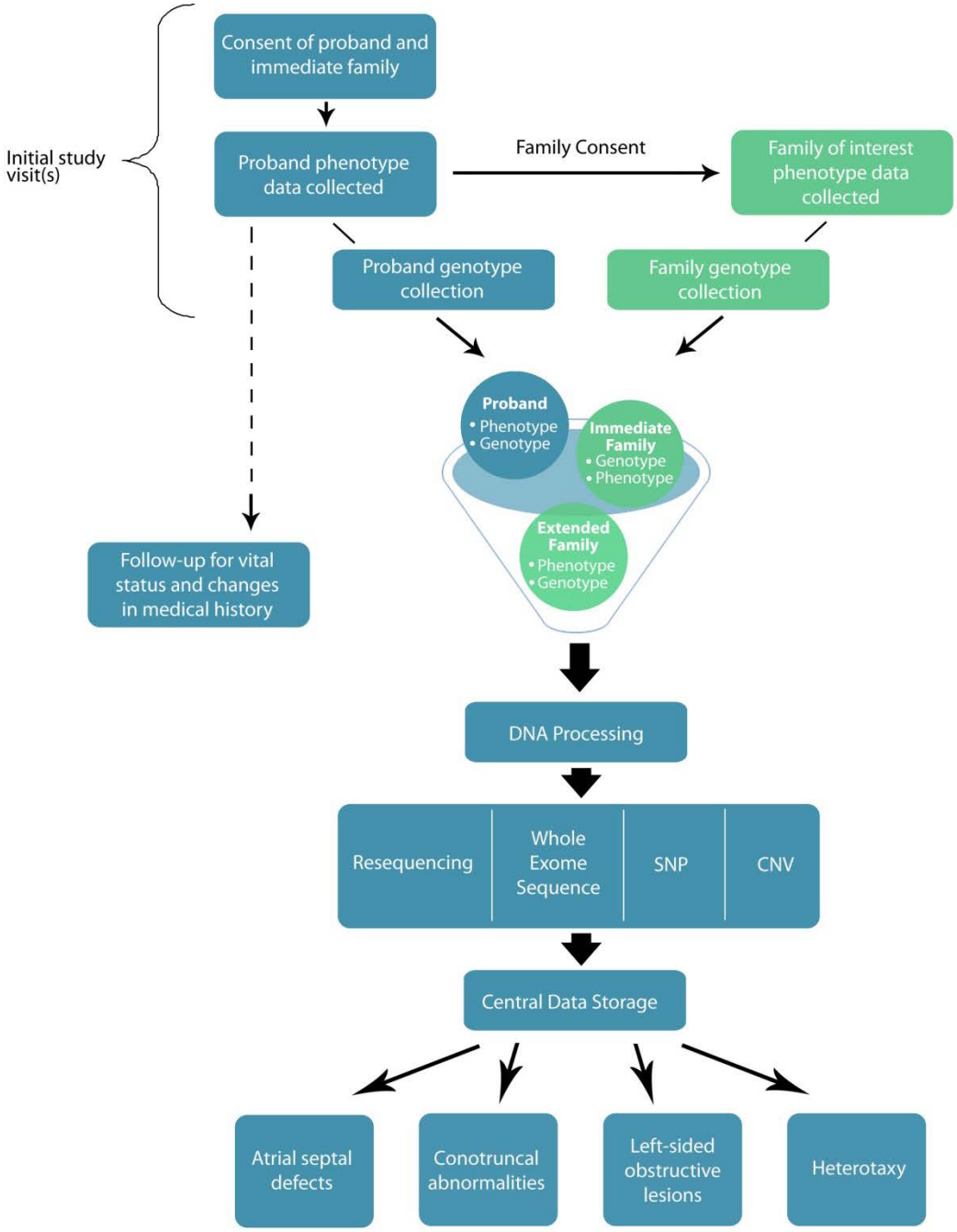
C. Study Design

C.1 Overview

This is a multi-center, prospective observational cohort study of those with CHD and those without CHD who have a genotype strongly associated with CHD. Participants will be recruited from the Pediatric Cardiac Genomics Consortium's centers, their satellite facilities, and additional

collaborating centers. Participants will undergo detailed phenotyping and biological specimens will be obtained for genetic analyses. Genomic technologies will be used to identify common genetic causes of CHD and genetic modifiers of clinical outcome.

Figure 1



C.2 Description of the Consortium

The National Heart, Lung, and Blood Institute-supported Pediatric Cardiac Genomics Consortium (PCGC) currently consists of 5 main centers located in Boston, New Haven, New York, Salt Lake City and San Francisco/Stanford. 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 participants 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). Clinical data will be entered into the Medidata Rave clinical data management system and will be stored within Medidata's clinical cloud computing solution. All data extracted from Rave for reporting or analysis will be stored at CHMC's secured servers. In some instances, with prior approval from the study Steering Committee, some data to be used for additional analyses may be entered and stored in a study specific REDCap or other appropriate database. Biospecimens will be stored at a central biorepository selected for appropriate expertise and operating standards. Extracted DNA will be sent to core laboratories for genotyping and sequencing. The resulting genetic data will be stored at a data repository, chosen by and agreed upon by the PCGC Steering Committee. Genetic data will be linked to the clinical data to allow for detailed correlation between genotype, phenotype, and outcome. The combined data sets will be made available to all investigators through a Consortium-wide request and access process.

The Consortium will also develop resources that will benefit the wider congenital heart disease research community. The de-identified clinical and genetic data 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.

C.3 Participant Recruitment and Screening

Participants with CHD or subjects with normal heart structure and function who have a genotype strongly associated with CHD (such as Trisomy or Chr22q deletion) will be recruited from prenatal centers, pediatric cardiology and adult congenital heart centers. Patients with genetic syndromes such as Trisomy or Chr22q deletion will have been diagnosed and well categorized. Participants with isolated prematurity-associated patent ductus arteriosus, isolated patent foramen ovale or isolated pulmonary stenosis secondary to twin-twin transfusion syndrome will be excluded from the

study. There will be no restrictions to participation based on age, sex or ethnicity. Existing published and preliminary data [28,29] suggests there will be a slight preponderance of male to female candidates for the study. Whenever possible, the study will recruit 'trios' of participants-- children, mothers and fathers-- as well as extended family members when appropriate and feasible.

Recruitment strategies will include publicizing the study on the Bench to Bassinet website and individual site websites, through brochures and other printed materials, formal presentations by study investigators to their divisions or departments, individual conversations between investigators and patients, and other standard recruitment tools.

C.4 Genetic Analyses

The study will use a variety of techniques to identify genetic characteristics associated with the specific CHDs of interest. Microarray analysis will be implemented to detect single nucleotide polymorphisms (SNPs) and copy number variants (CNVs). Genome wide association studies (GWAS) and evaluation of CNVs will seek to identify genetic variants associated with major categories of CHD (e.g., conotruncal defects, left-sided obstructive lesions) and, as numbers allow, within potentially more homogenous subsets (e.g., tetralogy of Fallot, hypoplastic left heart syndrome).

Whole exome sequencing will generate sequence data for all protein coding regions and whole genome sequencing will generate sequence data for the entire genome. Compared to publically available sequence data, rare variants with potential association to disease will be identified. Based upon data from GWAS, CNV analysis, whole exome sequencing, and whole genome sequencing, candidate genes or loci will be identified. Using capture based techniques and high throughput sequencing, subgenomic libraries consisting of these candidates will be resequenced in large numbers of patients. Such an effort will provide data that supports or refutes their causality in CHD and will define the spectrum of mutation type and location associated with CHD.

New genetic tools are developed frequently, so other genetic techniques that become available during the study period will be evaluated and used as appropriate to achieve the study aims.

To identify genetic variants that are associated with a specific CHD or clinical outcome, several analytic approaches will be undertaken. Two examples include a familial approach using case-

parent triad design and a case-control approach. The case-parent triad design will identify maternal genetic effects and de novo changes by comparing analyses of the proband's DNA with that of the proband's parents. In the case-control design, frequencies of identified genetic variants will be compared between cases and controls. As the technology and science advance, newer analytic approaches are likely to be added.

C.5 Return of Genetic Testing Results

Those enrolled in the study prior to the protocol modification to share genetic testing results will be contacted by mail to ascertain their preference for return of results. The letter will explain the policy and process for return of results. Participants will be able to opt-in or opt-out for results and choose the kind of results they would like: pathogenic or likely pathogenic CHD genetic results and/or actionable, incidental results. Sites will follow their local IRB determination regarding whether the signed opt in response letter will serve as consent or if reconsent is required. A genetic counselor will be available to answer general questions. For those who choose to receive results, confirmation genetic testing will be arranged with a CLIA certified lab. by a central genetic counselor. The cost for the confirmatory testing will be paid by the study. Each site will have a designated clinician who will receive the confirmation results and, if needed, will arrange for a clinical visit after disclosure of the results. For those who opt not to receive results, we may ask the parent/participant to complete a short optional survey regarding the reasons for not wanting to receive results. The survey will be approved by the IRB prior to use.

Parents of deceased participants will be also be contacted using a differently worded letter.

We will report results for genes (CNVs and SNVs) that are related to CHD and/or incidental findings, based on the current ACMG Secondary Findings Gene List. A committee of study PIs will meet periodically to review specific genes and variants and designate those to be returned that meet criteria to be classified as pathogenic or likely pathogenic. An established CHD gene would require segregation (de novos) in at least two families and (ideally) from two independent scientific groups. This will be an on-going process for the duration of the study as scientific knowledge changes over time.

C.6 Study Timeline

This is a long-term study, defined by the period of funding (and any extensions granted). At a future point, when funding for the PCGC has ended, the biospecimens will be deposited and maintained in a de-identified manner in an appropriate NIH-funded biorepository to be used as a future resource for the congenital heart disease research community. Similarly, information from DNA analyses and the clinical data sets will be placed into a central data repository, such as the National Center for Biotechnology Information (NCBI) repository.

C.7 Stopping Rules

There are no planned stopping points, other than elective withdrawal from the study or cessation of active investigation.

D. Collection of Samples and Data

D.1 Sample Collection

The following samples will be collected.

D.1.1 Proband samples

1. A sample of whole blood will be obtained from the proband from an indwelling line or in conjunction with a clinically warranted venipuncture whenever possible. Otherwise, trained technicians will perform phlebotomy to obtain a sample of blood from the willing child/family. A sample will be acquired only if drawing said sample will not in any way harm the participant's clinical status or precipitate the need for a blood transfusion. The blood sample will be used to extract DNA and may be used to make a cell pellet and/or immortalized lymphoblastoid cell line to allow for a renewable resource. Approximately 3-10 ml of blood will be drawn from an infant while 10-15 ml will be drawn from anyone older than one year (the volume will not exceed that permitted by weight and will not exacerbate any existing medical conditions). If blood cannot be drawn or the enrollee is unwilling, then a saliva sample (age permitting) will be obtained. In the event that the proband dies following consent, an autopsy specimen will be obtained according to local institutional guidelines. We may also request permission to

use blood samples collected for clinical procedures that would otherwise be discarded.

2. For fetal cases that are terminated or spontaneously abort, cardiac and other tissue will be collected from the products of conception. We will ask permission to use excess/discarded samples taken during medically indicated procedures during pregnancy including, but not limited to, excess/ discarded DNA from amniocentesis, chorionic villus samples, or percutaneous umbilical blood sampling. [OPTIONAL, according to local institutional procedures/guidelines]
3. If the proband is undergoing a surgical procedure, then tissue that during the course of surgery would have been discarded (such as discarded atrial tissue resulting from cannulation for cardiopulmonary bypass) may be acquired for research purposes. [OPTIONAL, according to local institutional procedures/guidelines]
4. The study will request and obtain consent for a tissue sample from probands who have an atrial septal defect and are undergoing an open procedure for repair. A small atrial septal biopsy sample (less than or equal to 1/8 inch X 1/8 inch) will be sufficient for extracting DNA. A tissue sample will only be taken if doing so presents no added risk or complication to the successful closure of the atrial septal defect. [OPTIONAL, according to local institutional procedures/guidelines]

D.1.2 Family samples

1. Up to 15 ml of whole blood will be drawn from consenting immediate and/or extended family. Blood draws will be done on site or a packet containing instructions for off-site phlebotomy will be sent. If a grandparent, sibling, or distant relative is unwilling or unable to undergo a venipuncture, then a sample of saliva will be acquired. In some instances, samples will be collected by mail. In this event, we will provide a self-addressed stamped return kit for the sputum or blood sample which will be in full compliance with standards for shipping biological materials.
2. For fetal cases, immediate and/or extended family, that are terminated or spontaneously abort, cardiac and other tissue will be collected from the products of conception. We will ask permission to use excess/discarded samples taken during medically indicated procedures during pregnancy including, but not limited

to, excess/ discarded DNA from amniocentesis, chorionic villus samples, or percutaneous umbilical blood sampling from prenatal cases. [OPTIONAL, according to local institutional procedures/guidelines]

3. If an affected relative is undergoing a surgical procedure, then tissue that during the course of surgery would have been discarded (such as discarded atrial tissue resulting from cannulation for cardiopulmonary bypass) may be acquired for research purposes. [OPTIONAL, according to local institutional procedures/guidelines]
4. The study will request and obtain consent for a tissue sample from affected relatives who have an atrial septal defect and are undergoing an open procedure for repair. A small atrial septal biopsy sample (less than or equal to 1/8 inch X 1/8 inch) will be sufficient for extracting DNA. A tissue sample will only be taken if doing so presents no added risk or complication to the successful closure of the atrial septal defect. [OPTIONAL, according to local institutional procedures/guidelines]

D.2 Clinical and Phenotypic Data

Baseline clinical and phenotypic data will be collected as outlined in detail below. In addition, participants will be asked to agree to future contact for the purposes of obtaining additional information.

D.2.1 Proband clinical data collection

Proband data will include:

1. Cardiac anatomy
2. Additional congenital anomalies or diseases
3. Demographics
4. Medical history, including pregnancy and birth history of the affected proband, information on selected medical conditions and common pregnancy exposures, neurodevelopmental history, and medications
5. Cardiac history, including surgeries and procedures, hospitalizations, complications, and co-morbidities
6. Family history of CHD and other conditions
7. Pedigrees
8. Dysmorphic features

9. Participants will be recontacted during the study period (not more frequently than annually) to document vital status and any changes in medical or cardiac history.

D.2.2 Phenotypic data collection techniques

Data collected on the proband will be compiled by the following methods:

1. Interviews with the proband and parents
2. Review of all medical records, including inpatient and outpatients records, images and reports from echocardiograms, electrocardiograms, cardiac MRIs, cardiac catheterizations, and operative reports
3. Request for copies of outside records and collection of available pertinent data
4. Proband photograph [OPTIONAL, according to local institutional procedures/guidelines]
5. Physical examination reports by a geneticist, if available

D.3 Family Data Collection

D.3.1 Parents data collection

Data collected on the proband's consenting parents may include:

1. Cardiac diagnoses (if any) and cardiac history
2. Relevant medical history including congenital anomalies
3. Demographics
4. An ECG and/or echocardiogram may be obtained, if the parent has a family history of interest to the research.

D.3.2 Extended family data collection

Data collected on the consenting extended family may include:

1. Cardiac history and diagnoses
2. Relevant medical history including congenital anomalies
3. Demographics
4. An ECG and/or echocardiogram may be obtained, if the family member has a family history of interest to the research.

D.3.3 Clinical data collection techniques

1. Interviews and survey
2. Medical record review

E. Study Subjects

While all patients with pediatric cardiovascular disease and adults with congenital heart disease are of interest, the study will initially focus on four CHD anatomic classifications:

- Atrial septal defects
- Conotruncal abnormalities
- Left-sided obstructive lesions
- Heterotaxy

Additionally, subjects without CHD who have a genetic syndrome strongly associated with CHD (such as Trisomy or Chr22q deletion) will also be recruited.

E.1 Inclusion Criteria

- Affected Individuals with CHD or subjects without CHD who have a genetic syndrome strongly associated with CHD (such as Trisomy or Chr22q deletion)
- Age 0 – 99 years of age
- Males and females
- No ethnic or race restrictions
- Sporadic and familial cases will be screened
- Pregnant women who have a fetus with diagnosed CHD

E.2 Exclusion Criteria

Patients who meet any of the following criteria will be excluded from the study:

- Isolated patent foramen ovale (except in confirmed cases of trisomy 21)
- Isolated prematurity-associated patent ductus arteriosus
- Lack of consent
- Isolated pulmonary stenosis secondary to twin-twin transfusion syndrome

E.3 Source of Subjects

Patients will be recruited from participating facilities' outpatient clinic and inpatient services and wards that service pediatric cardiology patients, adult cardiology patients, and women (and their biologic partners) pregnant with fetuses diagnosed with CHD. Sources of potential participants other than the five main centers and their auxiliary sites include interested investigators, health care facilities and organizations outside of the PCGC, and self referral.

Probands will be identified through mechanisms including, but not limited to:

- Daily screening of in-patient admission lists and out-patient clinic schedules
- Health care provider referrals
- Screening of medical databases
- Screening rosters of prenatal patients
- Clinician or collaborator referrals from outside institutions
- Self referral.

At each Center, an ascertainment (screening) log will include minimal data on all screened patients who are known to have an inclusion diagnosis and are approached for consent.
The data will include:

- Date of ascertainment
- Gender
- Age
- Race/ethnicity
- Cardiac diagnosis

De-identified data from the screening logs may be used for general study reporting related to demographic reports and report to the OSMB.

E.4 Subject Availability

The five main participating centers and their auxiliary sites have a current roster total of over 20,000 patients with CHD that includes one of the eligible CHD diagnoses. In addition, over 2500 new patients with these diagnoses are estimated to enter the systems of these centers annually. Based on previous institutional experience, we assume that 65-75% of these patients will consent to participate in this study. With these numbers, the proposed study has high feasibility to achieve its aims.

E.5 Subject Recruitment and Consent

At each clinical center, the Principal Investigator or other study staff will review cases and conduct subject recruitment. In compliance with local procedural requirements and good clinical practice, the parent(s) or legal guardian of potential study participants or the potential study participant (if 18 years or older) will be approached. The initial approach may be as early as during the prenatal period. To capture known patients, an introductory letter and/or brochure describing the study may be sent to families before a scheduled cardiology encounter through an out-patient visit, cardiac

catheterization procedure, etc. A website will publicize the Pediatric Cardiac Genomics Consortium and provide contact information for patients interested in the study.

Patients or parents of patients with CHD or a genotype strongly associated with CHD(such as Trisomy or Chr22q deletion) will be approached for the study. The study will be presented and the consenting process begun. If there is a family history of CHD, the investigators will attempt to recruit other family members into the study. Those families of interest will be approached only after the parent of the minor proband or the adult proband has given approval. Recruitment of other family members will comply with local standard procedures and good clinical practice. These procedures may include asking the parents of the proband if they would allow the study team to contact the family member or asking the parents to have the family member contact the study team directly. Approaching extended family may involve sending a letter, sending the study brochure, or a study staff telephone call. The proband's immediate family will be made aware that in contacting extended family, the proband's diagnosis of CHD will most likely be revealed. However, no other information about the proband will be discussed with extended family. Immediate and extended family members will be informed that the study team will **not** reveal each person's decision about participation, medical history, or) genetic results. Confidentiality rules restrict sharing of study data. However, each family member will be informed that the family pedigree may be published in scientific journals and reports. Published pedigrees do not list names but would detail clinical and genetic findings.

Consent and assent (according to local institutional guidelines when appropriate) will be obtained by the PI or designee or the Research Coordinator. The consenting process will comply with good clinical practices and local standard procedures. Whenever possible, written consent will be obtained; however, in some centers phone consent will be allowed. The study will be explained to potential subjects using age-appropriate language and easily understood lay terms. To obtain consent for subjects who are minors, the program will be discussed in detail with at least one parent or guardian able to consent for the minor.

Whenever a developmentally appropriate older child (older than 8 years or as permitted locally) or adolescent is the potential study subject, parental permission from a parent or guardian and assent from the subject will be obtained. Participants who reach the age of consent during the study period, as determined by local Institutional Review Board (IRB) policies, will be re-consented with the adult informed consent form upon recontact.

All sites will use a study consent and assent that is IRB/Ethics Committee (EC) approved. When telephone consenting is used, the IRB/EC procedures will be followed and appropriate documentation will be captured. After consenting, a copy of the fully signed consent will be given back to participants for their records.

All consents will include language requesting permission to contact the participant at a time in the future to collect additional data that may be useful in the research.

E.6 Voluntary Subject Withdrawal

If a subject should wish to withdraw from the study, data and samples collected prior to the withdraw will remain in the database. The subject might wish to have all research being conducted on his/her sample stopped. These requests will result in the sample being destroyed, the subject's name removed from the site- maintained enrollment log, and all data de-identified in the database. Clinical and genetic data already obtained would remain in the databases. If a subject wishes to have all research being conducted on their samples stopped and their samples destroyed, the participant will be asked to provide this request in writing to the Investigator.

Participants who are unable to give the requested volume of blood will be kept in the study database. All data collected will remain in the database, except as noted above.

E.7 Early Termination of a Subject's Participation

The study PI may choose to terminate a subject's participation if it is felt to be in his/her best interest for clinical or other reasons.

E.8 Study Termination

The study may be terminated only by the Sponsor or other related oversight organizations.

F. Statistical and Analytical Approaches

Various state-of-the-art technologies will be used to generate data on genetic variation that may be related to CHDs. As the technologies and methods for assessing genetic variation are rapidly

evolving, it is likely that the most appropriate statistical approaches for analyzing the data will also change with time. However, examples of current approaches are discussed below.

Public databases will be used to provide information on pediatric controls. Depending on the available data and the specific hypothesis being evaluated, statistical analyses will be based on data from either case-parent triads or cases and controls.

In general, analyses will be conducted within major categories of CHD (e.g., conotruncal defects, left-sided lesions) and, as numbers allow, within potentially more homogenous subsets (e.g., tetralogy of Fallot). Additional subgroups defined by clinical outcome may also be analyzed. Analyses of subsets of cases defined by race/ethnicity may also be conducted to ensure that findings based on the full case group are not biased due to population stratification (i.e., systemic differences in the ancestry of cases and controls that are unrelated to disease status). As illustrated by the sample size calculations presented below, very large sample sizes will be required to detect genetic variants that have only a modest impact on disease risk, whereas much smaller sample sizes will be required to detect genes that have a more dramatic impact.

F.1 Genome-Wide Association Studies (GWAS)

Genotype data from GWAS can be analyzed using data from cases and controls or from case-parent triads. Per case, the power to detect associations using case-parent and case-control approaches is similar. Although both approaches have strengths as well as limitations, the triad design has two major advantages over the case-control design:

- Genotype data from case-parent triads can be used to evaluate both maternal and inherited genetic effects, whereas case-control data can be used to evaluate only inherited genetic effects.
- Analyses of the inherited genotype using triad data are immune to bias resulting from population substructure, whereas this is a significant concern in the case-control setting.

Given these advantages, family-based analyses of GWAS data are likely to be preferred over case-control approaches. Hence, power calculations are presented based on analyses of data generated from case-parent triads. However, as noted above, the power of a case-control study with an equivalent number of controls will be comparable to that of a case-parent study.

Sample size and Power: Given the enormous number of statistical tests performed in any GWAS, it is necessary to set the p-value for declaring statistical significance quite low. It is generally agreed

that for GWAS, the threshold should be on the order of 10^{-8}. To illustrate the need for large sample sizes for these investigations, minimum sample size requirements to achieve adequate power (>80%) to detect genotype relative risks ranging from 1.2-2.0 under an additive model of inheritance are provided in Table 1. These estimates were obtained using the computer program Quanto (version 1.2.3, Gauderman and Morrison, http://hydra/usc.edu/gxe), and apply to both maternal and inherited genotypes.

Table 1: Minimum sample size requirements for GWAS using $p<10^{-8}$ as the threshold for significance.

Genotype Relative Risk	**Frequency of High Risk Allele**				
	0.10	**0.20**	**0.30**	**0.40**	**0.50**
1.2	13,444	7,711	5,988	5,338	5,219
1.4	3,726	2,177	1,720	1,559	1,548
1.6	1,821	1,082	869	799	804
1.8	1,118	676	550	512	522
2.0	777	477	393	371	381

F.2 Copy Number Variant (CNV) Analyses

Evaluation of CNVs will be conducted using a case-control approach, with controls drawn from publically available databases. In general, analyses of CNVs will be based on comparison of the frequency of an identified CNV or group of CNVs in cases and controls. These comparisons will be made by chi-square analysis or Fisher's exact test. Logistic regression analysis will be used to estimate the relative risks associated with groups of CNVs. Groups of CNVs that will be considered include:

- All CNVs
- All CNVs within a given gene or region
- All CNVs within a given pathway or gene family
- Individual CNVs

Where parental data is available, CNVs will be classified as inherited or *de novo*.

Sample size and Power: Given the potentially large number of statistical tests that will be performed to assess CNVs, it will be necessary to set the p-value for declaring statistical significance quite low. However, unlike GWAS studies, the number of tests to be performed cannot be specified *a*

priori. For illustrative purposes, sample size requirements are presented for a p-value threshold of $5x10^{-5}$ (accounting for 1,000 comparisons). However, this threshold will be reconsidered when CNV data are available, and can be used to estimate the number of comparisons that will actually be made. Minimum sample size requirements to achieve adequate power (>80%) to detect genotype relative risks ranging from 1.2-2.0 for common variants (minor allele frequency (MAF)≥0.10) and, from 1.2-5.0 for rare variants (MAF=0.001 and 0.01), under an additive model of inheritance, assuming a 1:4 case-control ratio are provided in Table 2. These estimates were obtained using the computer program Quanto (version 1.2.3, Gauderman and Morrison, http://hydra/usc.edu/gxe).

Table 2: Minimum sample size requirements for CNV analyses using $p<5x10^{-5}$ as the threshold for significance.

Genotype Relative Risk	**Frequency of High Risk Allele**						
	0.001	**0.01**	**0.10**	**0.20**	**0.30**	**0.40**	**0.50**
1.2	404,600	40,900	4,600	2,600	2,100	1,800	1,800
1.4	108,200	11,000	1,200	740	590	540	540
1.6	51,200	5,200	610	370	280	280	280
1.8	30,500	3,100	310	230	190	180	180
2.0	20,600	2,100	355	160	130	130	130
3.0	6,400	660					
4.0	3,400	350					
5.0	2,200	230					

F.3 Candidate Gene, Whole-Exome, And Whole-Genome Sequence Analyses

Evaluation of sequence variants will be based upon comparison with publically available sequence data. In general, these analyses will focus on identification of rare variants and, in particular, variants that are identified in as few as one case and are unobserved among a large number of control sequences. Power calculations are not provided for the proposed sequence analyses, since the identification of rare variants found only in cases is of interest regardless of the associated p-value.

F.3.1 Analysis and annotation of the exome sequencing data

We will use a mix of publicly available and privately developed tools. First, we will map sequence reads to the reference human genome (hg19) using the Maq program. Reads outside of the

targeted sequences will be discarded and statistics on coverage will be collected from the remaining reads. Once the likely mapping locations are identified, the reads will be aligned allowing gaps to the reference genome using BWA and comparable software packages in order to identify indels. Potential variants will be called using SAMtools, as well as custom tools already developed in-house at several of the participating centers. We will identify known variants from dbSNP, publicly available genomes and exomes, including the 1000 Genomes Project, and exomes previously sequenced at the participating centers.

After eliminating known SNPs, the remainder of the sequence variants will be filtered with these rules [30]:

a. Calls with a Phred-like quality score of less than 45 will be excluded

b. Reads supporting the major and minors alleles should have different start and end points

c. Total reads at variant bases should be > 10

For remaining sequence variants lying within exons, their predicted effect on the encoded protein will be determined and deleterious effects predicted with Polyphen or predicted premature termination of the protein. The degree of evolutionary conservation will be assessed for nonsynonymous changes using phyloP scores calculated from multiple alignments of >40 vertebrate species, which are available from the University of California Santa Cruz genome browser (http://genome.ucsc.edu) as well as comparison with invertebrate orthologues in C. elegans and D. melanogaster, which will be acquired from InParanoid (http://inparanoid.sbc.su.se). Gene expression patterns will be examined using the expression atlas at the Genomics Institute of the Novartis Research Foundation as well as data obtained within participating centers.

Rare exonic variants in genes expressed in cardiac tissues that alter conserved residues and are predicted to have significant impact on protein function will be studied further. When variants are demonstrated to be absent from ethnically matched control samples, segregation analyses within the family will be assessed and Lod scores determined. In addition, sequence analyses of that gene in unrelated affected samples will be performed, and newly discovered variants will be examined as above. The significance of rare variants found only in affected cases will be assessed by p-values.

G. DATA MANAGEMENT

An electronic clinical data management system (CDMS) (Medidata Rave clinical data management system) will be used for the study that is designed to support reliable and secure data entry for clinical research purposes. The system also provides seamless integration of electronic Case Report Forms (eCRF), implementation of protocol amendments, and SAS and XML study data exports. In some instances, with prior approval from the study Steering Committee, some data to be used for additional analyses may be entered and stored in a study specific REDCap or other appropriate database.

G.1 Data Entry

Medidata Rave - Data can be entered directly into Medidata Rave from multiple study sites via a fully validated and 21 CFR Part 11 compliant, secure Web application and stored centrally. 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.

REDCap (or other appropriate database) – Data entered into any other appropriate database 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.

G.2 Data Validation and Monitoring

Integrated into the Medidata Rave CDMS 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.

Data entered into REDCap or other appropriate database will be manually checked by the DCC for data entry errors or questionable values and queries manually issued to the sites to clarify discrepancies.

G.3 Specimen Tracking

Specimen tracking is started from the time of receipt at the site, through shipment to the central biorepository, during handling at the biorepository, and through shipment to various core laboratories. The individual sites and the biorepository will use the DCC's electronic clinical data management system (CDMS) for all specimen tracking including: acquisition, processing, storage, QA/QC, withdrawals, and distribution for utilization.

Each specimen will be labeled with a bar-coded label identified by a number consisting of a 3-digit site number followed by a unique specimen number that is different from the subject's unique study ID number. The master list linking the barcode numbers to the subject study ID numbers will be maintained under password protection in the CDMS at the DCC. This blinding code system will maintain the confidentiality of the specimens yet allowing linkage of the specimens with phenotypic data for analyses by the PCGC investigators.

G.4 Data Security and Integrity

G.4.1 Phenotype security and integrity

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.

G.4.2 Genotype data security and integrity

All genotype data will be transferred to a central data repository, chosen by and agreed upon by the PCGC Steering Committee.. All genetic data arising from collected specimens will have standard

safeguards in place to ensure participant confidentiality as per good clinical practices, state and local HIPAA rules and IRB/EC guidance.

- The data will be stored in password protected, secure computers and databases at the core laboratories and at the participating consortia centers.
- The data will be identified by study/biospecimen ID number only. There will be no use of patient identifiers.
- Access to the databases will be restricted to the PCGC investigators. Authorizations will be maintained on a study roster. Access will be granted to persons based upon the consortia's guidelines.
- Security, archiving, and backup systems will be in place to ensure data security.
- Access to linked data will be through an approval process at the steering committee level.

H. Quality Assurance and Quality Control Procedures

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

The key QC/QA activities are:

- Development of a study Manual of Operations;
- Clearly formatted and carefully constructed Data 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 DMS training and certification of Center data managers;
- 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 to Centers as required with pre-specified goals.

I. Human Subjects Considerations

I.1 Potential risks

I.1.1 Psychological distress

I.1.1.1 Genetic testing: There is a chance that participation in this study 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 may be passed on to their children. There is a possibility that the testing may detect instances of non-paternity. In such cases, this information will not be shared with the participant, but will be entered into the database.

I.1.1.2 Participating in a repository: There is a chance that participation in a repository may cause psychological stress or long-term anxiety. For this study, all data or specimens entered into a repository will be de-identified.

I.1.2 Blood draw

Minor temporary discomfort may be associated with the removal of blood by venipuncture. There is a risk of bruising and a very small amount of bleeding associated with the blood drawing. There is also a very small risk of infection at the site. Whenever possible, blood samples will be gathered when the participant is scheduled for routine blood testing or procedures.

I.1.3 Saliva collection

There are no known risks to collecting saliva.

I.1.4 Tissue collection [OPTIONAL, according to local institutional guidelines]

For individuals undergoing open-heart surgery for closure of a secundum ASD, subjects or their parents/guardians will be asked to consent to a study septal biopsy during the procedure. The risk of biopsying the atrial septum during open heart surgery on the septum is minimal, adding a trivial amount of time to the procedure and not interfering with the ability of the surgeon to achieve complete closure of the ASD.

There is no risk associated with the collection of otherwise-discarded surgical tissues taken during a medically indicated procedure for children.

For pregnant women with a spontaneous miscarriage, fetal demise, or elective termination of pregnancy, tissue from the fetus with CHD may be collected and may include cardiac and other non-cardiac tissues. There is no additional risk to the woman to collect these tissues.

I.1.5 Excess or discarded samples [OPTIONAL, according to local institutional guidelines]

For subjects enrolled prenatally, we will ask permission to collect left over DNA or excess/discarded samples from the laboratory if an amniocentesis, chorionic villus sampling, or percutaneous blood sampling was performed. There is no risk associated with the collection of otherwise discarded samples taken during a medically indicated procedure during pregnancy.

I.1.6 Clinical data collection

There are no significant procedural risks associated with physical examination or testing by ECG or echocardiography. These evaluations, however, may rarely reveal previously unknown cardiac abnormalities.

I.2 Confidentiality, Protection against Risks

Investigators will take all reasonable measures to protect the confidentiality of subjects and their families.

- Investigators will arrange for counseling if anxious feelings arise in the proband or family at any time during the study.
- The results of the genetic tests performed for research purposes will not be placed in the medical record.
- Non-paternity information will be kept in the strictest confidence and will not be divulged to research subjects or their families.
- Each child and parent is assigned a subject identification number (SID). All interview and clinical research data are stripped of identifiers and labeled with the study number. The enrollment log with participant identifiers will be maintained at each site in a secured, locked location available only to the study staff.
- The study will follow good clinical practices at all times. Databases will be secured as previously discussed and samples will be secured in locked secure study freezers.
- All participating laboratories and analysis facilities will follow good clinical practices maintaining data integrity and participant confidentiality.
- Samples for DNA will be stripped of the study SID at the laboratory and assigned specimen numbers that are linked to the SID.
- 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 Data Coordinating Center (DCC) and NIH/NHLBI to ensure study safety and quality control.

- The subject's name and any other identifying information will not appear in any presentation or publication resulting from this study.
- The study team will contact extended family members for recruitment according to local guidelines. As per local requirements, contact will be made with those individuals who have expressed a willingness to at least learn about the research study. Other family members will not be informed of who is and is not participating. The subject will also be warned not to disclose their participation in order to protect their own privacy.
- Results of testing on biological specimens will be shared with the participants who consent to receive them, after confirmation in a clinical lab (CLIA certified lab). Only pathogenic or likely pathogenic CHD genetic results and/or actionable, incidental results, will be reported to participants. If indicated, a clinical referral will be made for long term care.
-
- 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 findings will result from this research effort. If findings are detected, it may be years before any utility of these findings are realized.
- 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's database of genotypes and phenotypes (dbGaP) 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 determined, research. The dbGaP resource 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 summarized data regarding the entire collection of study subjects that are not linked to medical or personal information. The controlled access database includes de-identified medical information and genomic data that are made available to researchers who have received approval from an NIH Data Access Committee (DAC). The NIH DAC reviews proposed submissions for consistency

with appropriate policies to protect the privacy of research participants and confidentiality of their data.

- If an incidental finding is found on a study clinical test such as an ECG or echocardiogram, 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 cardiology referrals as indicated. 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. The presumption is that detection of a potentially clinically significant finding will prove to be beneficial to the subject in the long run.
- The study will obtain 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 family from voluntarily releasing information about the subject's involvement in this research. If an insurer, employer, or other person obtains a subject's or family's written consent to receive research information, then the researchers will not use the Certificate to withhold that information.

I.3 Potential benefits

In most cases, the study will not provide direct benefit to individual participants or families. The benefits are those to society as a whole in the improvement of knowledge of the causes of CHD, in the development of new diagnostic tests and ultimately in the improvement of treatment and prognosis.

Research using samples and data may result in inventions or discoveries that could create new tests and medicines that may have commercial value. Although subjects and their families will not receive any compensation now or in the future for their samples or data, income may be derived from future research or sales of the grouped data and will be used to support biomedical research.

I.4 Risk/Benefit Ratio and Importance of Information to Be Obtained

The risk/benefit ratio is favorable for this study and adverse events are not anticipated. The baseline risk is minimal because there are no therapeutic interventions. In addition, although an individual subject may not benefit from participation, the results of this study will make important contributions to the improvement of knowledge of the causes of CHD, in the development of new diagnostic tests, and ultimately in the improvement of treatment and prognosis.

I.5 Safety Monitoring

Consistent with NHLBI policy regarding monitoring of clinical research studies, an Observational Study Monitoring Board (OSMB) has been established. This group is independent and serves in an advisory capacity to NHLBI. The OSMB will meet every 6 months to review data on enrollment, demographics, specimen counts and quality, data completeness and quality, protocol violations, unexpected events and adverse events; these data are prepared for review by the CC. As there are no interventions in CHD GENES, adverse events are not expected during the conduct of this study. However, if any adverse events are documented, they will be reported to the CC, OSMB and NHLBI. Adverse events should also be reported to IRBs in accordance with local policies and procedures.

I.6 Inclusion of Women and Minorities

All eligible subjects will be enrolled in this study without regard to gender, race, or ethnicity. The distributions of subjects according to gender, race, and ethnicity will reflect both the epidemiology of congenital heart defects and the referral patterns to participating Centers.

Epidemiologic studies have demonstrated that individuals of varying ethnic/racial backgrounds are affected with CHD with comparable frequency [3]. Participants of all races and ethnic backgrounds will be included in this study. The study will be offered to English- and Spanish-speaking people. Translated and back-translated Spanish consents that are IRB/EC-approved will be used for Spanish-speaking individuals along with the aid of an interpreter. The study will also be offered to those who speak different languages (not English or Spanish) when an IRB/EC-approved, translated consent and appropriate interpreter is available.

Epidemiologic studies have also demonstrated that approximately equal numbers of males and females are affected with CHD overall, although at birth there is a slight male preponderance.

However, left-sided obstructive lesions and conotruncal defects occur significantly more frequently in males (60%) than females for reasons that are not understood [28,29]. Although this study will approach all eligible subjects regardless of gender, a preponderance of males will likely result as a reflection of the population affected with left-sided obstructive lesions and conotruncal defects. The gender distribution of patients enrolled in this program with other types of CHD will be approximately equal between males and females reflecting the general affected population.

J. Compensation and Costs

J.1 Compensation

Individual centers will determine if, and how, participants will be compensated.

J.2 Cost

There is no cost of participation in this study.

K. References

1. Perry LW, Neill CA, Ferencz C, Rubin JD, Loffredo CA: Infants with congenital heart disease: the cases. In: Epidemiology of Congenital Heart Disease: The Baltimore-Washington Infant Study 19811989. Edited by Ferencz C, Rubin JD, Loffredo CA, Magee CA, vol. 4. Mount Kisco: Future Publishing Company, Inc.; 1993: 33-62.
2. Botto LD, Correa A, Erickson JD: Racial and temporal variations in the prevalence of heart defects. Pediatrics 2001, 107(3):E32.
3. Ferencz C, Rubin JD, McCarter RJ, Brenner JI, Neill CA, Perry LW, Hepner SI, Downing JW: Congenital heart disease: prevalence at livebirth. The Baltimore- Washington Infant Study. Am J Epidemiol 1985; 121(1):31-36.
4. Pierpont, M.E., et al., Genetic basis for congenital heart defects: current knowledge: a scientific statement from the American Heart Association Congenital Cardiac Defects Committee, Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. Circulation, 2007;115(23): p. 3015-38.
5. Ferencz C, Rubin JD, McCarter RJ, Brenner JI, Neill CA, Perry LW, Hepner SI, Downing JW: Congenital heart disease: prevalence at livebirth. The Baltimore-Washington Infant Study. Am JEpidemiol 1985; 121(1):31-36.
6. Petrini J, Damus K, Johnston RB, Jr.: An overview of infant mortality and birth defects in the United States. Teratology 1997, 56(1-2):8-10.
7. Rosano A, Botto LD, Betting B, Mastroiacovo P: Infant mortality and congenital anomalies from 1950 to 1994: an international perspective. J Epidemiol Community Health 2000, 54(9):660-666.
8. Brown MD, Wernovsky G, Mussatto KA, Berger S: Long-term and developmental outcomes of children with complex congenital heart disease. Clin Perinatol 2005, 32(4):1043-1057.
9. Goldmuntz E, Clark BJ, Mitchell LE, Jawad AF, Cuneo BF, Reed L, McDonald-McGinn D, Chien P, Feuer J, Zackai EH et al: Frequency of 22q11 deletions in patients with conotruncal defects. J Am Coll Card/0/1998, 32(2):492-498.
10. Goldmuntz E, Bamford R, Karkera JD, dela Cruz J, Roessler E, Muenke M: CFC1 mutations in patients with transposition of the great arteries and double-outlet right ventricle. Am J Hum Genet 2002, 70:776-780.Krantz ID, Smith R, Colliton RP, Tinkel H, Zackai EH, Piccoli DA, Goldmuntz E, Spinner NB: Jagged l mutations in patients ascertained with isolated congenital heart defects. Am J Med Genet 1999, 84(1):56-60.
11. Gaynor JW, Gerdes M, Zackai EH, Bernbaum J, Wernovsky G, Clancy RR, Newman MF, Saunders AM, Heagerty PJ, D'Agostino JA et al: Apolipoprotein E genotype and

neurodevelopmental sequelae of infant cardiac surgery. J Thorac Cardlovasc Surg 2003, 126(6): 1736-1745.

12. Mahle WT, Crisalli J, Coleman K, Campbell RM, Tam VK, Vincent RN, Kanter KR: Deletion of chromosome 22q11.2 and outcome in patients with pulmonary atresia and ventricular septal defect, Ann Thorac Surg 2003, 76(2):567-571.
13. Hobbs CA, James SJ, Persian A, Krakowiak PA, Jernigan S, Greenhaw JJ, Lu Y, Cleves MA: Congenital heart defects and genetic variants in the methylenetetrahydroflate reductase gene. J Med Genet 2006, 43(2): 162-166.
14. Michielon G, Marino B, Formigari R, Gargiulo G, Picchio F, Digilio MC, Anaclerio S, Oricchio G, Sanders SP, Di Donate RM: Genetic syndromes and outcome after surgical correction of tetralogy of Fallot. Ann Thorac Surg 2006, 81(3):968-975.
15. Cleves MA, Ghaffar S, Zhao W, Mosley BS, Hobbs CA: First-year survival of infants born with congenital heart defects in Arkansas (1993-1998): a survival analysis using registry data. Birth Defects Res A Clin Mol Teratol 2003, 67(9):662-668.
16. Gaynor JW, Wernovsky G, Jarvik GP, Bernbaum J, Gerdes M, Zackai E, Nord AS, Clancy RR, Nicolson SC, Spray TL: Patient characteristics are important determinants of neurodevelopmental outcome at one year of age after neonatal and infant cardiac surgery. J Thorac Cardlovasc Surg 2007, 133(5):1344-1353, 1353 e1341-1343.
17. Kyburz A, Bauersfeld U, Schinzel A, Riegel M, Hug M, Tomaske M, Valsangiacomo Buchel ER: The fate of children with microdeletion 22q11.2 syndrome and congenital heart defect: clinical course and cardiac outcome. Pediatr Cardiol 200Q, 29(1):76-83.
18. Ziolkowska L, Kawalec W, Turska-Kmiec A, Krajewska-Walasek M, Brzezinska-Rajszys G, Daszkowska J, Maruszewski B, Burczynski P: Chromosome 22q11.2 microdeletion in children with conotruncal heart defects: frequency, associated cardiovascular anomalies, and outcome following cardiac surgery. Eur J Pediatr 2008,167(10): 1135-1140.
19. Hyett J, Moscoso G, Nicolaides KH. Abnormalities of the heart and great arteries in first trimester chromosomally abnormal fetuses. Am J Med Genet1997;69:207–16.
20. Mazzanti L, Cacciari E. Congenital heart disease in patients with Turner's syndrome. Italian Study Group for Turner Syndrome (ISGTS). J Pediatr. 1998 Nov;133(5):688-92.
21. McElhinney DB, Geiger E, Blinder J, Benson DW, Goldmuntz E: NKX2.5 mutations in patients with congenital heart disease. J Am Coll Cardiol 2003, 42(9): 1650-1655.
22. Goldmuntz E, Geiger E, Benson DW: NKX2.5 mutations in patients with tetralogy of fallot. Circulation 2001,104(21):2565-2568.

23. McElhinney DB, Krantz ID, Bason L, Piccoli DA, Emerick KM, Spinner NB, Goldmuntz EG. Analysis of cardiovascular phenotype and genotype-phenotype correlation in individuals with a JAG1 mutation and/or Alagille syndrome. Circulation 106:2567-2574, 2002.
24. Gebbia M, Ferrero GB, Pilia G, Bassi MT, Aylsworth A, Penman-Splitt M, Bird LM, Bamforth JS, Burn J, Schlessinger D, Nelson DL, Casey B (1997) X-linked situs abnormalities result from mutations in ZIC3. Nat Genet 17:305–308.
25. Garg V, Muth AN, Ransom JF, Schluterman MK, Barnes R, King IN, Grossfeld PD, Srivastava D. Mutations in NOTCH1 cause aortic valve disease. Nature. 2005 Sep 8;437(7056):270-4. Epub 2005 Jul 17.
26. Benson DW. Advances in cardiovascular genetics and embryology: role of transcription factors in congenital heart disease. Curr Opin Pediatr 2000;12:497-500.
27. Momma K, Kondo C, Ando M, Matsuoka R, Takao A. Tetralogy of Fallot associated with chromosome 22q11 deletion. Am J Cardiol 1995;76:618-21.
28. Ferencz C, Loffredo CA, Correa-Villasenor A, Wilson PD. 1997. Malformations of the cardiac outflow tract. Genetic and Environmental Risk Factors of Major Cardiovascular Malformations: The Baltimore-Washington Infant Study: 1981-1989. Armonk: Future Publishing Company, Inc. p 59-102.
29. Ferencz C, Loffredo CA, Correa-Villasenor A, Wilson PD. 1997. Left-sided obstructive lesions. Genetic and Environmental Risk Factors of Major Cardiovascular Malformations: The Baltimore-Washington Infant Study: 1981-1989. Armonk: Futura Publishing Company, Inc. p 166-225.
30. Choi M, Scholl UI, Ji W, Liu T, Tikhonova IR, Zumbo P, Nayir A, Bakkaloğu A, Ozen S, Sanjad S, Nelson-Williams C, Farhi A, Mane S, Lifton RP. Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. Proc Natl Acad Sci 2009, Nov 10;106(45):19096-101.