PEDIATRIC HEART NETWORK

LONGITUDINAL FONTAN STUDY

(FONTAN 3)

Date: March 5, 2012

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

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03/05/12

Pediatric Heart Network

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ABSTRACT

The Fontan Cross-Sectional Study (Fontan 1) created a large and unique dataset that was used to investigate the relationship of functional health status of these subjects to various laboratory measures of cardiac function. The cross-sectional design of Fontan 1 limits the ability to assess whether observed differences between older and younger subjects are related to the length of time one lives with Fontan physiology, or to changes in medical, catheter-based or surgical management strategies that have occurred since the oldest subjects in this study underwent their Fontan procedures. A follow-up study (Fontan 2) at an average of 7 years after enrollment in Fontan 1 included a limited re-evaluation of the original Fontan 1 cohort utilizing the following outcomes: vital status, functional health status, interim medical events, access to health care and self-reported availability and willingness to participate in future studies. The Fontan 2 study enrolled 85% of the Fontan 1 survivors and found 87% of these willing to participate in a future study.

For the present study, we will collect vital and cardiac transplant status data from medical records and other public records as required on the 546 subjects screened for the Fontan 2 study, and then approach those alive with a Fontan circulation for enrollment in Fontan 3. Repeat administration of age-appropriate health status questionnaires, maximal exercise testing, echocardiography and B-type natriuretic peptide (BNP) analysis will be performed prospectively during a visit to a participating PHN center and compared to data obtained during the previous Fontan studies. Additional data will be collected by medical record review and questionnaires to assess socioeconomic status, family functioning, and access to health care. The Fontan 3 study will also collect biological specimens for storage in a central repository for future genetic studies. Longitudinal assessment of functional health status and repeat testing of laboratory measures of ventricular function an average of 9 years after initial enrollment of this multicenter, well characterized cohort is of significant clinical importance and will provide useful comparisons to the single ventricle subjects being followed prospectively from birth in other PHN studies.

A. SPECIFIC RESEARCH AIMS AND HYPOTHESES

A.1 Primary Aim

To determine relationships between laboratory measures of ventricular performance and functional health status over time in a cohort of surviving Fontan subjects and explore predictors for poorer outcome

Hypotheses:

1. *Ventricular performance will be associated with current functional status and both will worsen with increasing age (time from Fontan).*
2. *Certain clinical and sociodemographic factors will predict greater decline in functional health status over time.*

Primary outcome:

- Longitudinal change from Fontan 1 to Fontan 3 in age-appropriate functional health status instruments

Secondary outcomes:

- Change over time in exercise capacity (anaerobic threshold, oxygen, maximal work rate, and maximal oxygen consumption)
- Change over time in echocardiographic measures (systolic and diastolic ventricular function, valvar regurgitation, mass to volume ratio)
- Change over time in brain natriuretic peptide
- Change over time in underlying heart rhythm (predominant rhythm, intervention for atrial tachyarrhythmia)

Potential predictors:

- Sociodemographic factors
  - Age
  - Gender
  - Socioeconomic status
  - Access to health care
- Functional health status assessed at Fontan 1 and Fontan 2
- Cardiac performance assessed at Fontan 3
  - Exercise performance
    - Echocardiographic assessment of ventricular function

        - B-type natriuretic peptide (BNP)
- Baseline cardiac performance at Fontan 1
    - Exercise performance
    - Echocardiographic assessment of ventricular function
    - MRI assessment of function
    - B-type natriuretic peptide (BNP)
- Interventions
    - Catheter-based intervention
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- Clinical Complications
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    - Protein-losing enteropathy
    - Thrombotic complications
    - Stroke
    - Seizure
    - Cirrhosis
    - Plastic bronchitis

A.2 Secondary Aim 1

To correlate current functional health status/quality of life with simultaneously derived measures of cardiac performance

Hypothesis:

> *Current functional health status and quality of life in children and young adults assessed at Fontan 3 will be associated with cardiac performance as measured in Fontan 3*

Outcomes:

- Age-appropriate measurements of functional health status

Potential predictors:

- Exercise performance
- Echocardiographic assessment of ventricular function
- B-type natriuretic peptide (BNP)
- Underlying heart rhythm

Covariates:

- Demographics, past interventions, and clinical complications (similar to those in Primary Aim)

A.3 Secondary Aim 2

To evaluate the effects of increasing age on access to appropriate health care and related resources.

Hypotheses:

1. *The majority of subjects age 19 and older will not have transitioned care to an adult congenital heart clinic.*
2. *Subjects age 18 and younger will be more likely than subjects age 19 and older to have seen a cardiologist (pediatric or adult) in the past year.*
3. *The health insurance mix of subjects older than 18 years of age will be different from the insurance mix of subjects 18 years of age and younger, with older subjects having a higher frequency of public insurance or no insurance.*

Outcomes:

- Transition to adult congenital heart specialist for those older than 18 years of age
- Time since last cardiologist visit
- Health insurance coverage assessed at present time
- Characterization of mix of health care and related services used by young adults with congenital heart disease.

A.4 Secondary Aim 3

To collect biospecimens from subjects for banking in a biologic specimen repository (biorepository).
Purpose: To establish a collection of genetic material that will leverage the careful phenotyping of the Fontan cohort by making samples available for future hypothesis-driven studies aimed at identifying genetic determinants of outcome in single ventricle lesions.

B. BACKGROUND AND SIGNIFICANCE

B.1 Fontan 1 Design and Results

The PHN undertook the Fontan 1 study to obtain additional information about pediatric Fontan survivors and to provide the basis for subsequent clinically relevant, randomized clinical trials in the

Fontan population.[1] This was a cross-sectional study of 546 children 6 to 18 years (mean 11.9±3.4 years) who had undergone a Fontan procedure. The goal was to define the relationships between functional health status and health-related quality of life and laboratory measures of ventricular function and performance. We compared subjects within pre-specified anatomic and procedure subgroups. Functional health status was measured by the Child Health Questionnaire (CHQ) and health-related quality of life was measured by the Congenital Heart Adolescent and Teen Questionnaire. Ventricular function was assessed by maximal exercise testing, 2D and Doppler echocardiography, cardiac magnetic resonance imaging (CMRI) and measurement of BNP. All data were obtained within a single 3-month window for each subject. Fontan procedure characteristics, subject age, ventricular morphology, and clinical history were obtained to determine whether correlations between health status and measures of ventricular performance varied as a function of these subject and surgical characteristics.[1]

The Fontan cross-sectional study has generated several publications, the results of which are summarized briefly below.

- Ejection fraction (EF) was normal for 73% of subjects; diastolic function grade was normal for 28%. EF and EF z-score were lowest, and semilunar and atrioventricular (AV) valve regurgitation were more prevalent, in the RV subgroup. Older age at Fontan was associated with more severe AV valve regurgitation. CHQ mean summary scores were lower than for historical controls; however, over 80% of subjects were in the normal range.[2]
- Mean percent predicted maximal oxygen consumption (VO_2) was 65% and decreased with age and pubertal status. VO_2 at ventilatory anaerobic threshold (VAT) was better preserved (78% predicted for the total population) than was peak VO_2. Higher percent predicted oxygen pulse at maximal exercise (a surrogate for stroke volume at maximal exercise) was associated with greater percent predicted maximal VO_2, work rate, and VAT. Adolescence and male gender were associated with decreased percent predicted VO_2.[3]
- BNP concentration ranged from <4-652 pg/mL (median 13), and higher levels were associated with increased age, and decreased physical functioning and exercise capacity. Increased BNP concentration was associated with markers of adverse ventricular performance and increased mass.[4]
- Other abnormalities identified included: predominant non-sinus rhythm on ECG (30%), resting bradycardia (26%), low peak heart rate (HR) at the time of exercise testing (mean 155 ± 23 bpm), a pacemaker at the time of study participation (14%), and a history of atrial tachycardia (9.6%). Lower resting HR and higher peak HR were each weakly associated

with better functional status, as defined by higher VAT and higher CHQ scores for physical functioning.[5]

- Parent-reported subject morbidities included deficits in vision in 33%, speech in 27%, and hearing in 7%, as well as problems with attention in 46%, learning in 43%, development in 24%, behavior in 23%, anxiety in 17%, and depression in 8% of subjects.[6] Functional health status measured by CHQ summary scores were significantly lower as compared with US population norms for Physical Functioning (mean z-score, -0.47±1.19; *P*<0.001) and Psychosocial Functioning (-0.28±1.08; *P*<0.001). Parent-reported medical conditions and long-term and current medical problems explained the greatest amount of variation in the Physical Functioning scores. Parent-reported subject conditions, including behavior, learning, anxiety, and attention problems and depression, explained the greatest amount of variation in the Psychosocial Functioning scores. Subjects with lower family income reported worse Physical and Psychosocial Functioning than those with higher family income. In contrast, laboratory measures of ventricular performance accounted for a small proportion of the variation in functional health status.[7]

This study is the largest to date of children who have undergone the Fontan operation. The impact of the initial manuscripts which have emerged from the Fontan 1 study are in many respects due to inclusion of a large number of subjects from all over North America.[2, 3, 5, 8] Data from this large cross-section of Fontan patients can be considered a benchmark for future studies.

B.2 Rationale and Design for the Fontan 2 Study

Although the Fontan 1 study was recognized as a very successful and unique study, analysis of the data as well as comments from peer reviewers of all related manuscripts underscored the limitations of the study's cross-sectional design. The importance of serial clinical evaluation and laboratory testing was recognized by the PHN. The Fontan 2 study was designed to capture vital status, additional medical history and current quality of life and health information for all Fontan 1 subjects. An important secondary goal was to assess the subjects' willingness to undergo repeat laboratory testing in a future study. In addition, since access to specialized medical care may become difficult as the subjects reach adolescence and young adulthood, we also collected data designed to evaluate the effects of increasing age on access to appropriate health care and related resources.

B.3 Fontan 2 Study Results

Fontan 2 recruited 85% (n=428) of the surviving, non-transplanted Fontan 1 cohort. Moreover, 87% of those enrolled expressed significant interest in future studies that would include repeat laboratory testing at PHN centers. Although not completely analyzed, there does appear to be a decline in the summary scores of functional health status over time between Fontan 1 and 2.

B.4 Rationale for the Study Design and Outcome Measures for Fontan 3

Given the success of recruitment into Fontan 2 and the self-reported interest in future studies by the subjects, a second follow-up of this group is feasible. We have carefully considered which laboratory testing to include and these tests are listed below. Of note, we are not including MRI because: 1) relatively few patients completed this examination in Fontan 1 (unable to cooperate and presence of contraindications such as various devices); 2) MRI techniques have changed appreciably since Fontan 1; and 3) Fontan 2 subjects indicate less willingness to complete MRI testing than other planned tests. In addition, we believe that access to and type of health care may affect functional health status in this transitioning cohort, but little is known about this topic. We are currently analyzing Fontan 2 data collected on this topic. We plan to enrich those data by re-evaluating the effects of increasing age on access to appropriate health care and related resources. The rationale for collection of these data is detailed below.

B.4.1 Functional Health Status

Results from Fontan 1 suggest that differences in ventricular performance and health status may be associated with increasing age.[3] More definitive investigation requires a longitudinal design. Fontan 3 is designed as a follow-up study of this cohort at an average of 9 years after the initial evaluation and 2 years after Fontan 2. Data from this follow-up will permit useful comparisons to the data from single ventricle subjects being followed prospectively from birth in other PHN studies. Continued detailed investigation by groups such as the PHN will improve our understanding of these patients and will lead to surgical and medical therapies to improve their functional health status.[9] For the Fontan 2 study, several age-appropriate measures of functional health status and quality of life were added to the CHQ-P50 and CHQ-87 (which were performed at the Fontan 1 study). These included the PedsQL Core 4.0 Child Form (Age 8-12 years), PedsQL Teen Form (Age 13-18 years) and PedsQL Young Adult Form (≥19 years), and the SF-36 (≥19 years). In the Fontan 3 study, we will use the same age appropriate instruments used in Fontan 2, thereby allowing longitudinal comparisons in individual subjects.

B.4.2 Echocardiography

In the Fontan 1 study, ejection fraction (EF) was normal for 73% of subjects; diastolic function grade was normal for only 28%. EF z-score was lowest, and semilunar and atrioventricular (AV) valve regurgitation were more prevalent, in the RV subgroup. Older age at Fontan was associated with more severe AV valve regurgitation.[2] Ventricular diastolic function has been shown to be abnormal in patients following the Fontan procedure.[11] The available data on diastolic function in patients after the Fontan operation are based on indices derived from Doppler samples of atrioventricular inflow (primarily E:A ratio), tissue velocity (primarily E/E'), and rate of deceleration of early inflow. Largely unknown is data specifically describing longitudinal changes over time in the same population using the same echocardiography protocol. There are insufficient available data to establish which, if any, of these indices will prove more reliable and load-independent. Faced with this uncertainty, the ease of obtaining these indices during the echocardiogram, and the absence of additional expense to the study, we will obtain these three indices in addition to the two more conventional pulsed Doppler indices for comparison with previously published data.

B.4.3 Exercise Testing Measures of Ventricular Performance

Maximal exercise testing provides measuring oxygen consumption as a surrogate of cardiac output during exercise. It is very attractive as an outcome measure in a clinical trial designed to evaluate the impact of a therapeutic intervention on children with congenital heart disease because the exercise test itself approximates closely activities of daily living experienced by a child. The purpose of including exercise testing in this study is to test for a correlation between functional health status and maximal oxygen consumption, oxygen pulse, maximal work rate and ventilatory anaerobic threshold. Our previous work in Fontan 1 showed that mean percent predicted maximal oxygen consumption (VO_2) was 65% and decreased with age. VO_2 at ventilatory anaerobic threshold (VAT) was better preserved (78% predicted for the total population) than was maximal VO_2. Higher percent of predicted oxygen pulse at maximal exercise (a surrogate for stroke volume at maximal exercise) was associated with greater %predicted maximal VO_2, work rate, and VAT. Adolescence and male gender were associated with decreased %predicted maximal VO_2.[3]

Little information is available regarding the longitudinal changes in exercise performance in Fontan patients. In a study by Reybrouck et al.[10], twelve subjects tested an average of two years apart showed a significant decrease in VO_2 measured at the aerobic threshold. Analysis of the individual data points of this study provided to us by these authors show a decline of approximately 2.0% per year in aerobic capacity. In a cross-sectional study of exercise performance in patients following

Fontan, Mahle[11] also has shown a strong negative linear relationship between percent of predicted maximal VO_2 and the age at testing with a slope of 2.2% per year, similar to that of Reybrouck. A more recent study by Giardini et al showed a similar rate of decline of 2.6% per year in maximal VO_2 in a longitudinal study of adolescents with Fontan physiology.[12]

B.4.4 B-Type Natriuretic Peptide

B-type natriuretic peptide (BNP) is a neurohormone primarily secreted by the ventricular myocardium in response to volume expansion or pressure overload. Early studies demonstrated a correlation of the plasma BNP level with ventricular dilation in patients with dilated cardiomyopathy.[13] The actions of this peptide include natriuresis, vasodilation, inhibition of the renin-angiotensin-aldosterone axis and inhibition of sympathetic nerve activity. Studies in adult patients found plasma BNP to be an accurate predictor of the presence of congestive heart failure. In patients with dyspnea, a BNP blood concentration of > 80 pg/ml had a 95% positive predictive value and a 98% negative predictive value for heart failure[14, 15]). The plasma BNP level has also been reported to provide predictive information for use in risk stratification in patients with acute coronary syndromes[16] and chronic heart failure[17].

In the Fontan 1 study, BNP concentration ranged from <4-652 pg/mL (median 13) and higher levels were associated with increased age, and decreased physical functioning and exercise capacity. Increased BNP concentration was also associated with markers of adverse ventricular performance and increased mass.[4] Longitudinal follow-up of serum BNP in such a large cohort of Fontan patients has never been performed.

B.5 Rationale for Biorepository

The Fontan 3 study will collect biological specimens to bank in an established biorepository as a resource for future, hypothesis driven studies. This is a unique and timely opportunity to leverage the detailed characterization of cardiac anatomy and clinical outcomes available in this population of children with single ventricle lesions. The specific scientific questions addressed and the approaches used will necessarily be determined by the investigators who propose studies using the specimens. Therefore, the intent is to ensure maximal flexibility for future studies. Accordingly, when possible, blood or saliva will be collected and processed in a way to permit genetic studies. Genotype will almost certainly influence long-term cardiovascular and other clinical outcomes in these patients. A biorepository of subject DNA, when linked with previously derived phenotypic

information, will be a key resource to help elucidate determinants of cardiac, neurodevelopmental, and other outcomes in this high-risk population.

C. RESEARCH DESIGN AND METHODS

C.1 Study Overview

The Fontan 3 study is a second follow-up of an existing cohort of children between the ages of 6 and 18 years at time of enrollment in Fontan 1 (March 2003 - April 2004). Subjects will be assessed for vital status. Medical history data for Fontan 1 study participants who are identified as deceased or transplanted or have had a conversion to a two ventricle circulation since the Fontan 2 Study screening will be obtained using a waiver of informed consent and HIPAA authorization for restrospective chart review. These patients and/or their families will not be contacted. Those alive with a Fontan circulation who provide informed consent will be invited to participating sites for the following tests:

1. Echocardiogram
2. Exercise testing
3. ECG
4. Brain natriuretic peptide measurement
5. Interim medical history assessment
6. Age-appropriate functional health status testing- using same instruments used for Fontan 2 (e. g., CHQ-P50 and PedsQL parent report for parents of subjects <19 years of age; CHQ-87, PedsQL teen for subjects <19 years of age; SF-36 and PedsQL young adult for subjects ≥19 years of age)
7. Collection of biospecimens to be stored in a biorepository using procedures currently being used in other PHN studies (utilizing a separate consent).

C.2 Participants

C.2.1 Inclusion Criteria

All 428 subjects enrolled in Fontan 2 will have assessment of vital status and those currently alive with a Fontan circulation will be approached for consent. We will also contact Fontan 1 subjects who did not enroll in Fontan 2 to assess vital status and approach for consent.

C.2.2 Exclusion Criteria

Subjects who have died, had a cardiac transplant, or conversion to a two ventricle circulation since Fontan 2 will be identified for the vital and transplant status outcome and collection of medical

history data will be performed up to the time of death, transplant, or conversion. These subjects will be excluded from Fontan 3.

C.2.3 Subject Availability

Subject enrollment will be affected by death, cardiac transplant status, loss to follow-up, or refusal to participate. All subjects who enrolled in both the Fontan 1 and Fontan 2 will be approached for consent. Eligible Fontan 1 subjects who did not enroll in Fontan 2 will also be approached for consent. Utilizing data collected in the Fontan 2 study, we expect a loss to death or transplant at a rate of 0.75% per year (29/546 subjects over a 7 year period). With an average of 2 years from Fontan 2 to beginning of Fontan 3, we would expect a surviving eligible cohort of 421 subjects. Based on an 87% self-reported willingness to participate in a Fontan 3 study, we expect to enroll 373 subjects.

C.2.4 Recruitment Procedures

The principal investigator at each clinical center, his or her designees, and the study coordinator will have responsibility for subject recruitment. Many families will be expecting to hear about a follow-up study as this was discussed as a possibility during the Fontan 2 study. Study team members requesting informed consent will have a high degree of familiarity and rapport with subjects and families which should optimize recruitment.

Vital and cardiac transplantation status will be determined by medical record review supplemented by public records (e.g., the Social Security Index, National Death Index, or other local public death records) if required. Families of subjects (or adult subjects of $\geq$18 years of age) who have died, have undergone transplantation, or conversion to a two ventricle circulation will not be approached for consent.

The principal investigator or authorized research staff will obtain informed consent for participation in the follow-up study verbally via telephone, followed by return of the signed consent form by mail, or at a scheduled clinic visit. The consent process will require separate forms and signatures indicating consent for participation in the Fontan 3 study and to submit a sample to the biorepository. Assent will be required in minors according to local IRB regulations. The specific procedures will be in compliance with the requirements of each site's Institutional Review Board. The recommended procedures are as follows.

1. A brief letter describing the study, two copies of the consent form, and a postage paid, pre-addressed opt-out postcard, will be sent to all potential adult (≥ 18 years of age) subjects and to the families of potential minor subjects. The letter will be double-sided with English on one side and Spanish on the reverse side.
2. After two weeks, if the opt-out card is not received by the study team, a team member will contact the adult subject or family directly by telephone. During the telephone call, when the subject /parent has a copy of the informed consent form in front of him/her, the informed consent form will be read verbatim by the researcher with frequent pauses to elicit questions, gauge comprehension, and provide answers.
3. The entire informed consent process and outcome will be documented in writing and retained in the study file for all subjects/families contacted.
4. After all questions are answered and the research staff feels confident that the subject/parent understands the study, the subject/parent will be asked to sign and date the consent form (we will flag or highlight the correct signature line), mail one copy back in a stamped, self-addressed envelope provided for this purpose, and retain one copy.
5. Once the signed consent form is received, the study team member who explained the study will sign the appropriate signature line with the current date (not the date on which he or she spoke to the parent/guardian). This individual will specify to whom the study was explained, the date on which verbal consent was obtained, and the date on which the signed consent form was received in a section created for this purpose.

C.2.5 Human Subjects Considerations

Data collection will be performed by medical record review, echocardiogram, exercise testing, ECG, blood draw for BNP and the biorepository (if biorepository consent is obtained), an interview with the subject and/or parent and subject and parental self-completion of questionnaires on neurodevelopment, behavior, quality of life, and family impact. The echocardiogram will be analyzed by the Core Laboratory.

C.2.5.a Potential risks and procedures to minimize risk

1. There is a slight chance of contacting parents of an expired subject not known by study staff to have died. To minimize this risk, before contacting the family we will contact the subject's cardiologist and access the Social Security Death Index to ascertain vital status.

2. There is some inconvenience and burden of completing questionnaires and some families may feel uncomfortable answering questions. Approximately 60 minutes will be required for completion of all instruments. However, subjects' families will be under little time pressure to complete the questionnaires. Study coordinators will be available to answer questions and respond to concerns of families. All testing that is not part of routine care will be performed free of charge. Subjects and their families will be reimbursed for costs associated with participating in the protocol that would not have occurred as part of routine clinical care. The echocardiograms and electrocardiograms are part of routine care of children and young adults with a single ventricle. There are no risks associated with echocardiography or electrocardiography.
3. The exercise test is part of the routine care of children and young adults with a single ventricle. A small number of subjects who have had a Fontan operation develop an abnormal heart rhythm with strenuous exercise. A member of the exercise testing team will be present throughout the test to monitor continuously the heart rhythm.
4. Risks associated with drawing blood for the BNP level from a vein include momentary discomfort and/or bruising or lightheadedness.
5. Protection of confidentiality: Investigators will take all reasonable measures to protect the confidentiality of the medical records of subjects and their families.
    a. Information on study subjects and families obtained during this research will be maintained confidentially by the research staff. The risk of breach of subject confidentiality will be minimized by storage of all study materials in a locked, secure location accessible only to study investigators. All research related information will be maintained in a system completely separate from the hospital's medical record system. The informed consent form states that subject data will be made available to the Data Coordinating Center (DCC), NIH/NHLBI, Institutional Review Board (IRB) and Data and Safety Monitoring Board (DSMB) if necessary for study safety and quality control.
    b. The subject's name and any other identifying information will not appear in any presentation or publication resulting from this study.
6. For subjects participating in the biorepository: Subjects will sign a single informed consent form for any testing of stored DNA in future studies, i.e., we do not expect subjects to sign additional consent forms in the future for each study that uses DNA stored in the biorepository. Therefore, we have provided a detailed list of potential risks.

a. Collection of blood or saliva for the biorepository is performed only for research purposes, and families will indicate their consent for this part of the research study separately (i.e., one can participate in other aspects of the study without consenting to the biorepository).
b. Risks associated with a blood draw are minor discomfort, bleeding, bruising, and lightheadedness. We will draw blood for the biorepository at the time of drawing the sample for BNP.
c. There are no risks or discomforts associated with providing a saliva sample.
d. For those participating in the biorepository, biological specimens (DNA) will be assigned a distinct repository identification number without other identifying information. All research related information is maintained in a system completely separate from the hospital's medical record system. Informed consent will state that subject data will be made available to the DCC, NIH/NHLBI, IRB and DSMB if necessary for assessment of study safety.
e. To help us protect the privacy of subjects who provide biological specimens and to be compliant with current National Intitutes of Health (NIH) policy, we will obtain a Certificate of Confidentiality from the 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.
f. Information from DNA analyses and phenotypic data from clinical studies or medical records may be placed into a central data repository in the future, such as the National Center for Biotechnology Information (NCBI) repository. The purpose of a central data repository is to help researchers work together to find out more information about ways in which genes affect disease. 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 on the Internet 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 approval to conduct human genetic studies and who have received approval from an NIH Data Access Committee.

g. The results of future tests on biological specimens will not be released to the subject/family. At the end of the study, the results of the genetic testing may be published for all the subjects as a group, but it will not be possible to provide results for an individual subject and medical management will not be changed based on individual results. 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. Further, if samples are "annonymized" prior to release to other investigators for research, it may not be possible to trace the results back to the subject.

7. The 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 that may be derived from future research or sales of the grouped data will be used to support biomedical research.

C.2.5.b Potential benefits

1. It is possible that the echocardiogram, exercise test, or ECG obtained for research purposes may disclose a finding of importance to the subject's management. Results will be provided to the subject's cardiologist, and the subject's family will be informed about this information transfer.
2. Evaluation of functional status and quality of life may provide valuable information that would not otherwise be available. If abnormalities or disabilities are detected in a subject, this information may lead to appropriate intervention measures designed to maximize developmental and functional potential.
3. Currently, there is no known direct benefit from the participation of the subject and family in the biorepository. However, we hope that DNA donation will help investigators to learn more about the relationship between genetic factors or biomarkers and longer-term

cardiac and neurodevelopmental outcomes. This information may help physicians provide better answers to families' questions regarding causes, risk, and recurrence risks. It may also provide clues to future interventions and/or treatments.

4. An indirect benefit may also come from the awareness that the results of this study may serve to help improve the care of children with similar problems in the future. Families may derive a sense of altruism, accomplishment, and contribution to furthering understanding of the problem through their participation.

C.2.5.c Risk/benefit ratio and importance of information to be obtained

1. The risk/benefit ratio is reasonable for this study. All of the testing involved in this study is no more than minimal risk. Although an individual subject may not benefit from participation, the results of this study will make important contributions to the design of optimal management strategies for children and young adults with single ventricle.
2. Longitudinal changes in functional health status have never been studied in such a large population of children with single ventricle physiology.
3. Data generated from this study will be unique in terms of the breadth and depth of the future guidance that can be provided to parents and medical care providers of children with univentricular hearts who have undergone the Fontan procedure.

C.2.5.d Data and safety monitoring plan

The data and safety monitoring plan for the Fontan 3 study will follow standard PHN monitoring principles. Oversight of data and safety for all PHN studies is provided by the PHN's, NHLBI-appointed independent DSMB. The DSMB, which meets at least biannually, is composed of experts in pediatric cardiology, congenital heart surgery, biostatistics and study design, and ethics, as well as a lay member. The DSMB will monitor patient screening, study accrual, number of subjects undergoing each of the different types of evaluations in the protocol, adverse events, data quality, and protocol violations on a regular basis, and it will make recommendations about study conduct to the Director, NHLBI. A summary of study recommendations following each DSMB meeting is posted on the secure PHN Web site. An early stopping rule for efficacy is not indicated.

In addition to the DSMB, local IRBs are also responsible for the safe conduct of research at each study site. Participation in the Fontan 3 study cannot begin at a clinical center until the local IRB has approved the protocol. Per NHLBI policy, the consent form from each site, once approved, is

reviewed again centrally to ensure that no changes inconsistent with the Office of Human Research Protections policy of study design have occurred.

After each DSMB meeting, a summary report of adverse events will be prepared within 30 days and will be distributed by NHLBI staff to each principal investigator and study coordinator with instructions that each principal investigator forward the summary report to their local IRB. The summary report will contain the following information:

- A statement that a DSMB review of outcome data, adverse events, and information relating to study performance across all centers took place on a given date.
- A statement as to whether or not the frequency of adverse events exceeded what was expected and indicated in the informed consent.
- A statement that a review of recent literature relevant to the research took place.
- The DSMB's recommendation with respect to progress or need for modification of the protocol or informed consent. If the DSMB recommends changes to the protocols or informed consent, the rationale for such changes and any relevant data will be provided.
- A statement that if safety concerns are identified, the NHLBI Program Official will communicate these promptly to the investigators.

C.2.5.e Inclusion of women, minorities, and children

Single ventricle anomalies are congenital defects requiring surgery early in life. The incidence is somewhat higher in males than females. All subjects were recruited for enrollment in the Fontan 1 study without regard to gender, race, or ethnicity. The composition, which reflects both the prevalence of single ventricle anomalies and referral patterns to PHN centers, is as follows: 40% female; 80% White, 10% Black; 10% Other; 7% of subjects were Hispanic. The subjects in the Fontan 3 study will be both children and young adults (aged 15-28 years).

C.3 Study Design

The Fontan 3 study is a second follow-up of an existing cohort of children between the ages of 6 and 18 years at time of enrollment in Fontan 1 (March 2003 - April 2004).

C.3.1 Study Completion

The study will be completed when the last subject has completed the required surveys and laboratory testing and abstraction of medical history data is complete for the cohort. All testing ideally will be performed in a single day, but subjects will be given a 3 month window to complete all

tests in the event of test instrument malfunction (e.g., exercise machine broken). Based on prior experience, we estimate this interval to be approximately 18 months from study launch. However, we hope to continue to follow this unique cohort beyond the current study period. To facilitate continuity of follow-up during application for PHN or other funding for the next follow-up period (i.e., "Fontan 4"), the consent form is written to permit annual review of medical records and patient/family contact for ascertainment of vital and cardiac transplant status of each subject for 10 years from the time of the initial consent for this study.

C.3.2 Subject Withdrawal

Subjects may withdraw from participation in the study at any time. The reason for withdrawal and the circumstances of withdrawal will be documented for all subjects withdrawn from the study.

C.4 Measurements

C.4.1 Schedule of Measurements

Data will be obtained at the time of the initial medical record review and contact with the subject or subject's family. The target window for completion of all study tests is one day and the target window for completion of all data collection is 3 months from time of consent.

C.4.2 Outcome Measures

C.4.2.a Functional Health Status and Quality of Life Instruments (see Table 1 for summary)

Table 1: Summary of Self-Administered Questionnaires Completed (Higher=Better)*

Subject Age (years)	Subject Completes	N. Items	N. Scales	Range	Z-Score Y/N	Transformed Z-Scores Y/N	Summary (Aggregate) Scores Y/N
≤18	CHQ-87	87	14	0-100*	N	N	N
≤18	PedsQL Cardiac Module-Teen Version	22	6	0-100	N	N	N
≤18	PedsQL 4.0 Generic Core-Teen Version	23	6	0-100	N	N	Y (2 Scores)
≥19	SF-36 v2	36	8	0-100	Y	Y	Y (2 Scores)
≥19	PedsQL Cardiac Module-Adult Version	22	6	0-100	N	N	N
≥19	PedsQL 4.0 Generic Core-Adult Version	23	6	0-100	N	N	Y (2 Scores)
Subject Age (years)	**Parent Completes**	**N. Items**	**N. Scales**	**Range**	**Z-Score Y/N**	**Transformed Z-Scores Y/N**	**Summary (Aggregate) Scores Y/N**
≤18	CHQ-50	50	15	0-100*	Y	N	Y (2 Scores, 10 Scales)
≤18	PedsQL Cardiac Module- Parent Report for Teens	22	6	0-100	N	N	N
≤18	PedsQL 4.0 Generic Core-Parent Report for Teens	23	6	0-100	N	N	Y (2 Scores)

*Change in health item scored from 1-5.

- Functional Health Status in subjects 10-18 years of age

  Different instruments will be used based on the subject's age. The Child Health Questionnaire (CHQ) will be used for subjects ≤ 18 years of age and measures the physical and psychosocial (e.g., emotional, behavioral, and social) well-being of children five to eighteen years of age[18, 19] There are questionnaires for both the child and the parent. The Child Report form (CHQ-87), which has been validated only for children in the age range 10-18 years, has 87 items. The Parent Report form (CHQ-50) has 50 items and provides two summary scores: 1) physical functional status and 2) psychosocial status. In addition to summary scores, the CHQ- 50 assesses 14 domains (physical functioning, physical role or

social limitations, general health, bodily discomfort, parental impact, emotional role or social limitations, self esteem, mental health, general behavior, family, and change in health). The CHQ can be reliably administered in a variety of formats including child- or parent-report, interview, and mail back. This instrument requires approximately 15-20 minutes for completion.

The CHQ User's Manual provides age- and gender-specific normative data for 379 children in the general United States population, as well as for six clinical samples of children, including those with chronic illnesses (e.g., asthma and epilepsy) and psychiatric problems.[18] Based on these data, five to ten point differences from the normative U.S. sample for either summary score (physical or psychosocial) represent clinically meaningful disease effects. The CHQ-PF50 has been used to assess functional health status outcomes for children following the Fontan operation in the Fontan 1 study[8]. Use of the CHQ-PF50 in the Fontan 3 study will allow us to compare outcomes with those in this older group of Fontan subjects, as well as with published CHQ-PF50 outcomes in other diagnostic groups.[20, 21]

- Functional Health Status in subjects 19 years of age and older

  In subjects age ≥ 19 years, functional status will be measured with the Short Form Health Survey (SF-36 v2). The SF-36 is a multi-purpose, 36-item health survey that yields an 8-scale profile of functional health and well-being scores as well as psychometrically-based physical and mental health summary measures and a preference-based health utility index. It is a generic measure, as opposed to one that targets a specific age, disease, or treatment group. Accordingly, the SF-36 has proven useful in surveys of general and specific populations, comparing the relative burden of diseases, and in differentiating the health benefits produced by a wide range of different treatments. The usefulness of the SF-36 in estimating disease burden and comparing disease-specific benchmarks with general population norms is illustrated in articles describing more than 200 diseases and conditions.[22] This questionnaire takes approximately 15 minutes to complete.

- Quality of Life

  Measures of quality of life will also be age-appropriate. For subjects ≤18 years of age, data will be collected from both the subject and the parent. The Pediatric Quality of Life Inventory (Peds-QL) is a self report instrument with a parallel parent proxy report designed to assess

quality of life in both healthy and acute or chronically ill children. [23, 24] The Peds-QL system provides a general quality of life assessment which addresses physical functioning, emotional functioning, social functioning, and school functioning for children 2 to 18 years old. In addition, a cardiac disease specific module assesses issues of quality of life specific to children with cardiac disease. This system provides instruments for children (age 8-12 years), for teens (age 13-18), and for young adults (age 19-25). Each of the instruments includes 23 items. Together, the general Peds-QL and the cardiac-specific module require 15 minutes to complete.

C.4.2.b Vital Status Assessment

The occurrence of death or transplantation will be ascertained through data collected at the time of review of medical records of the primary cardiologist, surgeon and study site or telephone contact. The cause of death will also be collected for secondary analyses. The participant consent form will allow for medical record review of vital and transplant status for up to 10 years following enrollment. For subjects lost to follow-up, we will search the Social Security or National Death Index (or Canadian equivalents). To minimize bias in the estimated incidence of morbidities after Fontan, the Fontan 3 study medical history form, which is designed to capture medical events from the time of Fontan 2 study participation up to the time of death or transplant, will be completed for these patients by the study coordinator. This data collection will be completed using a waiver of consent for retrospective chart review to ensure representativeness of the study sample while maintaining sensitivity with regard to not contacting families of deceased children (and children who have undergone transplant, some of whom may also have died).

C.4.2.c Access to Health Care

Access to appropriate medical care as subjects transition into adulthood can be evaluated by determining the types of physicians (and other health resources) utilized, the frequency of medical visits, and the type of health insurance, if any, among other factors. Subjects and their parents will be queried and medical records examined to determine the type of physicians the patient has seen in the preceding 2 years. Specifically, we will determine whether a subject who is 19 years of age or older visited an adult congenital heart clinic and the date of the last visit with a cardiologist (pediatric, adult, or adult congenital). We will ask subjects (and/or their parents) to provide the name of the medical provider and the name of the providing clinic. We will match provider against existing medical specialty board specialty listings. Providing clinics will be differentiated into those included or not in the Adult Congenital Heart Association:

http://www.achaheart.org/for_members/clinicdirectory/index.php. Type of health insurance will be assessed through questionnaires. Corroboration will be sought from the subject or parent during the subject/parent interview.

C.4.2.d Echocardiogram

Echocardiograms identified by blinded ID numbers will be electronically transferred directly from the clinical site or via the DCC to the Echocardiographic Core Laboratory for interpretation. The echocardiogram will consist of a complete two-dimensional echocardiogram and Doppler evaluation. A complete assessment for valve function and intracardiac thrombi will be performed. Standard short and long axis views of the ventricle(s) will be recorded to assess regional wall motion. End-diastolic and end-systolic volumes, mass and ejection fraction will be obtained from two-dimensional images using a modified biplane Simpson's rule. Ventricular sphericity will be calculated as the ratio of short-axis area to long-axis dimension. Systolic, diastolic, and mean blood pressure will be measured using an automated vital signs monitor (such as the Dinamapp).

All measurements and derived indices for the study population will be expressed as z-scores relative to body surface area or to age in normal subjects. Z-scores indicate the position of each measurement relative to the normal population expressed as the number of standard deviations from the population mean. Reporting the data as z-scores adjusts for the effects of variation in age and body size.

Ventricular diastolic function will be assessed using several indices. Assessment of ventricular diastolic filling will be performed using indices derived from pulsed Doppler interrogation of each of the atrioventricular valves (if present) and of the pulmonary veins. The atrioventricular valve early deceleration time and E/A ratio will be calculated. The duration of pulmonary vein flow reversal during atrial systole will be measured from pulmonary vein Doppler. The rate of diastolic flow propagation will be obtained using mitral valve inflow color Doppler M-mode. Atrioventricular valve early diastolic annular velocity will be assessed using tissue Doppler.

C.4.2.e Exercise Testing Measures of Ventricular Performance

Each subject who is at least 132 cm tall and free of neurological or developmental defects that preclude exercise will undergo maximal exercise testing using a ramp cycle protocol. Blood or urine pregnany testing will be performed for females who potentially could be pregnant. Pregnant females will not undergo exercise testing. Subjects will be fasted for two hours prior to exercising.

Resting pulmonary function consisting of inspiratory and expiratory flow volume loops and maximal voluntary ventilation will be performed prior to each exercise test. A 12-lead surface ECG will be obtained at rest in the supine, sitting and standing position. The rhythm will be monitored throughout the study and a 12-lead ECG obtained during every minute of exercise and the first ten minutes of recovery. Blood pressure will be measured at rest and during every three minutes of exercise and recovery. Oxygen saturation will be measured continually by oximetry.

The subject will be exercised to maximum exhaustion using an electronically-braked cyclo-ergonometer. The protocol will consist of sitting quietly for three minutes on the ergometer followed by three minutes of unloaded pedaling. The work rate will then be increased using a ramp protocol with a slope chosen to achieve the subject's predicted maximal work rate in ten to twelve minutes of cycle time. Minute oxygen consumption, minute carbon dioxide production (VCO_2), minute ventilation (VE), respiratory exchange ratio (VCO_2/VO_2 - RER), tidal volume, respiratory rate, and O_2 pulse will be monitored continuously on a breath-by-breath basis using a metabolic cart during rest, exercise and the first two minutes of recovery. All subjects will be encouraged to exercise to exhaustion.

C.4.2.f 12-Lead Electrocardiogram

For subjects who do not undergo exercise testing, standard 12-lead surface ECGs will be obtained in both the supine and standing postures.

C.4.2.g B-Type Natriuretic Peptide

A resting BNP level will be measured in plasma. The sample for BNP will be drawn after the subject has been in a sitting/supine position in a quiet room for 30 minutes. A volume of no more than 5 cc of whole blood will be collected and placed into a pre-chilled lavender-topped EDTA tube. The tube will be inverted to mix thoroughly and immediately placed on ice. The Fontan Serology Core Laboratory will provide centrifugation method and timing instructions to the clinical sites. The resulting plasma will be dispensed into vials for storage and frozen immediately at –20° to –80° C. Each vial will have a label identifying the patient study number, protocol number, sample date, and study center. Samples will be batched and shipped to the Serology Core Laboratory on dry ice.

Although considered part of the minimal test set for an enrolled patient, if the patient or family has consented but refuses to allow blood to be drawn at the time of the visit, or if the blood draw attempt is unsuccessful, the patient will remain enrolled in the study for the remaining tests.

C.4.2.h Biospecimens

Samples will be acquired from subjects in accordance with Best Practices for Biospecimen Resources. Blood specimens will be drawn by the clinical site nurse or physician in conjunction with routine blood draws and procedures where possible. No more than 5 ml per 5 lb body weight up to a maximum of 10 ml will be drawn. Blood is preferred and will be obtained if at all possible. However, if this is not possible, and saliva collection is feasible, 2-4 ml of saliva will be collected in saliva kits.

Following acquisition, specimens for this study will be processed as necessary at the local clinical site, then packaged for shipping to the central biorepository. For each shipment sent, clinical sites will complete a shipping notification form, shipping log and a specimen information form.

The PHN biorepository at the University of Michigan and the DCC will follow standard procedures for ensuring appropriate identification, processing, aliquotting, and storage of specimens. Specimens will be tracked in ADEPT and in the repository database.

C.4.2.i Adverse events

The approach to reporting of adverse events concerning the activities for the Fontan 3 study is summarized below.

- Definition

  An adverse event is any untoward medical occurrence experienced by a study subject. An event can be any unfavorable and unintended sign, symptom, laboratory abnormality, or disease associated with study participation.
- Classification

  Monitoring of adverse events in a therapeutic trial requires that they be classified as to seriousness, expectedness, and potential relationship to the study intervention, which then drives the reporting process. In this study, adverse events should be rare, and monitoring should be commensurate with risk, which is minimal. The only procedures being conducted are an echocardiogram, exercise test, electrocardiogram, blood sampling, and, with separate consent, participation in the biorepository. These testing procedures are well established in children and young adults and are associated with a very low risk of adverse

events. In this study, adverse events will be recorded only for the duration of study-specific testing and 24 hours afterwards.

A serious adverse event is one that:

a) Results in death,
b) Is immediately life-threatening,
c) Requires inpatient hospitalization or prolongation of existing hospitalization,
d) Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,
e) Results in a congenital anomaly/birth defect in the offspring of a participant.

An event definitely or probably related to the study procedure is one that follows a reasonable temporal sequence from the time of study testing, and cannot be reasonably explained by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

- Data collection procedures for adverse events

  Events occurring within 24 hours of study-mandated testing will be recorded by the clinical center study coordinator, including the date and time of occurrence, seriousness, duration, treatment prescribed, and resolution. If classified as serious, this event information will be forwarded electronically within three working days to the DCC.

- Reporting procedures

  Serious adverse events related to the study procedures would all be unexpected, but if any occur, they will be reported to the DCC within 24 hours of learning of the event. The DCC will report any such events to the NHLBI, and the PHN Medical Monitor 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 adverse events occurring during the time period specified for this study will be reported quarterly to the DCC. The DCC will report these events at least twice a year to NHLBI and the DSMB. The site investigator or designee will report all serious adverse events to the local IRB according to local IRB policies.

C.4.3 Covariate or Predictor Measures

C.4.3.a Sociodemographic factors

Sociodemographic factors of interest include the subject's age, gender, race/ethnicity, and socioeconomic status as measured by the Hollingshead Index of Social Status. Additionally,

regardless of vital status, addresses and zip codes will be used to identify a subject's census tract which will then be used as a surrogate for family income and SES. Finally, access to health care will be measured as described above.

C.4.3.b Baseline Status: Function and cardiac performance

Baseline assessments of cardiac performance obtained at Fontan 1 included exercise performance, standardized echocardiographic and MRI evaluation, and measurement of serum BNP. Additionally, number and types of medications were recorded.[2]

C.4.3.c Growth measurements

The subject's current weight and height will be obtained by study staff using locally maintained equipment. These values will be used to calculate body mass index, body mass index z-score, weight-for-age z-score, height-for-age z-score, and weight-for-height z-score for all subjects <20 years old, using data available from the National Center for Health Statistics of the Centers for Disease Control.

C.4.3.d Interventions

Data regarding clinical interventions that have occurred since last study participation will be collected via medical record abstraction and from subject/parent self-report. Medical records will be obtained as required from non-PHN centers after consent and a medical record release form has been obtained. Interventions of interest include at least the following: cardiovascular interventions (surgical or catheter-based), permanent pacemaker, and medications.

C.4.3.e Heart failure class

This cohort is old enough to be assessed by the New York Heart Association (NYHA) heart failure class used for adults (Table 2). The NYHA Heart Failure Class will be determined by medical history and self-report.

Table 2. New York Heart Failure Class Definition	
NYHA Class	**Symptoms**
I	No symptoms and no limitation in ordinary physical activity, e.g. shortness of breath when walking, climbing stairs etc.
II	Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.
III	Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20-100 m). Comfortable only at rest.
IV	Severe limitations. Experiences symptoms even while at rest. Mostly bed bound patients.

C.5 Statistical Analysis

C.5.1 Sample Size and Power Analysis

All subjects alive and with a Fontan circulation from the original Fontan 1 cohort will be approached for consent. Study sample size will depend on the ability to contact families and subjects who participated in Fontan studies and on the consent rate for contacted subjects. Based on the data collected in the Fontan 2 study, a loss to death or transplant is expected to be at a rate of 0.75% per year (29/546 subjects over a 7 year period). With an average of 2.3 years from Fontan 2 to beginning of Fontan 3, we would expect to lose less than 2% of subjects to death or transplant. Based on self reported willingness of 87% of subjects enrolled in the Fontan 2 study to participate in a Fontan 3 study, we expect to enroll 85% of Fontan 2 subjects (N=364).

Table 3 presents the percentage of subjects (out of 546 enrolled) with valid results measured at Fontan 1, for each of the outcomes by age group. Because the age of the cohort at the time of Fontan 3 sampling will be > 15 years of age, we will use the rightmost column for our sample estimates.

Table 3: Percentage of subjects (out of 546 enrolled) with valid test results at Fontan 1 by outcome and age group

Outcome	Overall	Subjects ≤ 15 years of age	Subjects > 15 years of age
Exercise –all subjects	75%	74%	82%
Exercise –with Max effort achieved	41%	34%	65%
Echo – with mass-volume data	76%	78%	69%
BNP	93%	96%	96%
CHQ physical functioning summary score (PHS)	94%	93%	94%

Primary Outcome Sample Size

For the primary outcome (change over time in physical functioning summary score in CHQ-PF 50, PHS) sample size will depend on the age of the subjects (since CHQ-PF 50 is valid and completed by the parents of subjects who are less than 19 years of age).

We will provide 2 estimates for the number of subjects who will be less than 19 years old at the time of consent at Fontan 3. Optimistic consent schedule: Assuming that subjects approaching age 19 years will be given a priority in the consent process and that by the end of February 2012 signing of consent forms will actually start, we can expect 192 children under age 19 at the time of consent. A more conservative consent schedule (to be used in further estimates): Assuming that consent process will start by the end of February 2012 and it will follow the pattern of Fontan 2 with a shift by 2.3 years (from Nov 10, 2009 to Feb 28, 2012), we can expect 173 children under age 19 at the time of consent. All these children have PHS data at Fontan 1 or Fontan 2.

In addition, not all 193 children will enroll and have valid study data. Using a .80 deflation factor (85% consent rate and 94% calculable PHS), we expect 123 subjects with PHS measurements at 3 time points and another 15 subjects with PHS measurements at 2 time points, for a total of 138 subjects under age 19 with longitudinal PHS data (Table 4).

Table 4: Projected Number of Matching CHQ PHS Observations for Fontan 1-2-3 Studies. Based on number of subjects enrolled in Fontan 2 study with Fontan 1 data (2nd column), 85% consent rate, and projected number of Fontan 2 subjects < 19 yrs of age at Fontan 3 (4th column).

Outcome	N Fontan 1§	N Fontan 2	N3 Projected number of Fontan 2 subjects <19 yrs at Fontan 3	K Projected % with PHS†	Projected N with PHS at Fontan 3 .85 * N3 * K	Projected N with PHS data at 3 time points (F1+F2+F3)	Projected Additional N with PHS data at 2 time points only	
							F1+F3	F2+F3
PHS	404	258	173	94%	138	123	7	8

§ out of N=428 enrolled in Fontan 2

† based on % for subjects older than 15 years from Fontan 1

We know that the Pearson correlation for parent-reported functional status score between Fontan 1 and Fontan 2 is 0.50 (Spearman correlation =0.47). Taking into account that the mean time gap between Fontan 1 and Fontan 2 is 6.7 years, and the estimated mean time gap between Fontan 2 and Fontan 3 is 2.3 years (see Table 5) it is reasonable to assume that the correlation for parent-reported functional status score between Fontan 2 and Fontan 3 will be at least 0.5 (probably more) and correlation between Fontan 1 and Fontan 3 will be somewhat lower than 0.5.

Table 5: Mean age, time since Fontan surgery and time gap with the previous CHQ measurement at Fontan 1, 2 and 3

	Fontan surgery	Fontan 1	Fontan 2	Fontan 3§
Mean age (yrs)	3.4	11.9	16.1	17.4
Time since Fontan surgery (yrs)	0	8.4	13.2	14.5
Time gap between CHQ data at current and previous time points (yrs)	NA	NA	6.7	2.3
N *	511	511	258	173**

§ All numbers for Fontan 3 are estimated

* Calculated for subjects with PHS

** 138 (173 times 0.8) is used in Table 4

Note that subjects with a CHQ PHS score enrolled in each aubsequent study (Fontan 2 and 3) represent a subset of younger subjects from the previous study (Fontan 1 and Fontan 2)

Secondary Outcomes Sample Size

Secondary outcomes for the primary aim include change over time (between Fontan 1 and Fontan 3) in variables from exercise testing, echocardiography and brain natriuretic peptide (BNP). Power calculations are provided conditional on the estimated number of subject pairs for each outcome variable. The percentage of subjects enrolled in the Fontan 3 study with a valid measurement of a particular outcome will be estimated based on the percentage of subjects with this outcome among children over 15 years of age in the Fontan 1 study (right column in Table 3), except for the echocardiographic variables, where an estimate of 75% will be used. A lower percentage for successful echocardiographic imaging in older children seen in the Fontan 1 study may be related to poorer acoustic windows and less familiarity with the echocardiographic protocol; however, we expect that the improvement in technology over last 9 years may somewhat compensate for challenges encountered in Fontan 1.

The number of subject pairs is estimated (right column of Table 6) based on an 85% consent rate, the number of subjects with baseline (Fontan 1) measurements (2nd column of Table 6) and the expected percentage of valid test measurements (3rd column).

Table 6: Projected Number of Pairs for Fontan 1 and Fontan 3 Studies. Based on number of subjects enrolled in Fontan 2 study with Fontan 1 data (2nd column), 85% consent rate and % of Fontan 1 subjects with valid test results (3rd column) by test

Test	N Fontan 1§	K Projected % of subjects with valid test results†	Projected number of matching pairs at Fontan 1 and Fontan 3 .85 * N * K
Exercise – all subjects	310*	82%	216
Exercise – with max effort achieved	118	65%	65
Echo – with mass-volume data	310*	75%	198
BNP	383	96%	313
CHQ-P50 PHS	404	94%	N/A**

§ Out of N=428 enrolled in Fontan 2

† Based on % for subjects older than 15 years from Fontan 1 (except for Echo test)

* the identical number is a coincidence; only 243 subjects out of 310 have both tests

**estimated number of pairs is 130 (see more details in Table 4)

Power calculations

Next we will provide power calculations for one selected measure from each laboratory test. Table 7 provides descriptive statistics for selected variables at Fontan 1.

Table 7: Fontan 1 Descriptive Statistics for Selected Measures

Test	Variable	N	Mean± SD
Exercise – with max effort achieved	Percent predicted max work rate, %	157	66.2 ±16.1
Echocardiogram	End-diastolic volume z-score	389	-0.6±1.9
BNP	Log of BNP, pg/ml	483	2.7± 0.9
CHQ Parent Report	Physical Functioning Summary Score	511	45.3±11.9

Using data from Tables 3-7, we will provide power calculations to detect differences equal to one third of a baseline standard deviation in selected outcomes from Fontan 1 to Fontan 3 (Table 8). Typically, a one-half standard deviation difference is considered clinically significant.

Table 8: Power to Detect Fontan 1 to Fontan 3 Differences in Outcomes equal to one third of a baseline standard deviation, assuming two-sided α=.05, and correlation between 2 measurements 0.3 to 0.7

Test	Number of Paired Measurements	Outcome	Baseline Mean ± SD	Mean Change in Outcome	Fontan 1 to Fontan 3 correlation		
					0.7	0.5	0.3
Exercise	65*	Percent predicted max work rate, %	66.2±16.1	5.4	93%	76%	62%
ECHO	184	End-diastolic volume z-score	-0.6±1.9	0.63	>99%	>99%	98%
BNP	313	Log of BNP	2.7±0.9	0.3	>99%	>99%	>99%
CHQ	130	PHS summary score	45.3±11.9	4.0	>99%	97%	90%

*maximum effort is achieved

An alternative approach may be to assess power to detect a significant slope of an outcome in time (i.e. number of points that an outcome changes per increasing year since Fontan) over approximately 9 years (Table 5) between Fontan 1 and Fontan 3 measurements. For each outcome we will calculate slopes leading to half a standard deviation and a standard deviation difference over nine years between Fontan 1 and Fontan 3 measurements (i.e. if SD is a standard deviation at

Fontan 1, we will be looking for slopes equal to SD/18 and SD/9). Table 9 presents power calculations for these 2 scenarios.

Table 9: Power to detect a slope leading to half a baseline standard deviation and a standard deviation difference over nine years (baseline SD/18 to SD/9), where X is number of years since Fontan and Y is the mean difference in outcome from Fontan 1 to Fontan 3, assuming σ_x=2.24, two-sided α=.05, and correlation between 2 measurements = 0.5

Test	N Paired measurements	Outcome	Fontan 1 SD	Slope	Power
Exercise	65*	Percent predicted max work rate	16.1	0.9	17%
				1.8	51%
ECHO	184	End-diastolic volume z-score	1.9	0.1	35%
				0.2	89%
BNP	313	Log of BNP	0.9	0.05	59%
				0.1	99%
CHQ	130	PHS summary score	11.9	0.7	32%
				1.3	79%

* With maximum effort achieved

In the first scenario (SD/18, leading to .5 SD change over 9 years), power is low for all outcomes, while in the second scenario we have power above 80% for all outcomes except for exercise. Taking into account that actual correlations may be lower than 0.5 and that one baseline SD change may be very large clinically (e.g. for EDV z-score it implies change equal to almost 2 standard deviations in a normal population) we conclude that this study will have limited power to detect a significant slope for small clinical changes.

One of the study aims (A.2, Secondary Aim 1) addresses the contribution of independent cardiac performance variables to functional status scores at Fontan 3 study enrollment. Power calculations for multiple linear regression of Fontan 3 parent-reported functional status score and exercise test with the maximal effort achieved (controlling for initial score from Fontan 2 (or for a handful of subjects from Fontan 1) to detect an additional R^2 of 0.1 or higher attributable to 5 to 10 variables to be tested are presented in Table 10.

Table 10: Power to detect an additional R^2 for multiple linear regressions controlling for baseline score with correlation of 0.5 or 0.7

Number of subject pairs	Number of dependent variables	Additional R^2	Correlation of independent variable between baseline and Fontan 3	
			R=0.5	R=0.7
138	5	0.10	96%	>99%
138	10	0.15	99%	>99%
65	5	0.10	63%	85%
65	10	0.15	72%	93%

Of note, these calculations do not take into account missing predictor data (see Table 4 for specific estimates for missing data). However, because the power estimates above are very high, even a reduced predictor dataset will yield reasonable power for modeling.

C.5.2 Analysis Plan

C.5.2.a. Analysis for Primary Aim A.1

The primary aim of the study is to investigate changes in functional status in time and identify predictors of change in functional status. For the CHQ Parent reports, data will be available at 3 time points (Fontan 1, Fontan 2 and Fontan 3), while laboratory measures will be available at 2 time points (Fontan 1 and Fontan 3). Since CHQ Parent reports are collected only for subjects under 19 years of age each subsequent study dataset presents a subset of younger subjects from the previous one. A preliminary assessment will be made regarding whether to use all available data (including older patients without Fontan 2 or Fontan 3 CHQ data), or limit the analytic dataset to complete cases.

Linear mixed effects regression with CHQ Physical (Psychosocial) summary score as the outcome will be used to estimate a) age effects; b) period effects (i.e., whether outcome is associated with time since surgery) and c) associations with laboratory measures. Baseline functional status, patient age and time from Fontan surgery will be key design covariates. In particular, our goal is to determine whether changes in health status are due to time since surgery, independent of age. Second, we wish to learn whether there are other predictors of outcome independent of age and disease duration that may perhaps be modifiable. Other medical history, Fontan 1 and Fontan 3 study measurements such as cardiac function and BNP, and subject

demographic factors will also be examined in modeling, controlling for the key covariates noted above, to identify independent predictors of change in functional status. Other analysis steps (missing values, non-linear associations, normalizing transformations for outcome measures) will be identical to those mentioned in Section C.5.2.b. .

A secondary analytic approach to be explored is group-based trajectory modeling (GBTM) to split CHQ summary score trajectories over time into distinctive clusters. After clustering, a logistic or multinomial regression analysis would be used to assess associations between clusters of trajectories (outcome), the design effects, and the clinical/laboratorypredictors . This approach may be helpful in identifying factors associated with membership in a group that is defined by improvement, no change, or decline in CHQ summary scores in time.

Another secondary analytic approach for change in CHQ summary score to be explored is to apply recursive partitioning techniques (Classification And Regression Trees; CART) to assess factors contributing to change between the baseline and Fontan 3 time points. CART requires a dichotomous outcome, which in this setting could be a decrement of at least 3-5 points in CHQ summary score vs. a group comprised either of all other change scores or of all subjects with an increment of at least 3-5 points in CHQ summary score. The CART algorithm imposes interactions of candidate predictors that are dichotomized, creating nodes that represent high- and lower-risk patient subgroups that do not require prespecification. This non-parametric approach may lead to a more robust predictive model than classical regression.

C.5.2.b Analysis for Secondary Aim 1

This aim is focused on associations between functional status (CHQ Parent report; the outcome) and measures of cardiac performance (laboratory measurements including exercise testing, echocardiography, and BNP; predictor variables) at the time of Fontan 3.

To assess the generalizability of the findings, two comparisons will be made. First, a comparison will be made of the demographic and anatomic characteristics of those enrolled in Fontan 3 to those who were eligible but refused to participate. Second, a comparison of the subjects with CHQ summary scores at Fontan 3 vs. those enrolled but without summary scores who were in the appropriate age range will be conducted (the latter group is expected to be small). The two groups

will be compared with respect to their demographic profile and their medical/functional status profile from the Fontan 1-2 studies.

Missing measurements will be monitored for each group of the laboratory tests and subjects with missing measurements will be compared with the rest of the cohort in terms of key patient characteristics. If no significant differences between the 2 groups are detected, missing values for the predictor variables may be imputed.

Linear regression modeling will be used to assess associations between functional status and measures of cardiac performance. The distributions of the outcome and predictors will be inspected to assess normality and apply appropriate transformation as needed. In the cases where type 1 error may be inflated due to multiple comparisons of an outcome an appropriate adjustment will be performed. Because of the large number of potential predictors, assessment of their significance may need to be conducted in a staged approach to assess collinearities and to determine which medical events, Fontan 3 study measurements, and patient characteristics are most important within a particular class. It is possible that some variables are important risk factors only for selected subsets of subjects. Tests of interaction will be used with a limited number of patient characteristics to assess whether risk factor sets depend on subgroup (e.g., dominant single ventricle, age at Fontan, type of Fontan). Nonlinear associations between continuous predictors and outcome will be examined using generalized additive modeling and non-linear and categorical transformations will be applied, where appropriate.

To determine whether the risk prediction models will have utility for future patients, it is desirable to validate the model using an independent dataset. For this reason, the Fontan 3 study dataset may be divided into two components: a 'training' dataset (to derive the model) and a validation dataset (to apply the model and compare predicted status to actual status). The smaller validation dataset will be comprised of observations that are randomly selected, without replacement, from the overall dataset.

C.5.2.c Analysis for Secondary Aim 2

This aim focuses on current health care access and resource utilization as outcomes. It is primarily descriptive to understand the profile of health care practices for this patient population as it ages into adulthood. Assessments of interest are type of health care provider, frequency of cardiology follow-up, health insurance coverage, and utilization of support services. Regression and CART

techniques may also be employed to understand the profile of patients who have lost health insurance coverage, and those who have transitioned to a different type of health care provider (e.g., adult vs. pediatric cardiologist, or general practitioner). Age of the subject will be a critical covariate and/or variable defining a health care outcome. For example, age at loss of health insurance coverage may be considered a time-to-event variable and its distribution estimated using Kaplan-Meier methodology with Cox regression to identify factors associated with the timing of loss of coverage. Logistic and multinomial regression may be used to identify correlates of type of health care provider and whether support services are utilized. Important mediating variables for this aim are socioeconomic status (including the subject's level of education and employment status), and time since Fontan surgery. Tests of interaction can assess whether the association of age with health care status is dependent on these mediating variables.

C.5.3 Subgroup Analyses

To determine whether changes over time in functional health status and whether other outcomes vary according to patient subgroup, separate comparisons may be made of the primary and secondary outcomes within the following subgroups, similar to those subgroups investigated in the Fontan 1 study:

- Age of Patient
- Time since Fontan
- Dominant single ventricle (right, left, mixed)
- Fontan procedure type
- Age at Fontan
- Performance of a surgical fenestration at Fontan surgery
- Presence of a persistent fenestration assessed by core lab at Fontan 1
- Performance of a Stage 2 procedure

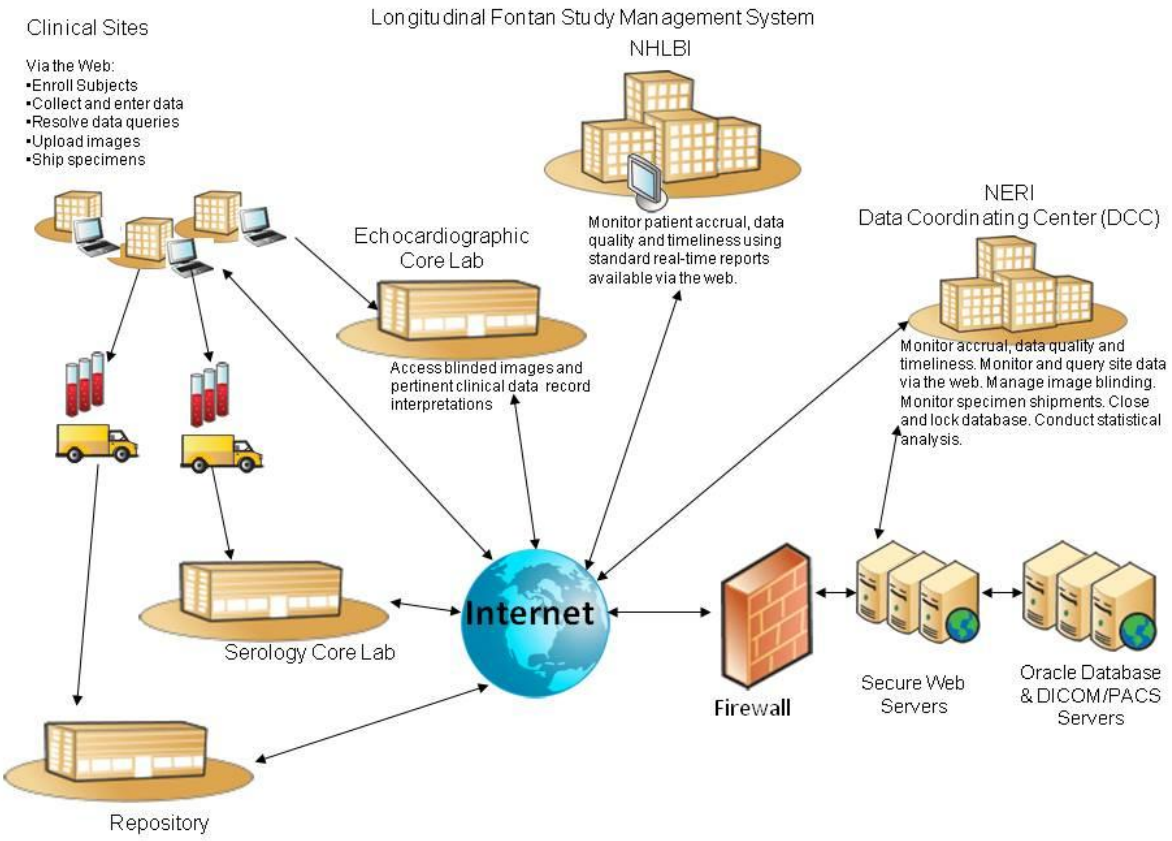
These subgroup comparisons will also be made through the regression analysis approach described earlier, by examining these pre-specified factors as candidate predictors of outcome.

C.6. Data Management

C.6.1 Information Flow

Data will be received from several sources, including clinical sites and Core Laboratories. Clinical sites will enter data over the Internet using the Advanced Data Entry and Protocol Tracking (ADEPT) software, a customized and secure Web application depicted in Figure 1 and described

below.



Sites will send samples directly to the Serology Core Laboratory and Biorepository. The DCC will electronically track the shipment and receipt of these specimens in the ADEPT Data Management System (DMS). Echocardiogram image files will be electronically transmitted directly to the Echocardiography Core Laboratory from the clinical sites or the DCC. Results of studies performed by the Echocardiography Core Laboratory will be directly uploaded to an Oracle database at the DCC.

C.6.2 Overview of Data Management System

ADEPT uses a browser-based user interface together with an Oracle relational database engine which allows direct data entry from multiple study sites or at NERI, and then stores these data centrally at the DCC. Information entered into the data entry system will be by subject study identification number; names will not be linked with subject data in the database. Clinical sites will maintain records linking the subject name with the main study identification number assigned for the study in secure areas. Sites will have full access to their own data and be able to view these data

remotely, over the Internet. A separate biorepository identification number will be generated by the DCC or the Biorepository for specimens, and the link will be maintained at the DCC only.

The ADEPT data entry system will include real-time field level validations and context sensitive help. Electronic data entry forms will be formatted to resemble closely the paper-based study instruments or worksheets, where applicable, or to enable direct data capture. These forms will be enhanced with client side code to ensure rapid data entry, proper validations of all data fields, and proper skip patterns within study data forms. Data will be saved at regular intervals during data entry to prevent loss of information in the event of a disruption of the Internet connection. In the unlikely event of a major disruption of the Internet infrastructure, the ADEPT system has a dial-in backup system to allow for access to the DMS.

The Web-based components of the data management system utilize several levels of security to ensure privacy and integrity of the study data. Access to data from both inside and outside the data center is controlled by Oracle's extensive security features. The Oracle archiving and back-up system ensures minimal data loss, even in the most catastrophic system failure. ADEPT's Oracle database is backed up nightly with backups rotated to a secure offsite facility on a routine basis. Data will not be stored on laptop computers.

C.7 Quality Control

This section describes the quality control program that will be implemented to ensure standard implementation of the protocol, protocol compliance, and data integrity. The DCC will develop and update the Manual of Operations in collaboration with study investigators and directors of the Core Laboratories and Biorepository. In addition, an ADEPT Manual will be developed for personnel at the clinical sites, Core Laboratories, and Biorepository who will be using the ADEPT data management system. The two manuals will serve as both training and reference manuals and will be accessible on PHN administrative website.

C.7.1 Clinical Center Coordinator Training and Certification

The DCC Project Manager, in collaboration with the study PI, will provide central training of clinical center staff in the areas of protocol implementation, data collection and management, collection and handling of imaging studies and biological specimens, medical records abstraction, and quality control expectations. Training slides will be prepared that reflect clearly and succinctly the learning goals for clinical coordinators and represent the skills and protocol components required to collect

quality data. Study PIs will be given additional training on the use of electronic signatures for signing electronic data capture (EDC) forms. Training follow-up will be completed through conference calls and site visits, as appropriate.

Before the study is started, each center must complete certification requirements, which include demonstration of familiarity with study procedures, methods for endpoint measurement, use of the database management system, and designation of staff to conduct the study. The DCC will work with each center on certification and notify NHLBI when it is achieved.

Echocardiography personnel at each center will undergo sessions on standardization of technique and complete certification requirements, as specified by the Echocardiography Core Laboratory.

C.7.2 Data Monitoring/Site Visits

Each clinical site may be visited once by representatives from the DCC and the NHLBI during the Fontan 3 study period. The primary roles of the site visit team will be to evaluate general protocol compliance and adherence to IRB requirements, review site data files for correct filing of copies of consent forms and study forms, audit a random sample of records to assess data integrity, and identify and resolve general problems with study progress. At each site visit, the site monitor will review procedures, observe form completion and data entry (where applicable), and assess adherence to protocols and flow. A random sample of medical records will be reviewed in order to determine whether reporting of data has been accurate and complete. Follow-up actions by the site coordinator or investigator and a schedule for completion will be identified at each site visit. An evaluation checklist will be completed at each site visit for inclusion in a Site Visit Report to the investigators. New staff will be trained and existing staff will be retrained, if necessary. Site coordinators will be expected to provide materials and answer questions prior to and during these visits.

Data monitoring for Fontan 3 will also be conducted remotely by the DCC. The remote data audit will be conducted by comparing data entered in study forms or directly into ADEPT to data in source documents for a random sample of study subjects. To conduct the audit, sites will be asked to submit copies of CRFs and a subset of appropriately de-identified source documents to the DCC. Any resulting discrepancies will be forwarded to the study team for resolution. All findings will be presented in writing to the study team in a Summary Report. The Summary Report will also be provided to the NHLBI Program Office.

The DCC may conduct site visits to the Core Laboratories and/or Biorepository to review in-house quality assurance (QA) and quality control (QC) procedures and data transfer to the DCC. Review of central laboratory-related reports will be conducted at least monthly to identify overall or site-specific problems in data or specimen acquisition and reporting of results.

D. STUDY LIMITATIONS

Outcome findings are conditional on the characteristics of the cohort who were enrolled in the original Fontan 1 study and on the cohort that could be recruited for this study, whose representativeness of all children with a Fontan circulation is unknown.

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