TRIAL OF ACE INHIBITION IN INFANTS

WITH SINGLE VENTRICLE

10-24-08

APPENDICES

Appendix A.

Sample Informed Consent Form for Trial

SAMPLE **INFORMED CONSENT FOR RESEARCH**

CONSENT TO PARTICIPATE AS A SUBJECT
IN MEDICAL RESEARCH
TRIAL OF ACE INHIBITION IN INFANTS WITH SINGLE VENTRICLE

PI:

IRB #

The Nature and Purpose of this study

We are asking you to let your child take part in a research study because your child has a heart abnormality – a one ventricle (lower chamber of the heart) instead of a two ventricle heart. This study is being done to find out whether a drug called Enalapril (an Angiotensin Converting Enzyme Inhibitor--ACE-I) will improve your child's growth and heart function in the first year of life. Enalapril is a drug that has been shown to improve heart function in adult patients with heart failure. This medication has not been studied in infants with one ventricle.

Approximately ____patients will be studied at _____. This study is also being conducted at all the Pediatric Heart Network sites and it is planned that a total of 230 patients will be enrolled from all of the sites. This study is being funded by the National Heart, Lung and Blood Institute (NHLBI) of the National Institutes of Health. Portions of Dr. _____and his(her) research team's salaries are being paid by this grant.

Explanation of Procedures to be followed

In order for your child to participate in this study, he/she must meet certain specific criteria, and you must sign this Consent Form. Your child's primary cardiologist has been responsible for the review of the medical information and has determined that your child may be eligible for this study. If you agree to allow your child to participate in this study, we will review and record information from your child's medical chart to get background information about your child's heart problem, how the heart condition was treated, and what other medical problems your child measured. Your child will also have blood drawn to check blood cell counts, blood chemistry, and kidney function.

If your child is eligible for the study and you have agreed to allow your child to participate in this study, he or she will receive either enalapril or an equal amount of placebo (an inactive substance that contains no active medication) along with his/her routine monitoring, medications, and care. The study medication will be determined by random assignment (like flipping a coin). Neither your child's doctor, the study staff, nor you will know which medication your child will receive. However, this information can be obtained easily by the study staff in the event of an emergency.

The study medication will be started at a low dose and will be increased over a period of time until the highest-tolerated dose, or the target dose is reached. This is called the up-titration phase of the study. Your child will take the study medication by mouth and all doses of the study medication will be based on your child's weight and how well

he/she tolerates each dose. During the uptitration phase your child's blood pressure, heart rate, and oxygen saturation (amount of blood oxygen, checked by placing a small band around your child's finger or toe) will be checked right before the dose of the study medication and every 30 minutes for the first two hours after the study medication is given.

If your child's blood pressure, heart rate, and oxygen saturation remain stable, your child will be considered to have tolerated the dose of the study medication. We will try to get your child up to the maximum study medication dose within 2 weeks after starting the study medication, either while he or she is still in the hospital or during his or her office visits. Once your child reaches this dose, he or she will continue the same dose for body weight throughout the maintenance phase of the study. The dose of the study medication will be adjusted as your child gains weight. The highest possible dose your child will receive is 0.4 mg/kg/day, given by mouth twice a day.

The study medication will not be given right after your child has the Glenn shunt surgery. Your child's doctors will restart your child on the study medication before your child goes home from the hospital after the surgery.

If your child is hospitalized for another illness or procedure, your child's cardiologist may want to stop the study medication and restart it once your child is better.

Your child will be evaluated for the study at the following time points:

- 4 days after starting the study medication
- 2 weeks after starting the study medication
- Before the Glenn shunt surgery
- After the Glenn shunt surgery
- At 10 months of age
- At 14 months of age

The evaluations may be performed while your child is in the hospital or during an office visit.

In addition to taking the study medication, your child will have several tests to evaluate how your child's heart is functioning during the 14 months your child is in the study. The next section explains these tests.

Echocardiogram: An echocardiogram is a painless test using sound waves that takes a 2-dimensional picture of your child's heart. Your child will need to lie quietly on a table

for about 30 minutes while the test is being done. In addition to the regularly scheduled echocardiograms that your child will have as part of his or her routine care, he or she may have an echocardiogram performed at entry into the study and will have an echocardiogram at the end of the study when he or she is 14 months old if there is no regularly scheduled echocardiogram between 13 and 15 months.

Your child may need medication to help him/her lie still during the echocardiogram performed when he or she is 14 months old. His/her breathing, heart rate, and oxygen level saturation in the blood will be monitored to be sure there are no side effects from the sedation medicine. The sedation medicine is a standard medication used to sedate infants for echocardiograms.

Questionnaires: As part of the study, you will be asked to fill out two questionnaires when your child is 14 months old. The first questionnaire, called the Functional Status IIR instrument, describes your child's general health and level of physical function. The second questionnaire is called the MacArthur Communicative Developmental Inventory. It measures your child's ways of communicating (talking and gestures). The study coordinator will explain the questionnaires to you and show you how to fill them out. You may ask him/her questions about how to complete the form, but he/she cannot help you decide what the answers are. The questionnaires will be given to you, and you can fill them out before your clinic appointment or during the time your child is at the center undergoing testing.

In addition, the Bayley Scales of Infant Development will be used to measure your child's level of development when he or she is 14 months old. This is a test that will be administered by a specially trained person to evaluate your child according to their developmental milestones - how well your child is able to do different things related to how old they are. For example, the specialist will evaluate whether your child can walk and talk, and how they play with toys. You will be told the results of this evaluation and if and your child has any delays in his/her development. The early identification of any delays will allow for early intervention.

Blood Samples: Your child will have the following blood tests drawn as part of this study:

- Complete blood count - to check the number of blood cells your child has;
- Electrolytes - to check your child's body salts;
- Serum creatinine - to check your child's kidney function;
- B-type natriuretic peptide level (BNP) - The blood level of BNP, a protein made by the heart, may be higher in infants with heart failure.

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The first three tests (complete blood count, electrolytes and serum creatinine) will be performed after 2 weeks on the study medication, after 7 days from the restart of the study medication following the Glenn shunt surgery and at 14 months of age. The total volume of blood required for all three of these tests at one visit is 1 milliliter, a little less than one quarter (1/4) of a teaspoon. These blood tests are also performed as part of routine care after 4 days of starting the medication and before the Glenn shunt surgery and the results will be recorded. Blood levels of BNP will be measured before your child's Glenn shunt surgery and at the visit when your child is 14 months old. Prior to drawing a blood sample at the 14 month visit, your child will need to lie quietly for 15 minutes and a cream that numbs the skin will be placed in the area where the blood will be drawn. Then a small needle will be placed into a vein and 1 milliliter, or a little less than one quarter (1/4) of a teaspoon of blood will be drawn from the needle.

The total amount of extra blood drawn during the whole study will be <5 ml (<1 teaspoon).

Cardiac Catheterization: If your child has a routine cardiac catheterization ordered by your child's cardiologist before the Glenn shunt surgery, the information from the test will be recorded for the study. Your child does not need to have a cardiac catheterization done to participate in this study.

Responsibilities of parents and subjects

During the study, you should tell Dr. _____or _____your child's medical history, all of the medicines your child is taking and about any pain or signs of illness experienced during the study. You must keep all of your child's scheduled visits with the study doctor.

Foreseeable Risks and Discomforts

Enalapril is an ACE inhibitor drug and it is approved by the Food and Drug Administration for use in children. ACE inhibitors are generally well tolerated in infants and children with heart failure. There is a small risk of renal insufficiency (kidneys not working as well as they should). Your child's kidney function will be closely monitored by drawing blood samples for serum blood urea nitrogen and creatinine levels as described above. Enalapril may lower the blood pressure in your child. In infants with only one ventricle, lower blood pressure may decrease the amount of oxygen in your child's blood. Your child's blood pressure, heart rate and oxygen saturation (amount of blood oxygen) will be closely monitored. A small number of children with single ventricle are known to experience a range of heart-related events, including sudden death. The effect of enalapril on these events is unknown. (NOTE: These last 2

sentences, in their entirety, must be included in your local consent form as mandated by the DSMB.)

Risks associated with drawing blood from a vein include momentary discomfort and/or bruising. Infection, excess bleeding, clotting, and fainting are also possible, although unlikely. To minimize discomfort, every effort will be made to obtain the blood samples needed for all research laboratory tests at the same time as other blood tests that are routinely performed for your child's care.

There are no risks associated with echocardiography. To obtain the echocardiographic pictures needed for this study, your child may need to be sedated for the examination at age 14 months. The risks of sedation include lower oxygen saturation, decreased breathing and low blood pressure. The heart rate, blood pressure and oxygen saturation will be monitored during the examination, and your child will be closely observed by his/her caretakers during the examination.

Benefits

If your child receives Enalapril, your child's growth and heart function may improve. Some children in this study will receive an inactive medication (placebo) and may show signs of benefit from the placebo. It is also possible that your child may experience no benefit from this study regardless of the medication to which he/she is assigned or even get worse.

We will be able to determine how your child's heart is functioning through the use of the tests described above. Your child may receive no direct benefit from study participation. However, the information obtained from the study may prove useful to other children who have decreased heart function.

You will be told if your child is found to have a delay in his/her development after the Bayley Scales of Infant Development evaluation is completed. You may then choose to arrange intervention for your child.

Alternative Treatments

The alternative to participating in this study is to have your child receive standard treatment for patients with a single ventricle (one chamber) heart.

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Voluntary Participation/Withdrawal

If the study medication is discontinued for a medical reason or at the request of you or your cardiologist, your child can remain in the study.

You may choose not to have your child be in the study, or, if you agree to have your child be in the study, you may withdraw your child from the study at any time. If you withdraw your child from the study, no new data about your child will be collected for study purposes unless the data concern an adverse event (a bad effect) related to the study. If such an adverse event occurs, we may need to review your child's entire medical record. All data that have already been collected for study purposes and any new information about an adverse event related to the study will be sent to the study sponsor.

Your decision not to have your child participate or to withdraw your child from the study will not involve any penalty or loss of benefits to which your child is entitled and will not affect your child's access to health care at _____. If you do decide to withdraw your child, we ask that you contact Dr. [PI] in writing and let [him/her] know that you are withdrawing your child from the study. [His/her] mailing address is [address].

Your child's participation in this study will be stopped if at any time it is determined by your doctor to be in your child's best interest.

A copy of this consent form will be provided to you for your records.

New findings

Any important information about how this drug affects children, or any new information that we learn during this study which may affect your child's condition or your decision to have your child continue taking part in this study will be given to you by Dr. ____or _____.

Confidentiality and Privacy of Information

The study results will be retained in your research record for at least six years or until after the study is completed, whichever is longer. Your child's medical record may also be reviewed in the six-year period after the study is completed to add medical events and measurements related to your child's heart condition to the research record. At that time either the research information not already in your medical record will be destroyed

or information identifying you will be removed from such study results at _____. Any research information in your medical record will be kept indefinitely.

Study records that identify you and your child will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. Except when required by law, you and your child will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of_____. All study information sent outside of _____ will be linked to your child through a study identification (ID) number and through a combination of your child's initials. The link between the study ID and your child will be kept in locked files at _____.

The tapes or disks with your child's echocardiogram will be sent to the Pediatric Heart Network Data Coordinating Center for submission to laboratories outside of _____ for reading. These tapes or disks, which may have your child's name on them, will be kept in locked files at these laboratories. Your child's name will not be recorded in any other records kept outside of _____.

Information gathered during this study and your child's medical records may be inspected and verified by staff representatives of the study sponsor (the National Institutes of Health), _____ Institutional Review Board, or the Pediatric Heart Network Data Coordinating Center. Medical records for this study and medical records from other institutions that contain your child's identity will be treated as confidential by the National Institutes of Health and will be shared only with these agencies, or as required by law.

<u>Costs</u>

There will be no additional costs to you as a result of your child's participation in this study. Tests required by the study and not part of your child's standard care will be provided to your child free of charge. You will be responsible for all other costs related to your child's medical care such as hospitalization, surgery, drugs, laboratory tests, diagnostic procedures and physician fees which are considered standard medical care for patients with your child's condition.

The following paragraph will be modified by each study site according to local guidelines. The study investigators will pay for travel expenses and food for your child and two family members/others on the days your child is scheduled for tests related to the study. If you live far away from the study center and an overnight stay is necessary

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to complete the study testing, you will be reimbursed for one night's lodging and meals. You will receive $_____ for the time required by your family to participate in the study.

Responsibility for Research-Related Risks

Immediate necessary medical care is available at _____ in the event that your child is injured as a result of your participation in this research study. However, there is no commitment by _____, or your [Institution] physicians to provide monetary compensation or free medical care to you in the event of a study-related injury. Further information concerning this and your child's rights as a research subject can be obtained from the _____ Office of Risk Management at _____.

Offer to answer questions about this study:

If you have any questions about this study, you should contact:
Dr. _____ Phone_____ Pager_____
Study nurse_____ Phone_____ Pager_____

For information or questions regarding your child's rights as a study subject you may contact:

_____

_____

To obtain information about study procedures, report a research related injury or ask questions about this research study, you should contact:

_____	_____	_____
Contact Name	Phone	Pager
_____	_____	_____
Contact Name	Phone	Pager

Agreement to Participate in the Study:

"The purpose of this study, procedures to be followed, risks and benefits have been explained to me. I have been allowed to ask questions, and my questions have been answered to my satisfaction. I have been told whom to contact if I have additional

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questions. I have read this consent form and agree for my child to be in this study with the understanding that I may withdraw my child at any time. I have been told that I will be given a signed and dated copy of this consent form."

_____ _____
Parent/Legal Guardian Signature Date

_____ _____
Signature of Person Obtaining Consent Date

****

Appendix B

Sample Informed Consent Form for Genotyping

The purpose of this consent form is to provide the information you need in considering whether to allow your child to participate in this research study.

Study Title: Trial of ACE Inhibition in Infants with Single Ventricle: Genotyping Sub-study

IRB study number:

Study Purpose

Your child is being invited to participate in this research study because your child has a heart abnormality – a one-ventricle (lower chamber of the heart) instead of a two-ventricle heart and he or she is participating in the "Trial of ACE Inhibition in Infants with Single Ventricle". The type of genes that a person carries can change his or her response to a disease or to the medications and procedures used to treat the disease. This sub-study will help to determine whether certain genes contribute to the way the heart adapts in infants with single ventricle hearts. The hearts of adult patients have been shown to respond differently to disease and medications, depending on the types of genes that are present. We will study all infants with single ventricle to determine if certain genes can affect the thickness and function of the heart and also how well the heart responds to treatment with the study medication, enalapril. This knowledge will help us understand the genetics of human disease, and may lead to progress in the prevention and treatment of heart disease in children.

Study Procedures

If you decide to allow your child to participate in this study, a blood sample will be drawn, if possible either during your child's cardiac catheterization procedure or during the Glenn shunt surgery or at another time that a blood sample is being drawn for other studies. Your child's sample will be sent to a centralized Core Laboratory for analysis. No more than 3 ml, or a little more than a half of a teaspoon, of blood will be collected for this purpose. The results of this testing will be analyzed along with the information about your child's medical history, heart function and the other data that is being gathered as part of the larger study.

Additional Procedures

None

Study Risks

None

Informational Risk

Your child's blood sample will be examined for genes that affect how his/her heart responds to treatment. Your child's name and other identifying information will not be sent to the laboratory performing the analyses. The sample will be analyzed along with samples from other children. The results of your child's genetic tests will not be released to you or your family and no formal genetic counseling can be provided, because the clinical importance of the genes being tested is not known. Your child's medical management will not be changed based on these results. 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 child.

You should be aware that insurance companies sometimes use information on genetic testing to deny coverage to applicants. This study involves research in genetics that could be used to develop such genetic testing in the future. The information obtained in this research study cannot provide any meaningful information about individual participants. Since this is the case, if you are asked, your child has not had a genetic test.

Under some circumstances, medical research, including genetic research, can lead to the association of a specific medical illness with a particular group of persons. This association could be viewed as harmful, but may also benefit the group if such research ultimately leads to earlier detection and treatment of the condition.

Study Benefits

Your child will not benefit personally from this study. He/she will not receive immediate medical benefit from participation, but the information will help us learn more about genes that may affect your child's heart condition. It is possible that in the future the study could help children with a similar heart condition by improving the way we treat children with heart disease

Alternatives

The alternative would be not to participate in this study. Your child's future care will not be affected by your decision.

Costs and Compensation

There will be no costs to you or payments made to you for participation in this study. (Institution) does not have any program to provide compensation for persons who may experience injury while participating in research projects. Further information about research-related injuries is available from the Office of the Institutional Review Board (Phone:_____).

Confidentiality

Confidentiality is a central concern of this sub-study of "Trial of ACE inhibition in infants with single ventricle". Any genetic information obtained during this study and associated with your child will remain strictly confidential. Once we take your child's blood sample, we will assign the specimen a unique identifier (a combination of letters and numbers). We will separate your child's name and any other information that points to your child's specimen. The results of the testing will only be sent to the Data Coordinating Center. Genetic information will not be part of your child's medical record. Your child's identity will not be revealed when research findings are presented or published.

Although every reasonable effort will be made to protect the confidentiality of your child's records, such protection cannot be guaranteed. Government regulatory agencies such as the Food and Drug Administration (FDA) and the Office of Research Protections (OHRP) may inspect the research records if needed. Information gathered during this study and your child's medical records might be inspected and verified by staff representatives of the study sponsor (the National Institutes of Health), _____ Institutional Review Board, or the Pediatric Heart Network Data Coordinating Center. We remind all persons participating in this research that the maintaining of complete confidentiality is a responsibility of both the investigator and his/her staff, and the participant. You should consider these issues carefully before consenting to allow your child to participate in the study.

Participation is Voluntary

Your child's participation in this study is completely voluntary. You can refuse to participate or withdraw from the study at any time and such a decision will not affect your medical care at _____ (Institution) now or in the future. The investigator is also free to terminate the study, or your child's participation in it, at any time. You may at any time request that your child's blood sample collected (or the materials in it, including genetic materials) be removed from our collection and destroyed. Signing this form does not waive any of your legal rights.

Storage of Research Samples

In addition to the studies that are planned, we would like to keep any unused DNA for future research. We may want to use the sample you have provided for future studies of cardiovascular disease. We may also want to analyze your child's DNA sample as part of other cardiovascular disease research activities or share portions of it with other cardiovascular disease researchers working in other institutions. The unused DNA sample will be given a unique genetic sample identifier (that is different from the identifier used for your child in the main study) and the sample will be stored at the Genetics Core Laboratory. The Data Coordinating Center will have a list that links the main study identifier and the genetic sample identifier. Your child's name will only be stored at _____Hospital and therefore no hospital staff member or investigator will have information that links your child's name to the genetic sample identifier. If we distribute your child's sample to other individuals who have an interest in the genetic causes of this disease, it will be released with the unique genetic sample identifier and without your child's name, medical record number, or main study identifier. This will make it very difficult for the doctor receiving the sample to find out the identity of the patient who provided the sample. You may choose not to have your child's sample stored for future research and still be part of the research study. Also, you may agree to have your child's specimen stored and later decide that you want to withdraw it from storage. If you make that decision, you should notify Dr. _____ in writing requesting that your specimen be discarded.

The following check boxes allow you to choose whether or not you agree to the storage of your child's sample for future research. Please read the following statements and check and initial one or more of the following:

- ❑ I AGREE to allow my child's DNA sample to be stored for future cardiovascular disease studies that are related to this research study.

_____ Initials of Parent or Legal Guardian

- ❑ I AGREE to allow my child's DNA sample to be stored for future cardiovascular disease studies that are not related to this research study.

_____ Initials of Parent or Legal Guardian

- ❑ I AGREE to allow my child's DNA sample to be shared for cardiovascular disease research by other investigators who are related to this research study.

_____ Initials of Parent or Legal Guardian

- ❑ I AGREE to allow my child's DNA sample to be shared for cardiovascular disease research by other investigators who are not related to this research study.

_____ Initials of Parent or Legal Guardian

- ❑ I DO NOT AGREE to allow my child's DNA sample to be stored for future research.

_____ Initials of Parent or Legal Guardian

Questions

If you have any questions about this study, you can reach

Dr. _____ Phone_____ Pager_____

Study nurse _____ Phone_____ Pager_____

If you have any questions on your child's rights as a research subject, you can call the Institutional Review Board at _____ for information.

STATEMENT OF CONSENT

"The purpose of this study, procedures to be followed, risks and benefits have been explained to me. I have been allowed to ask questions, and my questions have been answered to my satisfaction. I have been told whom to contact if I have additional questions. I have read this consent form and agree for my child to be in this study with the understanding that I may withdraw my child at any time. Signing this form does not waive my legal rights. I have been told that I will be given a signed and dated copy of this consent form."

_____ _____
Parent/Legal Guardian Signature **Date**

_____ _____
Signature of Person Obtaining Consent **Date**

INVESTIGATOR'S STATEMENT

I have fully explained the nature and purpose of the above-described procedures and the risks involved in its performance. I have answered and will answer all questions to the best of my ability. I will inform the subject/family of any changes in procedure or the risks and benefits if any should occur during or after the course of the study. I have given a copy of the consent form to the subject/family. I have offered an opportunity for further explanation of this procedure to the individual whose signature appears above.

_____ _____
Investigator eliciting consent **Date**

The solicitation of subjects into this study has been approved by the _____(Institution) Institutional Review Board.

APPENDIX C

MEASUREMENTS OF SOMATIC GROWTH

APPENDIX C. MEASUREMENTS OF SOMATIC GROWTH

Measurements of weight, recumbent height, and head circumference will be performed according to the guidelines developed by the U.S. Department of Health and Human Services, Health Resources and Services Administration, Maternal and Child Health Bureau (MCHB) and the Centers for Disease Control and Prevention (CDC). All study personnel who will obtain height and weight measurements will complete the Growth Charts Training provided on the HRSA website:
http://128.248.232.56/mchbgrowthcharts/module4/text/mainintro.htm

Equipment to determine accurate and reliable recumbent height, weight and head circumference will conform to the MCH/CDC specifications described on the HRSA website:
http://128.248.232.56/mchbgrowthcharts/module4/text/page1a.htm

Weight, recumbent height, and head circumference will be normalized to the patient's age using data from the National Health and Nutrition Examination Survey available on the CDC website:
http://www.cdc.gov/nchs/about/major/nhanes/growthcharts/zscore/zscore.htm

APPENDIX D

ROSS HEART FAILURE CLASS

APPENDIX D. ROSS HEART FAILURE CLASS [1]

Infants will be examined in a quiet room after they have been in a steady, resting state for 15-30 minutes. The severity of heart failure will be measured using the Ross classification.

Ross' Classification of Congestive Heart Failure:

Class I: No limitations or symptoms

Class II: Mild tachypnea and/or diaphoresis with feeds in infants; dyspnea on exercise in older children. No growth failure.

Class III: Marked tachypnea and/or diaphoresis with feeds or exertion and prolonged feeding time with growth failure

Class IV: Symptomatic at rest with tachypnea, retractions, grunting or diaphoresis

Reference

1. Ross, RD, Daniels, SR, Schwartz, DC, et al. Plasma norepinephrine levels in infants and children with congestive heart failure. Am J Cardiol 1987; 59:911-914.

APPENDIX E

B-TYPE NATRIURETIC PEPTIDE

APPENDIX E. B-TYPE NATRIURETIC PEPTIDE

A volume of 1 ml of peripheral venous blood will be collected into a pre-chilled lavender-topped tube containing EDTA after the subject has been in a sitting/supine position in a quiet room for 15 minutes. The pre-Glenn shunt BNP level will be drawn after the patient has been sedated for either a cardiac catheterization or open heart surgery. At age 14 months, a topical anesthetic will be applied to the area of the venipuncture in order to minimize patient agitation.

Once drawn, the tube of blood will be inverted to mix thoroughly and immediately placed on ice. The Serology Core Laboratory will provide the centrifugation method and timing instructions to the clinical sites. Typically, the sample is centrifuged at 3000 rpm for 15 minutes at 4^{o}C. A minimum of 0.5 ml plasma is required. Plasma aliquots will be shipped and stored at -80^{o}C until analysis. Each vial will have a label identifying the patient study identification number and sample date.

The Serology Core Laboratory will determine the specific type of immunoradiometric assay (Shiniogi BNP-32 Human Assay versus Biosite Triage) that will be used to measure B-type natriuretic peptide levels in plasma. The units are pg/mL. Results are reported with up to 4 significant digits. If values are < 100, results will be reported with one decimal place. Values > 100 will be reported without decimals. The lower detection limit is 4 pg/ml ~~and the upper detection limit is 1300 pg/ml~~. The within-assay coefficient of variation is 2% at a concentration between 12.5-150 pg/ml, and 3.7% at a concentration of between 150 and 680 pg/ml.

APPENDIX F

NEURODEVELOPMENTAL AND FUNCTIONAL STATUS

APPENDIX F. NEURODEVELOPMENTAL AND FUNCTIONAL STATUS

Bayley Scales of Infant Development®—Second Edition **(BSID–II)** offers a standardized assessment of cognitive and motor development for children ages 1 month through 42 months. BSID–II was renormed on a stratified random sample of 1,700 children (850 boys and 850 girls) ages one month to 42 months, grouped at one-month to three-month intervals for the variables of age, sex, region, race/ethnicity, and parental education. BSID–II computes two scores, the Mental Development Index and the Psychomotor Development Index. A behavior rating scale is also incorporated into the testing to assess the degree of patient cooperation.

The Mental Development Index yields a normalized standard score evaluating a variety of abilities: sensory/perceptual acuities, discriminations and response; acquisition of object constancy; memory, learning, and problem solving; vocalization, beginning of verbal communication; basis of abstract thinking; habituation; mental mapping; complex language; and mathematical concept formation.

The Psychomotor Developmental Index yields a normalized standard score that assesses these skills: degree of body control, large muscle coordination, finer manipulatory skills of the hands and fingers, dynamic movement, dynamic praxis, postural imitation, and stereognosis. This score reflects a child's progress in large muscle activities (e.g., sitting, crawling, walking, climbing) and in prehension skills (e.g., visually-directed reaching, grasping).

The BSID-II will be administered by a trained developmental specialist at each institution using the standard kit available through the Psychological Corporation, San Antonio, Texas. The estimated time for performing the BSID-II ranges between 1-2 hours depending on patient cooperation.

MacArthur Communicative Development Inventory/Words and Gestures (CDI):
The MacArthur Communicative Development Inventory/Words and Gestures (CDI) is a parent-report instrument for assessing early language skills, designed for use in children 8 to 16 months of age. Use of the CDI will complement the Bayley Scales by providing a detailed assessment of several important aspects of early cognitive development (specifically symbolic) that are difficult to measure in a brief developmental assessment. The instrument is attached. This instrument has been validated in both English and Spanish, and will be administered in both English and Spanish depending on the language preference of the parent/primary caregiver.

Functional Status II-Revised Questionnaire: The version of this questionnaire designed expressly as a parent report for parent/caregivers of children less than 5 years old is included in this Appendix. This questionnaire has been modified after consultation with the author of this instrument to be self-administered in this trial, rather than completed in an interview format, and requires 15-20 minutes to complete. This instrument will be administered in both English and Spanish depending on the language preference of the parent/primary caregiver.

APPENDIX G

ECHOCARDIOGRAPHIC PROTOCOL

APPENDIX G. ECHOCARDIOGRAPHIC PROTOCOL

Study Equipment:

1. Echocardiographic imaging system equipped with transthoracic transducers appropriate to patient size.
2. Studies will be recorded in either sVHS format with standard 1/2" sVHS videotape or in any of the several full-motion digital formats in common use (MPEG-I, MPEG-II, MPEG-IV, motion JPEG, AVI) with a minimum clip length of 10 seconds.

Timing of Studies:

Three echocardiograms per subject will be evaluated by the Core Laboratory. At the local clinical center, an echocardiogram performed before study enrollment will be reviewed by the site investigator to evaluate ventricular function and atrioventricular valve regurgitation to evaluate trial eligibility, and then submitted to the Core Laboratory. Two additional echocardiograms, performed according to the acquisition protocol below, will be obtained: prior to the Glenn shunt surgery and at age 14±1 months.

Study Acquisition (Pre-Glenn and age 14-month study):

1. Height and weight: Patient height in centimeters and weight in kilograms will be measured at the time of echocardiography.
2. Blood pressure: An automated blood pressure device (such as the Dinamap) will be used to record multiple right brachial blood pressures during echocardiographic assessment.
3. Moderate (conscious) sedation may be required to obtain the echocardiographic images necessary for analysis. If sedation is needed, the echocardiogram will be performed under moderate sedation according to the Sedation Policy at each study center and will conform to the "Practice guidelines for sedation and analgesia by non-anesthesiologist" (1).
4. Two-dimensional echocardiography: In addition to complete orthogonal sweeps from subxiphoid, apical, parasternal, and suprasternal notch windows, the following specific information pertinent to derivation of the indices of systolic and diastolic ventricular function is required:

A. *Two-dimensional recording of the ventricular short axis:* The short axis image will be obtained at the position of the largest short axis cross-sectional area in a plane orthogonal to the long axis of the systemic ventricle.
B. *Two-dimensional recording of the ventricular long axis:* The long axis image will be recorded in the plane transecting both atrioventricular valves (if both are present) and intersecting the true apex of the ventricle.
C. *Two -dimensional recording and measurement of the semilunar annulus :* Parasternal long axis images of the semilunar root(s) will be recorded with zoom mode activated to maximize resolution of the semilunar annulus.
D. *Color Doppler assessment of the severity of atrioventricular valve regurgitation:* In subjects with atrioventricular valve regurgitation, color Doppler images of the proximal jet width including the *vena contracta* are to be recorded from apical transverse and parasternal long-axis views (2).
E. *Color Doppler assessment of the semilunar valve(s):* From apical and parasternal long axis views, color Doppler samples of the ventricular outflow tract(s) are recorded with the color sector placed in the ventricular outflow tract below the semilunar valve(s).
F. *Spectral Doppler recording of the atrioventricular valve inflow jet:* 2D color Doppler mode is used to direct spectral Doppler recording of the atrioventricular valve inflow. If two atrioventricular valves are present, the assessment is carried out on each.
G. *Spectral Doppler recording of the pulmonary vein inflow jet:* Color Doppler directed sample of pulmonary vein Doppler just proximal to the point at which the jet emerges within the left atrium.
H. *Spectral Doppler recording of the semilunar outflow jet(s).*

<u>Core Laboratory Data Processing and Analysis:</u>

1. Original recording of analog or digital recordings will be submitted to the Core Laboratory. Each echocardiographic study from each subject must be submitted on a separate tape/CD.

2. Data primarily recorded on sVHS video tape will be converted to digital format using a video digital capture system at a temporal and spatial resolution sufficient to avoid any information loss in the data conversion.
3. Measurements will be performed on a microcomputer-based workstation custom programmed for electronic caliper overlay of captured digital images for recording

The following measured and derived parameters will be obtained:

Ventricular size and function: End-diastolic (frame at which atrioventricular valve closure occurs) and end-systolic (frame preceding atrioventricular valve opening) endocardial and epicardial borders of the ventricle on long and short axis images, excluding the papillary muscles but including outflow tract(s), will be used to compute volumes using a modified Simpson's rule algorithm (3) to provide end-diastolic and end-systolic volumes, ejection fraction, ventricular mass, and mass:volume ratio. In patients with two ventricles that contribute to systemic outflow (for example, mitral atresia with ventricular septal defect), the ventricular volumes will be calculated separately and subtracted from total epicardial volume to obtain total ventricular mass. The shape of the ventricle(s) is quantified as eccentricity from the end-diastolic long axis dimension (L) and short axis area (A) as Eccentricity = $[L^2 - (4A/L)^2]^{0.5}/L$ (2).

Cardiac index and systemic resistance: The aortic velocity-time integral (VTI_{aortic}) is obtained from the aortic outflow Doppler and measured aortic valve diameter is used to calculate flow area (FA) to obtain stroke volume (SV) as SV = (VTI_{aortic})(FA), Cardiac output (CO) as CO = (SV)(Heart Rate), and cardiac index (CI) as CI = CO/BSA, where BSA = body surface area calculated from height and weight according to the method of Haycock (6). Cardiac output will not be determined in patients with two sources of aortic outflow. Systemic resistance is calculated as (CO)/(mean arterial pressure).

Doppler indices of diastolic function: Numerous derived variables have been reported from these tracings, but those which are considered of most interest are the ratio of peak early velocity (E_P) to peak atrial velocity (A_P), the early deceleration time, and

the duration of the atrial contraction-related retrograde pulmonary vein Doppler signal (7).

Atrioventricular valve regurgitation

1. *Color Doppler grade:* the degree of atrioventricular valve regurgitation will be graded by the area of regurgitant jet vs. area of atrium and the degree of extension into pulmonary vein.
2. *PISA calculation of atrioventricular regurgitant volume:* An isovelocity hemisphere is identified within the proximal flow convergence zone positioned a distance of between 2 and 3 times the diameter of the *vena contracta* from the plane of the regurgitant orifice. The radius of the hemisphere is measured frame-by-frame over the course of systole and the per-frame regurgitant volume is calculated as the flow velocity at the hemisphere times the hemispheric surface area. The regurgitant volume is then obtained by integration over the cardiac cycle (8).
3. The proximal jet width will be measured directly.

Semilunar valve regurgitation: The degree of semilunar valve regurgitation will be graded by the width of proximal color Doppler jet at the level of the semilunar valve.

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~~APPENDIX H~~

~~CARDIAC MAGNETIC RESONANCE IMAGING PROTOCOL~~

NOTE: The MRI has been dropped as a study test from the trial and this appendix was deleted on 5-2-05.

APPENDIX I

GENOTYPING STUDIES

APPENDIX I. GENOTYPING STUDIES

A volume of 3-5 ml of peripheral venous blood will be collected in an EDTA tube. The samples will be labeled with the patient study number and sample date and and shipped overnight at room temperature.from the participating institution to the Genetics Core Laboratory.

DNA will be extracted from whole blood using the QIAmp Blood Kit 250 (Qiagen Valencia, CA). Polymerase chain reaction of genomic DNA segments is achieved using a standard thermocycler. Details of the amplification protocols, oligonucleotide primer sequences, and diagnostic restriction enzymes have been described [1, 2]. DNA sequence polymorphisms in the candidate genes will be tested and an additional set of primers will be used to evaluate the I/D polymorphism of the ACE gene to prevent misclassification of I/D as D/D. The Genotyping Core Laboratory will determine the techniques to be utilized to evaluate the multiple alleles. The following genes will be evaluated: ACE gene, angiotensinogen gene, angiotensin II receptor type 1 gene, aldosterone synthase gene, and cardiac chymase A gene.

DNA samples will be stored frozen at -70°C in the Core Laboratory and may be used in the future specifically for evaluation of newly-identified genetic polymorphisms related to ventricular hypertrophy or other cardiovascular diseases.

References

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