

Jackson Heart Study

Manual 3

Family Study

Version 1.1

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For Copies, Please Contact
Jackson Heart Study Coordinating Center
Jackson Medical Mall
350 W. Woodrow Wilson Dr.
Jackson, MS 39213

FOREWORD

This manual is one of a series of protocols and manuals of operation for the Jackson Heart Study (JHS). The complexity of the JHS requires that a sizeable number of procedures be described, thus this rather extensive list of materials has been organized into the set of manuals listed below. Manual 1 provides the background, organization, and general objectives of the JHS Study. Manuals 2 and 3 describe the operation of the Cohort Procedures and Family Study Components of the study. Detailed Manuals of Operation for specific procedures, including those of reading centers and central laboratories, make up Manuals 3 through 9. Manual 10 describes the Cohort Surveillance Component of the study and Manual 11 details the Data Management System.

JHS Study Protocols and Manuals of Operation

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|---------------|--|
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| 2 | Cohort Procedures |
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| 4 | Blood Pressure |
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1.0 INTRODUCTION, BACKGROUND AND HYPOTHESES

It is generally accepted that most common cardiovascular diseases and associated risk factors are the result of the complex interplay between multiple environmental and genetic factors. The excess CVD risk experienced by African-Americans relative to their white counterparts in the United States may be the result of disproportionate exposure to harmful environmental factors (e.g., diet and stress), increased frequency of deleterious genes or the complex interplay between these risk factors. Improvement in molecular techniques and findings from the Human Genome Project makes it possible to study the contributing influence of genetic factors to the pathophysiology of common complex diseases including most forms of CVD. Evidence from ongoing studies of the genetic epidemiology of CVD shows that differential distribution of CVD and associated risk factors may in part be due to ethnic differences in gene variants. Recent molecular evidence shows that the influence of some gene variants is similar across ethnic groups while other variants are more important in specific ethnic groups including African-Americans. The Jackson Heart Study (JHS) promises to contribute to our understanding of the genetic variants relevant to increased susceptibility and/or resistance to heart, lung and blood disorders in African-Americans and other ethnic populations in the US and across the globe.

Extensive work has been undertaken to better explain the genetics of CVD and associated risk factors including hypertension, obesity, and diabetes. Findings from the renin-angiotensin aldosterone system, an important pathway in water and salt regulation, have identified several genetic variations that may increase susceptibility to hypertension in certain ethnic groups. In comparison to Caucasians, some of these genetic variations are more common in ethnic minority populations including African-Americans. However, the significance of these genetic variants in the pathophysiology of hypertension and other CVDs has been inconsistent within and between populations.

In addition, several investigators have uncovered specific single-gene defects leading to hypotension and hypertension in small populations. These defects, which obey simple Mendelian principles, include glucocorticoid remediable aldosteronism, apparent mineralocorticoid excess and Liddle's syndrome. However, these and other forms of inherited hypertension account for only a small fraction of essential hypertension. Similarly, work in diabetes and obesity has uncovered rare forms of disease that are explained by a single gene defect. Despite explaining only a small proportion of the observed genetic variations, these advances have provided invaluable insight into the pathophysiology of these diseases, which may lead to novel therapeutic and preventive strategies in the future.

The proposed JHS genetic research seeks to identify new genes important to heart, lung and blood disorders in African-Americans. In addition, knowledge of candidate genes will be used to investigate the effect of known variants on disease risk in African-Americans. To date there have been few large biomedical studies involving families of African origin. Therefore, replication of genetic findings from other populations of African origin is also an important goal of the JHS. Because of the complex interaction of risk factors for heart, lung and blood disease, a major goal of the JHS is investigation of genotype-environment interaction in determining disease risk.

The JHS genetic research is further defined to focus on heart, lung and blood disorders where there is a disproportionate risk in African-Americans. For example, hypotheses focus on genetic and environmental risk for obesity, particularly in African-American women. The JHS will have a unique opportunity to identify new genes involved in the development of renal disease and hypertension. Other areas of interest include identification of new genes predisposing to left ventricular hypertrophy and disentangling the genetic and environmental factors leading to diabetes in African-Americans.

1.1 Cultural Sensitivities

There is a long history of mistrust of the medical scientific community among African-Americans. This mistrust manifests itself in several ways including low rates of involvement of African-Americans and other minorities in medical research and skepticism in adopting the resulting recommendations. In addition, the willingness of some investigators to use genetics to explain ethnic differences (e.g., performance on IQ tests and mental illness), especially in the absence of rigorous evidence, has unquestionably contributed to further alienation of ethnic minority populations with respect to participation in biomedical research activities.

It is obvious that any investigations of racial/ethnic differences in cardiovascular or other diseases (like cancer or mental illness) have to be undertaken in such a way as to preserve the dignity of all participants. This may be particularly true of genetic studies given the widespread belief that genetic determinants of disease cluster in racial/ethnic groups. In this regard, the JHS must be conducted in a manner that adheres to the desires and concerns of the greater Jackson area African-American community. The JHS investigators have engaged in dialogue with the community concerning this issue and will continue this dialogue as the study progresses and newer genetic techniques evolve.

The JHS investigators will pay particular attention to issues of race and privacy whenever dealing with genetic materials. The primary use of the data will be to attempt to better explain CVD and to identify potential preventative and therapeutic strategies. As there may be differences from one racial or ethnic group to another in the genetic and environmental contributions to CVD, we may discover genes that are more or less important in African-Americans than in other groups. There must be care to foresee what may be implied or inferred, yet not stated, when genetic data are reported. These steps are absolutely necessary for the JHS data to be accepted and effectively applied for the reduction of CVD morbidity and mortality in African-Americans.

2.0 DESCRIPTION OF THE JHS FAMILY STUDY

The JHS Family Study is designed to detect new genes influencing risk factors for a variety of heart, lung and blood disorders. To this end, family enrollment is based on a random ascertainment strategy. In addition, the Family study will be nested within the JHS population-based sample, such that each *participant eligible to participate in the JH cohort study is a potential proband (initial family contact) for the family study*. This will ensure that results obtained from the Family Study are generalizable to the greater JHS community.

1. To be eligible, an index family member must be between the ages of 35-84 years;
2. Live in the Jackson tri-county area;
3. Self identify as African-American; and
4. Have an appropriate family structure and family members who are willing to participate in the JHS family study.
5. In addition, attempts will be made to enroll equal numbers of male and female index family members to avoid subtle biases in family structures.

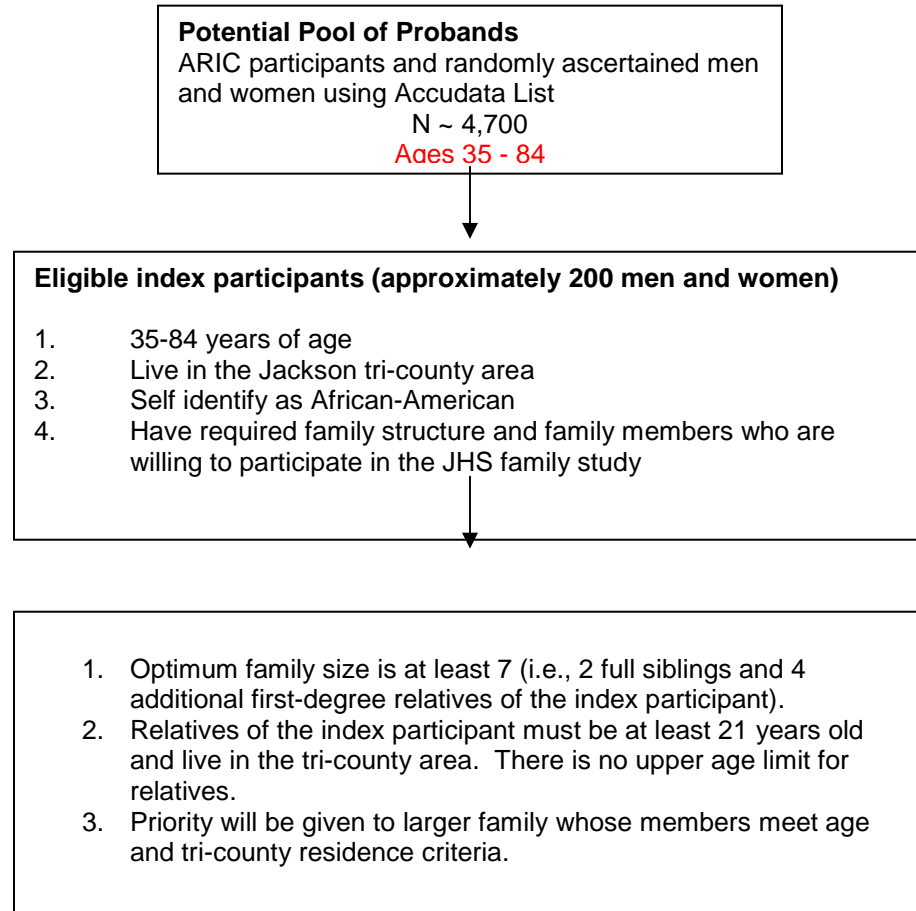
Figure 1 shows a schematic diagram of the relationship between the random population-based sample and the family sample. It may be necessary to sample outside of our age range to obtain families of sufficient size. There will be **no upper age limit** for relatives of index participants. However, **to be included in the Family Study, secondary family members must be at least 21 years old**. In addition, some family members may not be of African descent. These members will be included in the family study as they clearly bear on familial determinants of CVD.

The total number of individuals in the family study is expected to be 2,000. Efforts will be made to recruit the largest families available with an **optimum family size of at least twelve**, resulting in approximately 160 families depending on the family size.

3.0 COMMUNITY INVOLVEMENT IN GENETIC RESEARCH

The success of the Family Study of the JHS rests, in large measure, on the co-involvement of the Jackson metropolitan community as partners with the research team. This partnership and sharing of power includes sharing in decision-making on central aspects of the design, ongoing evolution and monitoring of the Family Study. Creating opportunities for the community and research partners to think together about new solutions for the age-old issues of suspicion and mistrust surrounding the gathering and use of genetics materials is central to informed community co-participation. Two exemplar opportunities are addressed below as community decision-making and community education. Additional opportunities will be incorporated as appropriate throughout the course of the JHS.

Figure 1. Jackson Heart Study Sampling Plan



Total number of participants for the JHS

| | |
|----------------------------|--|
| Randomly ascertained | 4,500 (including ~ 200 index participants) |
| Family members of probands | <u>2,000</u> |
| | <u>6,500</u> |

3.1 Community Decision-making

A process for community decision-making in the JHS is described in Manual 1 in the section defining the “Partnership for Community Awareness and Health Education (PCAHE)”. The PCAHE is designed to serve as the “voice” of the community with power to influence decision-making on JHS issues vested in its two voting Steering Committee members. In keeping with this process model, one or more representatives of the PCAHE will participate in the Genetics Committee (GC). This representative(s) will act as a liaison between the GC and the PCAHE to assure community co-participation in all decisions regarding the Family Study. Such level of involvement will allow the representatives attending the GC meetings to relay information on issues, discussions and recommendations reciprocally between the GC and the PCAHE. The process will make provisions for sufficient time for input and dissemination of information to the full PCAHE in order to provide its two Steering Committee voting representatives the opportunity to express the desires of the community during Steering Committee meetings.

A specific process for time-sensitive decisions will be developed by the GC and PCAHE to assure community co-participation within realistic time constraints, which will not jeopardize elements of study protocol.

3.2 Community Education

Community capacity building is a major goal of the JHS. Improving community knowledge and understanding of key issues in cardiovascular health through ongoing interactive community education is an expected outcome of this study. Providing opportunities for educational dialogue regarding issues of family history and genetics creates another opportunity for shared learning and new research partnerships with the community at-large. As researchers and community come together to learn more about genetic and environmental aspects of disease, possibilities for new conversations about community concerns such as cloning, opening themselves to DNA identification for other than research purposes, the ongoing legacy of Tuskegee, and others emerge.

Education and community dialogue about issues of family history and genetics will be enacted as one component of the Community Mobilization Health Education Plan. In keeping with the overall design of this plan, education will occur on multiple levels including dissemination of educational materials, “train the trainer” family/genetics sessions, community education forums on family history and genetics as CV risk factors, among others. As few materials are available for this type of programmatic effort, initial efforts will be directed to developing culturally sensitive materials with input from collaborating consultants and community co-investigators. Sample educational materials from other projects including a family/genetics component will be sought as models. Input regarding community education needs and desires specific to the JHS will be obtained from the CRC, the Community Mobilization Advisory Committee and the Council of Elders throughout the process. These materials will be disseminated widely to the JHS cohort and the community at-large.

Genetics training workshops were initiated in March, 2000 as an ongoing component of community education. These workshops, which will include a train the trainer component, will be conducted periodically by genetics collaborators and consultants. These efforts will include JHS investigators and staff and select community members. The PCAHE, as both representatives of and liaisons to the community at-large, along with interested members of the Council of Elders, will be invited to take part in the next series of family/genetics workshops. These sessions will serve to both increase understanding of the key issues among the co-investigators and to establish a “train the trainer” model for conducting ongoing community forums for genetics information and discussion. It is anticipated that at least two rounds of these sessions will be conducted to create a large enough cadre of community/investigator teams to conduct these forums.

Ongoing community education and dialogue forums will be offered throughout the study area in places where the local community gathers. These forums will be co-led by a community trainer and a JHS investigator/staff to provide opportunities for information dissemination about family/genetics and community discussion of issues of importance. A record of community discussions and issues will be

kept from these sessions with a summary provided to both the CC and the GC on a quarterly basis to assure ongoing consideration of community concerns in the Family Study.

4.0 COLLECTING FAMILY INFORMATION

Information regarding potential eligibility for the Family Study will be gathered from all eligible JHS participants (ARIC, random community sample and family sample) during the initial Home Induction Interview. Select questions (items 10-19) on the Eligibility (ELG) Form (see Manual 2) will ascertain the number of family members (grandparents, parents, siblings, children, aunts and uncles, nieces and nephews) living in the tri-county sampling area for the JHS who are 21 years of age or older. If a participant has at least two siblings and four other first-degree relatives (parents, siblings, children) who are ≥ 21 years old and live in the tri-county area, his/her record will be flagged, indicating eligibility for the family sample. The participant will be told that s/he is eligible for the family study and will be asked to bring family contact information to the initial clinic visit.

The recruiter will inform clinic staff by telephone of the upcoming appointment of each "family study eligible" participant, and will indicate this information in the participant's profile sheet. Clinic staff is thus alerted to gather additional family information during the clinic visit, including production of a graphical pedigree (using Progeny2000®; see "Data Management") and completion of a Parental Identification Form (PIF) (Appendix 1). This process also alerts laboratory staff that blood is to be drawn for cryopreservation of lymphocytes. The graphical pedigree will include the full name and age of each person in the pedigree, to the extent known by the participant. "Jr.", "Sr.", "III", etc., will be included where appropriate. Members of the pedigree who live in the tri-county area will be identified in the graphical pedigree and this information will be recorded automatically to a tabular data field in Progeny2000®. If family members other than the informant have participated in JHS, their name(s) will be recorded in designated spaces on the PIF. Before the participant leaves the clinic all available family contact information will be recorded in Progeny, with emphasis on first-degree relatives. Permission to contact each family member will be sought and responses will be recorded. In some cases the participant may be asked to locate additional contact information that will be collected when 24-hour exam components are picked up.

4.1 Instructions to Interviewers

4.1.1 Gathering Family Information

For the African-American community the family has a wider circle of members than the word suggests in European cultures. In traditional European society, the family includes children, parents, grandparents, uncles, aunts, brothers and sisters who may have their own children, and other immediate relatives. In many other cultures, including African and African-American the most common concept is extended family. The range of family members can be extensive and even include non-blood/biological members. In this kind of familial relationship, family members, often children are sent to live with relatives, and these children are counted as members of the families where they happen to live. Also the blending of families has always been prevalent in the A/A communities, with there being children from prior relationships from either parent, common-law relationships, grandparents raising their grandchildren or an array of configurations of the extended family unit. Terms such as adopted, step or half are generally not used because the sense of family diffuses beyond the Western concept of blood or biological kinship. This kinship system is like a vast network stretching laterally (horizontally) in every direction, to embrace everybody in any given local group. This means that each individual is a brother, or sister, father or mother, grandmother or grandfather, or cousin, or brother-in-law or sister-in-law, uncle or aunt or something else to everybody else. That means that everybody is related to everybody else. That means that the precise relationship existing between two individuals. Thus understanding the dynamic definition of family and relationships is key in completing a graphical pedigree for the African-American community.

In these kinds of alternative family arrangements, terms such as adopted, step and half are not conceptualized for the average African-American. Below are the terms defined by Webster's dictionary in reference to the terminology used by researchers:

- Stepfather-one's mother's husband who is not one's natural father
- Stepmother-one's father's wife who is not one's natural mother
- Stepson-the son of one's spouse by an earlier marriage
- Stepdaughter-the daughter on one's spouse by an earlier marriage
- Stepbrother-the son of one's stepparent by an earlier marriage
- Stepsister-the daughter of one's stepparent by an earlier marriage
- Half brother-a brother related through only one parent
- Half-sister- a sister related through only one parent
- Adopted-to-take a child into one's family legally and raise as one's own.

Most of these terms are based upon the ideal of a legal marriage. In the African-American community this is not necessarily a prerequisite for defining the official family structure. There may be blended families, common-law relationships, or children/adults inherited (by various ways and raised as one's own, but not necessarily involving the formal legal procedures). There are no literal word substitutions for the aforementioned terms; however there will be stories and phrasing for the same concepts. And these concepts can be captured through a culturally relevant understanding of the dynamic familial relationships that exist in the African-American community.

This concept of African-American family was discussed with several African-American psychologists (Drs Cynthia Ford, Pamela Banks, Mary Ann Jones-Gali) and a clinical psychologist, Dr. Althea Henry from Syracuse. Dr. Henry commented on the search to define family relationships by saying, "It's not until you dig a little deeper do you find that siblings have different mothers or fathers. So people will usually say as an example, 'my sister on my father's side'". One has to probe to get further clarification. They all agreed that using the Western terminology of the traditional family unit was not useful in discovering blood relationships.

They all thought that asking people initially "name your children/siblings" then asking each person's parents name would be a non-invasive and indirect way of getting the same information without using the non-descriptive, and insensitive terminology of adopted, half and step. This would allow people to become comfortable with the subject matter, feel at ease to disclose private information, and tell the story of their complex and extended pattern of family relationships. With the information the interviewer and researcher would be able to appropriately discern the graphical pedigree or family tree for the individuals.

By changing the way in which you ask the family tree or graphical pedigree questions, such as administering the table framework and allowing the participant to include all siblings and children (as the participant defines) you can then query on the possible differences in the family framework and relationships. Phrasing such as "there are different ways in which people define and call family and we want to know how distinct and unique your family is..." can be utilized to frame the discussion.

A full and accurate understanding of the African-American family for the purposes of the JHS will occur only when it is conceptualized, studied and evaluated in terms of its own intrinsic definition. This task requires in itself, new theory, new analytical frameworks and new research models.

Questions about family relationships should be asked with sensitivity to each family member's background. Caution must be used if the interviewer has information of which the family member is unaware. It is important that the interviewer identify questions that need to be asked with care (or not at all if information can be gained elsewhere) at the start of the interview.

4.1.2 Constructing the Family Tree

A preliminary graphical pedigree will be produced by the clinic staff during the initial clinic visit using Progeny2000®. A definitive pedigree will be generated automatically by Progeny2000® from information in a spreadsheet derived from the PIF. A brief description of symbols used in family trees is provided below:

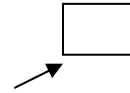
males are represented by squares:



females are represented by circles:

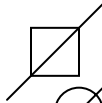


Arrow pointing to a symbol identifies the **index family member**:



Deceased individuals are represented with a line through the symbol:

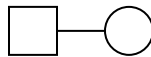
Square for male:



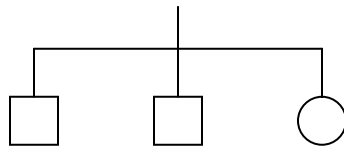
Circle for female:



Partners who share biological children are represented by a horizontal line joining a square & circle:

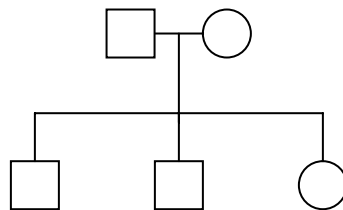


A **sibship** is represented by a horizontal line with vertical lines dropping from it to the symbols:



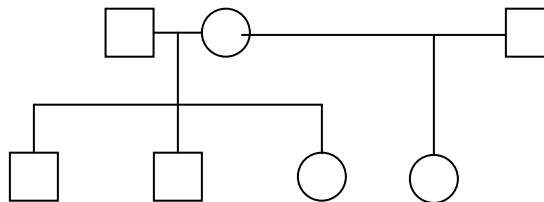
The example above represents two brothers and a sister.

A vertical line connects **parents with their children**:



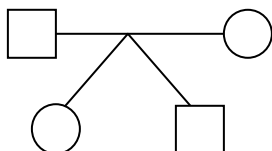
The example above represents a family that includes both parents and their three children, who are full siblings (all have the same mother and father).

Multiple unions and half siblings (siblings who only share one parent) are represented as follows:

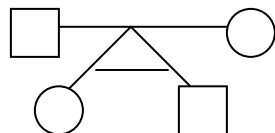


The example above represents a family where the mother has children from two different relationships. One relationship resulted in three full sibs and the other resulted in one daughter who is a half sib to the previous three full sibs.

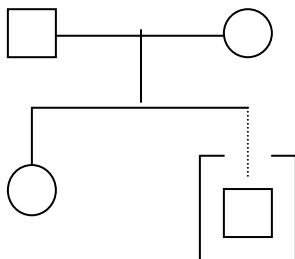
Twins (dizygotic):



Twins (monozygotic):



Adoption:



4.2 Sample Script for Clinic Staff Drawing an Initial Graphical Pedigree

“As you know, the JHS is trying to help scientists understand why diseases like high blood pressure, stroke, diabetes and heart disease run in some families. By studying the occurrence of these diseases across family generations, scientists may be able to identify factors in our environment (e.g., diet and stress) and factors that we may have inherited from our parents (genes) which put us and family members at greater risk of developing these diseases. To be successful, JHS scientists need to know exactly how family members are related. To do this we need to draw a diagram that shows as clearly as possible how the members of your family are related by blood.”

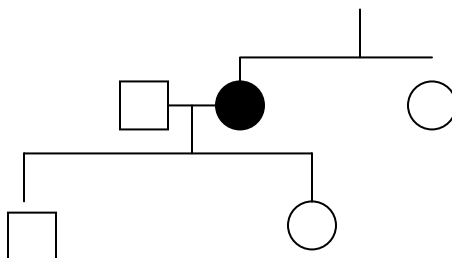
4.3 Considerations Regarding Family Size and Structure

Families of all JHS participants are potentially eligible for the JHS family study. Families will be selected for recruitment based on data in participants' ELG, and on preliminary graphical pedigrees. Relatives to be recruited do not necessarily need to meet the other recruitment requirements for JHS participants. Parental partners should be counted among the first-degree relatives if they have eligible, natural children with a JHS participant. A family size of twelve may be expected fairly frequently based on an average sibship size of three in the ARIC Jackson cohort. For example, a simple pedigree might consist of the participant and two siblings (sibship of three) each having three offspring and a parental partner, for a total family size of 12-15 (with all partners). Data from the first 1826 ELGs were used to develop criteria for determining which participants should have pedigree information collected in the clinic. The chosen criteria are that index participants for the family study should have two siblings and at least four other first-degree relatives. It is anticipated that this will generally yield families of ≥ 12 members when second-degree and other relatives are included (aunts, nieces, etc.).

There are other considerations in choosing families for the study. We would like to recruit families that are as large as possible; thus priority should be given to families with twelve or more members. If we have difficulty recruiting families of this size, we will then consider recruiting smaller families. In addition, willingness to participate in the study will influence our choice of families. The initial study participant will be asked for permission to contact his or her relatives. We will choose to recruit those families first where we have permission to contact all members.

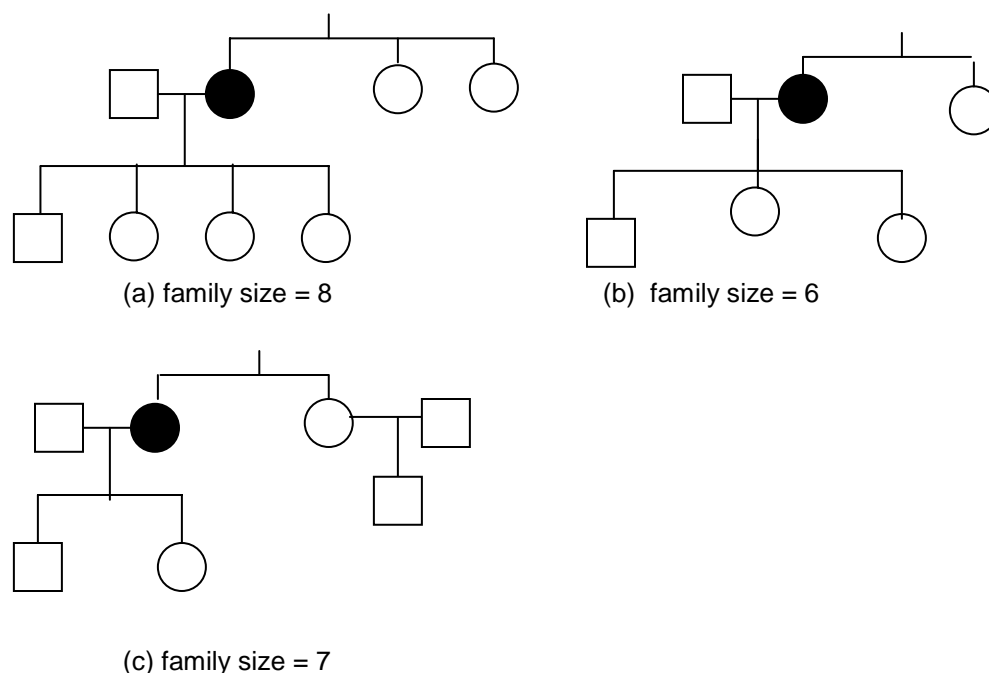
In case we are not able to collect enough families of 12 or more members, we have set a minimum acceptable family size. A family size of five may be acceptable if the family consists of: the index participant, their parental partner, one sibling and 2 offspring. The family tree would look as follows:

Figure 2: Minimal Family Structure



For family sizes of fewer than nine, the pedigree should consist of the minimal family structure (Figure 2) plus one to three first-degree relatives of any pedigree member. These additional relatives should be blood relatives of the index member (not relatives by marriage) if possible. In all pedigrees of fewer than nine individuals both parents of each child should be collected. Possible pedigree structures are shown below:

Figure 3: Examples of Family Structures



As stated, the criteria identifying participants who will have pedigree information collected in the clinic are that the index participant has two siblings and at least four other first-degree relatives. However, as noted above, families of index participants with fewer than two siblings may sometimes be recruited if family structure is otherwise desirable. For families of size nine to eleven individuals, examples of desirable family structures include the following (in this priority):

- 1) An index participant eligible for the JHS with 2 full siblings who meet recruitment criteria AND six to eight additional first degree relatives of the index participant and siblings who live in the JHS recruitment area.
- 2) An index participant eligible for the JHS with 1 full sibling who meets recruitment criteria AND seven to nine additional first degree relatives of the index participant and siblings (including their parents) who live in the Jackson recruitment area.

5.0 FAMILIES SELECTION AND RECRUITMENT

5.1 Family Selection

Recruiters will identify JHS participants who are eligible to become index participants in the family study based on information in the Eligibility Form. Every JHS participant who has two siblings and four other first-degree relatives (parents, siblings, children), all ≥ 21 years old and living in the tri-

county area, is to be designated "Family Study Eligible." This information will be indicated on the participant's profile sheet and will be conveyed to clinic personnel. Every such participant will complete a graphical pedigree and a Parental Identification Form (PIF) during his/her clinic visit. Complete contact information will be collected on the nuclear family (e.g. parents, siblings, spouse/partner and children) and the names of these and all other family members in the pedigree will be recorded in the Progeny2000® database. The Clinic Manager/Nurse or Family Study Research Associate will review the graphical pedigree during the participant's visit. If the pedigree is determined to be structurally sound, based on Family Study eligibility requirements, the participant will be informed during the clinic visit that her/his family has been selected for recruitment. With consent, blood will be drawn for cryopreservation of lymphocytes from this index family member. Once a family has been selected for recruitment the Family Study Research Associate will (1) assign a family ID number (P number: "P" + unique six-digit number); (2) complete a Family Linking Form (FLK) for the index participant; and (3) record the "P number" in the Progeny2000® database. When there are questions about whether a family should be recruited, the Family Study Research Associate may delay selection pending discussion with Drs. Wyatt and Wilson.

Initial family contact information gathered during the clinic visit will be provided to the Family Study Patient Representative (FSPR) who will make exhaustive efforts by telephone and accessing public records to obtain complete contact information for every family member depicted in the pedigree. The FSPR may make efforts to extend the pedigree while trying to ascertain additional family information (section 5.2). **All** efforts made to obtain **any** contact information will be tracked using the Family Study Contact Record of Calls form (Appendix 2). The Family Study Contact Record of Calls form is a non-DMS form that will be filed in each family member's recruitment folder. When either all contact efforts have been exhausted or all information has been collected on an eligible family by the FSPR, the information will be reviewed and entered into Progeny2000® by the Family Study Research Associate. When this data entry step is complete, the FLK of the index family participant will be entered into Clintrial and the family will be released to the EC for recruitment, after completion of the cross-checking procedure described below. In cases where a family has been deemed genetically uninformative after FSPR efforts (i.e. adequate contact information is not available or family members are determined to be ineligible) the FAMID will be changed to a "PX" format and stored in an "inactive" folder in the Progeny2000® database. The index family member will be informed that her/his family was not selected for recruitment based on ineligibility or inadequate contact information.

For families that are to be recruited, the CC will perform an automated cross-check of names and addresses to identify family members who are already JHS participants and will note the "J" numbers of these participants. The family will be released to the EC by the CC for recruitment, with (1) a copy of the graphical pedigree; (2) a spreadsheet containing contact information for family members; and (3) the index participant FLK form with a sheet of "P number" stickers attached. Recruitment letters will be sent informing family members that they will be contacted by a recruiter. Recruiter Assistants will assign a "J number" (unless already assigned) and complete and data enter an FLK form for each family member who is released into the family sample.

5.2 Family Recruitment and Pedigree Extension

Each family will be assigned to a Family Study Recruiter. In most cases initial contact with family members (after letters have been sent) will be by telephone. Family Study Recruiters will track contact efforts using the Individual Record of Calls (IRC). During the Home Induction Interview of each secondary family member, questions #10-19 of the Eligibility Form will be omitted (as family study eligibility has been established). Instead, the participant will be asked to list his/her grandparents, parents, siblings, and children/grandchildren (if likely to be age eligible) and to provide age, tri-county information, and contact information/permission for each. This process will allow extension of the pedigree and progressive completion of contact information in the field. This information will be recorded on a supplemental, non-DMS data collection form (Appendix 3). This form will be transmitted to the Family Study Research Associate on, at minimum, a weekly basis. The Research Associate will append contact information collected on newly-identified family members to that family's "working pedigree" in Progeny2000® (see below) and will forward this information to the

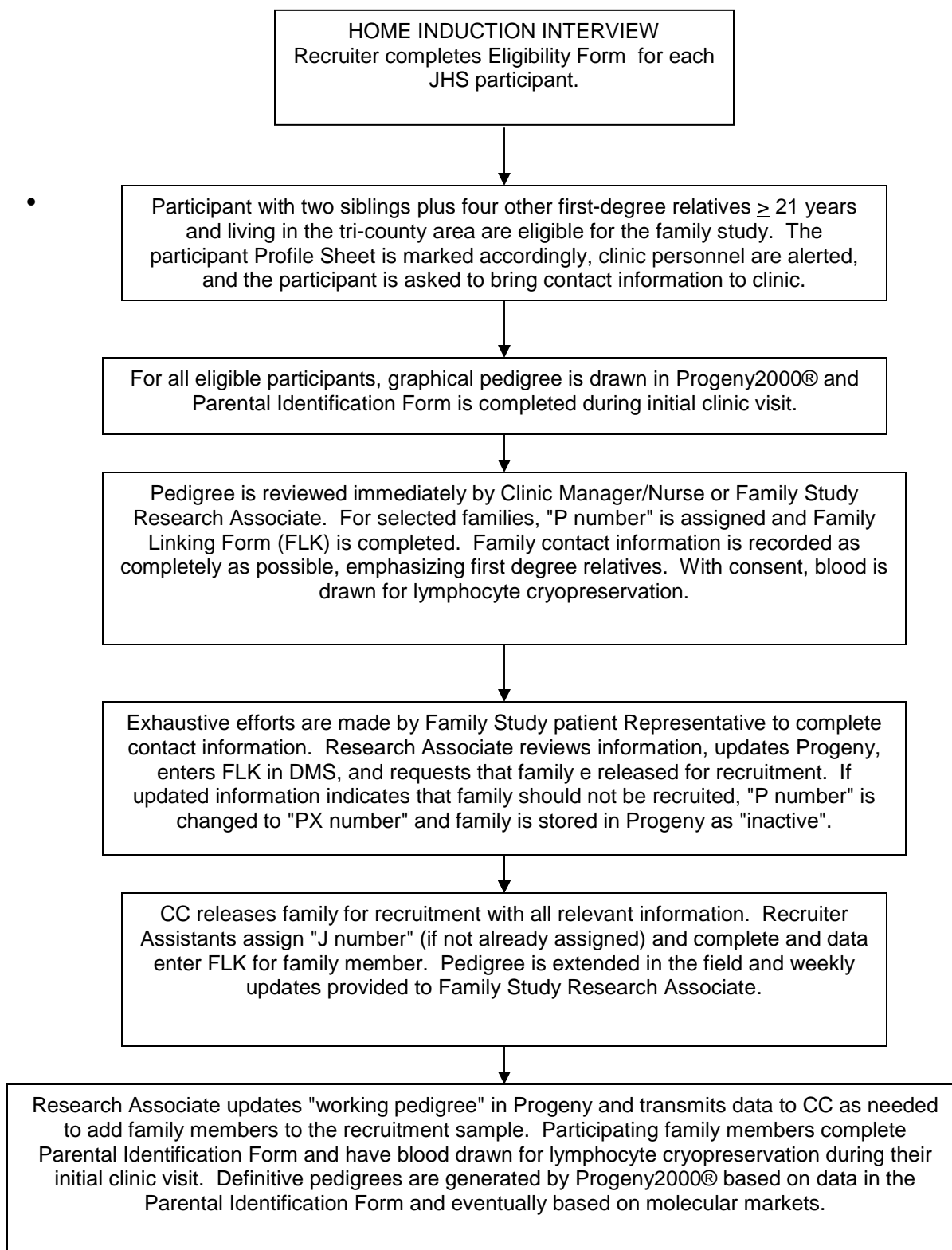
CC for inclusion of the additional family members in the recruitment sample. The hard copy of the form will be filed in the recruitment file of the secondary family member providing the information. Family Study Recruiters will meet with the Research Associate weekly to update information for all families in active recruitment. Following the usual protocol, the CC will release the names of newly-identified family members to the EC for recruitment. After recruitment of a family has been closed out, the "working pedigree" will be finalized based on PIFs of family members who have actually come through the clinic. Family relationships will eventually be verified by molecular markers.

When recruiters identify additional qualifying family members during a home visit who are either represented in the initial pedigree or are first-degree relatives of those represented, these family members can be assigned a "J number" and recruited immediately, with completion of an FLK and all other relevant paperwork. When individuals are identified who were not represented in the initial pedigree, and are relatives but not first-degree relatives of those listed, their names, relationship, age, tri-county information, and contact information will be recorded. This information will be reviewed with Drs. Wyatt and Wilson in weekly family recruitment meetings and the individuals will be recruited subsequently if appropriate.

For each family, the initial pedigree as provided by the index participant will be stored in Progeny2000® under the family "P number." Only in the case of pedigree extensions will the initial pedigree be archived. In this case, the P number will be changed to a "PA" format ("PA" indicating an archived status), and the amended pedigree will be stored in a "working pedigree" file with the same family ID number, but changed to a "PW" format. All subsequent changes to a pedigree will be recorded in the "working pedigree." Family Study Recruiters will receive a copy of the pedigree, on which they will hand-draw their modifications. As noted, the recruiters will update the Family Study Research Associate regarding pedigree changes at least weekly.

When two pedigrees that were initially identified separately are found to share family members, data from the smaller of the two will be copied/merged to the larger pedigree through a "drag-and-drop" mechanism. The combined family will retain the family ID number of the larger family, and that of the smaller family will become inactive. Family merger history will be tracked by Progeny2000®. Definitive pedigrees will be derived from data in the PIFs of JHS participants having the same final family ID number (See Section 8.2, "Progeny2000® Database"). In certain cases during generation of definitive pedigrees study participants may be contacted to determine whether a parent may sometimes go by another name. If inconsistencies cannot be reconciled, data for the pedigree will be referred to the Genetics Committee or to a designated committee member for review.

Figure 4. Family Study Data Collection Flow Chart



5.3 Additional Notes for Family Study Recruiters

While working on a family, the recruiter will keep an organized “notation of adoptions” log, in which all information about adoptions in the family will be recorded, including the source of the information and the stated relationship of the source to the adopted person. A copy of this log will be given to the Family Study Research Associate when that family is “closed out” by the recruiter, and information in the log will be used to generate an edited version of the pedigree after incorporation of data from PIF but before analysis and incorporation of molecular markers. There will be circumstances in which adoption information from other persons may over-ride the PIF of the adopted person. This will be decided on a case by case basis in consultation with members of the Genetics Committee.

Adopted children will be recruited as part of the family if they live in the household with biologic members of the family or if they live in the tri-county area and request to participate as part of the family. They will be given a “P number,” have an FLK completed, and undergo the same blood sampling as other family members.

When a household is identified through the Family Study, potentially eligible household members include blood relatives, spouses, and adoptive relatives only. No one else in the household is eligible, though they could conceivably be recruited as part of the random or volunteer sample. For example, if an otherwise ineligible household member expresses interest in participating, the recruiter can record name, address, date of birth and interest in volunteering in the study and return that information to the Data Entry Specialist at the CC for inclusion in the volunteer sample list.

Spouses of participants who do not have biological children with the participant are not eligible for participation in the family sample; thus they should not be actively recruited. However, if the spouse of a participant is available during a home visit and expresses desire to participate, s/he can be accepted into the study, be assigned a “P number” and a “J number,” and have an FLK completed, irrespective of whether s/he has children with the biologic member of the family being recruited. Data for this spouse will neither add nor detract from the overall genetic analysis. Recruiters are to provide a list of these “special cases” to Dr. Wyatt on a regular basis. These persons should not have cryopreserved blood drawn in the clinic. The Participant Profile Sheet for each such individual should be marked (in all capital letters): NO CRYOPRESERVED BLOOD DRAW. Recruiters should note that this situation is considered to be an exception that should not be pursued unless it is clear that the family will be lost to recruitment if the otherwise ineligible family member is not included.

For any secondary family member, if the index family member has answered “No” to permission to contact, we can still have other family members who are in contact with the individual ask them to call in. However, we would consider them as a “No Permission to Contact” unless they contacted us voluntarily.

6.0 RECRUITING FAMILIES

Our success in recruiting families depends on our ability to explain the goals of the Family Study in a way that makes its value clear. The following narrative may help.

“Heart disease and high blood pressure are serious health problems for African-Americans and other Americans. Research has established that heart disease and high blood pressure run in families, although the reasons why are not clear. Family members tend to live together for part of their lives and as a result share eating habits, exercise patterns and exposure to cigarette smoke. Family members also share genes and we think that some of these genes may increase the risk of heart disease and high blood pressure. The pattern of heart disease and high blood pressure in a family is the result of both shared genes and environmental factors such as diet and smoking.

“One of the goals of the JHS is to study the causes of family patterns of disease in African-Americans. The participation of families in your community, including parents, children, brothers, sisters, aunts, uncles, and possibly grandchildren or grandparents in the JHS can help us learn about

family patterns of disease. For each participating family we will draw a family tree, give each member an extensive physical exam and ask questions about health-related habits that may influence risk of heart disease and high blood pressure. All of the information collected will be coded so that confidentiality of individuals and families can be strictly maintained.

"We will analyze family patterns of traits related to heart disease and high blood pressure to determine what factors, including genes, influence these traits. If the effects of a gene can be detected we will try to locate the gene by studying genetic material (DNA) from your blood sample.

"Discovering genes that contribute to the risk of developing heart disease and high blood pressure is very important for reducing the burden of these diseases on African-American families and communities. If these genes can be identified, new treatments and ways of preventing disease can be found and individuals and families at risk of developing high blood pressure or heart disease can be helped before they become ill. We will also be able to help people change their lifestyles so that the effect of harmful genes is lessened. In keeping with the JHS legacy of health, these genetic discoveries will enhance our knowledge of cardiovascular disease risk factors and help in developing new treatments for future generations of African-Americans."

7.0 CLINIC EXAM

7.1 Sample Collection

During the clinic exam two 10 ml ACD tubes will be collected for isolation of genomic DNA. With the participant's specific consent, two 5 ml CPT tubes will be collected for lymphocyte cryopreservation and either immediate or selective transformation of lymphocytes at a later date. Genomic DNA will be prepared for all JHS participants. Lymphocyte cryopreservation will be done for all participants whose families are selected for recruitment. Immortalized cell lines will provide essentially unlimited amounts of DNA from a single sample, eliminating the need for additional blood draws for research where DNA is needed. Tubes will be bar coded and will not be identified by the participant's name. Samples will be shipped to the Central Laboratory at ambient temperature the same day, by overnight carrier. Any blood drawn for lymphocyte cryopreservation of participants who are not ultimately selected for inclusion in the Family Study will be discarded.

7.2 Tracking of Samples

Each sample will receive a bar code and will be recorded into a database prior to shipping to the Central Laboratory. Details of sample data base management can be found in the Manual 9, Specimen Collection and Processing.

7.3 Destruction of Samples

As provided in the informed consent, some study participants may choose to withdraw from JHS genetic studies and may request that all samples of their genomic DNA and cryopreserved cells be destroyed. Upon receipt of such a request, the contractor responsible for handling and storing these samples will be contacted by electronic mail, will destroy the designated samples and record their destruction in the sample log, and will notify the JHS by electronic mail that the samples have been destroyed. Paper copies of these electronic mail messages will be printed and retained in the participant's JHS file, and a letter will be sent notifying the participant that her/his samples have been destroyed. Genotype data for the participant that have been stored in the JHS Database will be deleted, and the participant's genotype will be recorded as "deleted by request." Published or submitted manuscripts that have included analysis of the participant's DNA will not be altered.

8.0 DATA MANAGEMENT

8.1 Progeny2000® Master File

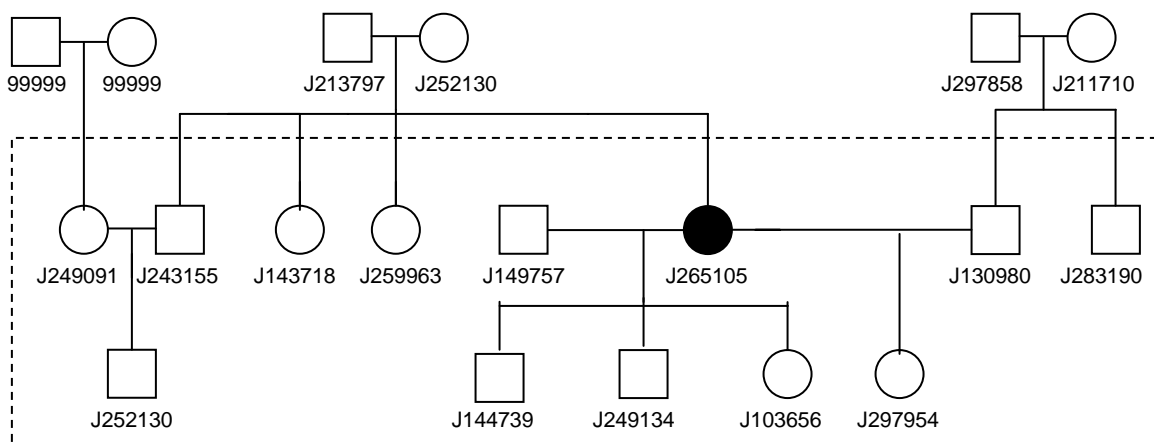
Each participant's information will occupy one line.

Required columns:

FAMID
JHS ID
Participant age
Participant gender
Mother first name
Mother middle name
Mother last name
Mother maiden name
Mother age
Father first name
Father middle name
Father last name
Father age
Mother ID (MA)
Father ID (PA)

8.2 Progeny2000® Database

The master file of all tabular pedigree data items will be maintained in Progeny2000®. The Progeny2000® database will be used to generate and analyze preliminary and definitive family trees. In addition to other variables, the tabular master file will contain the following standard genetic analysis identifying information: Family ID number, participant ID (J+ six digit unique identifier for each participant in the JHS), MA (mother ID - identifies the mother of each person within a family), PA (father ID - identifies the father of each person within the family), SEX (male=1, female=2). All data except MA and PA will be entered automatically from the graphical pedigree and PIF. MA and PA will be entered manually based on other data in the spreadsheet to allow for differences in spelling, etc. (e.g. Johnny vs. Johnnie). Any number of independently developed data files bearing the unique ID is easily merged as needed with the Master File. Furthermore, the Progeny2000® software generates printable ASCII characters, which makes it very easy to import data developed on other database systems and to export data to other programs or computers for analysis. The following example will illustrate the ID numbering system.

Figure 5. Example of ID Numbering System

Only those individuals within the dashed line box are available for clinical exam.

The ID numbers for the above pedigree are shown below. Family ID (“P number”) is assigned during the index participant’s initial clinic visit, when the family is selected for recruitment (see section 5.1).

| P123456 | | | |
|---------|---------|---------|-----|
| JHS ID | MA | PA | SEX |
| J265105 | J252130 | J213797 | 2 |
| J259963 | J252130 | J213797 | 2 |
| J143718 | J252130 | J213797 | 2 |
| J243155 | J252130 | J213797 | 1 |
| J249091 | 99999 | 99999 | 2 |
| J149757 | 99999 | 99999 | 1 |
| J130980 | J211710 | J297858 | 1 |
| J283190 | J211710 | J297858 | 1 |
| J297954 | J265105 | J130980 | 2 |
| J103656 | J265105 | J149757 | 2 |
| J249134 | J265105 | J149757 | 1 |
| J144739 | J265105 | J149757 | 1 |
| J252130 | J249091 | J243155 | 1 |
| J213797 | 99999 | 99999 | 1 |
| J252130 | 99999 | 99999 | 2 |
| J297858 | 99999 | 99999 | 1 |
| J211710 | 99999 | 99999 | 2 |

Note: A JHS ID will be assigned to every person included in the recruitment sample for a family that is selected for recruitment.

Note: 99999 are missing value indicators. J213797, J252130, J297858 & J211710 are dummy parents (founders who are not available for clinical exam) linking two siblings who both had a clinical exam.

The above information is what is needed for linkage and other family analyses. The table also contains all the information you need to know how individuals are related and therefore the ability to correctly deduce the family tree. It is remarkably straightforward.

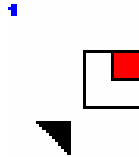
8.3 Annotation of Pedigrees in Progeny2000®

Contact information and tri-county residence status for each family member are stored in the Progeny2000® database. A color-coding scheme has been created to present such information about family members in the graphical pedigree (see appendix 4). Each color, located in one of three quadrants of an icon, indicates a specific status of that individual's contact information.

All tri-county eligible family participants are identified by black shading in the upper left quadrant of their icon in the graphical pedigree. The lower right quadrant can assume any of the four conditions listed below.

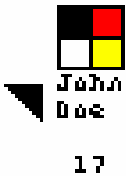
- Green: Contact information is complete
- Yellow: Contact information is partially complete
- Blue: Tri-county eligible, no contact information provided
- Gray: Tri-county residence unknown, no contact information provided

If it is determined that a family member no longer resides in the tri-county area, her/his permission to contact status will be changed to “No” (i.e., no permission to contact provided by the index participant). This change in status will be indicated graphically by a red box in the upper right corner of the individual’s icon in the pedigree (see below).



There are several other circumstances in which an individual may exhibit a red quadrant in his/her icon, as shown below:

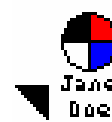
Age ineligible



Refused to Participate



Index Participant did not provide permission to contact



8.4 Interface of Progeny2000® with Clintrial

Family data will be entered in Clintrial only if a family is to be released by the CC for recruitment. At that point, each eligible person listed in the pedigree and having adequate contact information is assigned a JHS ID number. Data sharing between Progeny2000® and Clintrial can occur at two levels. At the level of primary data management, Progeny2000® data will be accessed by Clintrial through a “Central Agency” function. Alternatively, data sharing can occur downstream in the analytic process as a SAS function. To allow database interactions at the level of primary data management, a single relational table has been created in ClinTrial, having three data fields, including (1) Family ID number; (2) JHS ID number; and (3) a code indicating whether the participant is the index family member (code=1) or a secondary family member (code=2). When it occurs that more than one family member has been recruited through ARIC or by random selection, the appropriate “P number” will be assigned to that participant via the FLK. In this situation, there will be some overlap between the family and the random/ARIC samples. However, only one person in a family will be designated as the index family member.

Certain functions, including contact tracking and rates of recruitment for the Family Study, will occur at the primary data management level. Efforts to contact family members will be logged using the same Individual Record of Calls (IRC) form that is used for ARIC, volunteer, and randomly ascertained participants. No Household Record of Call (HER) forms will be used for family study participants. Most contact efforts involving family members will be by telephone rather than home visit, and appropriate codes for telephone contacts are included in the IRC. Standing reports based on data in the IRC will be created in ClinTrial to allow family-specific contact tracking and analysis of rates of recruitment for the Family Study.

8.5 Quality Assurance

8.5.1 Pedigrees

Family structure programs (including S.A.G.E®, Progeny2000®, Pedsys®, etc.) will be used for error checking on the pedigree data structure. Several structural errors may be detected including married persons with the same sex code, an individual who is his or her own ancestor, or more than one person having the same ID. These programs may also identify certain types of consanguineous matings and loops in the pedigree.

8.5.2 Samples

See Manual 9, Sample Collection and Processing for quality assurance in handling DNA and blood samples for immortalized cell lines.

8.5.3 Genotypes

As genotype information becomes available, marker-typing incompatibilities will be checked. Given an individual's genotype, inconsistencies with parents and offspring data will be verified. For example, we will consider the following inconsistencies:

- a. Parent and child alleles are incompatible.
- b. There more than 4 alleles in a sibship (brothers and sisters).
- c. A sibship has more than 3 alleles in the presence of a homozygous child.
- d. Males homozygous for an X-linked allele.

Mendelian consistency will be verified for all pedigrees. It may be necessary to set the genotype of some individuals to missing to save a family.

9.0 DATA SHARING POLICIES

9.1 Sharing Genetic Information with Participants

Results generated from the Family Study component of the JHS will be handled in the same manner as other JHS results considered to be of research value only. They will not be routinely reported. If a participant requests them, these values will be provided on an *ad hoc* basis only after completion of a written request form (Appendix 5).

If during the course of the JHS a genetic polymorphism is discovered which has clear clinical relevance for a treatable condition, participants will be notified by study wide approaches. A description of the polymorphism, its health risk and treatment will appear in the JHS newsletter along with a phone number to receive more information and a referral for gene testing if available. All referrals will be at the cost of the participant.

9.2 Sharing Data with non-JHS Scientists

Genetic data (pedigrees, genotypes and DNA samples) will be shared with JHS- approved investigators who agree to maintain participant confidentiality and follow JHS publication clearance procedures for ancillary genetic studies of heart, lung and blood disorders and their risk factors. All investigators will be subject to the procedures outlined for ancillary studies prior to access to JHS data. A specific process for ancillary genetic studies involving JHS samples will be developed by the Genetics Committee within the first year of the study. Participants have the option in the informed consent to specify that their samples not be used for research by non-JHS investigators.

APPENDICES

Appendix 1. Parental Identification Form (PIF)

Appendix 2. Family Study Contact Record of Calls Form

Appendix 3. Pedigree Extension Form

Appendix 4. Description of Pedigree Legends

Appendix 5. Request for JHS Results Not Previously Reported

REQUEST FOR JHS RESULTS NOT PREVIOUSLY REPORTED

Participant's name

Date of request

Result requested

Date exam performed

Reason for request (to be completed by participant)

I understand that the requested result was not originally reported to me as it was considered by JHS investigators to be of insignificant or undetermined clinical usefulness. Although the Exam Center will not provide an interpretative explanation of the requested result, I will be provided the result as it was reported to the Exam Center.

Participant's signature

____ Approved
____ Not Approved

Exam Center PI