Coordinating Center For Implementing Familial Hypercholesterolemia Cascade Screening: A Feasibility Pilot

Supported by: Duke University Center for Applied Genomics and Precision Medicine (CAGPM), Medical College of Wisconsin, and NIH National Human Genome Research Institute

Principal Investigator(s):	Lori Orlando, MD, MHS Duke University Department of Medicine Center for Applied Genomics & Precision Medicine 307 Trent Dr. Durham, NC 27710
Co-Investigators:	Geoffrey Ginsburg, MD, PhD Duke University Department of Medicine
	Joan Neuner, MD Medical College of Wisconsin

Responsible Study Personnel

Project Manager	Tejinder Rakhra-Burris, MA
Data Manager(s):	Marshall Nichols
Responsible Regulatory Coordinator:	Seung-Hye Jung

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Coordinating Center:

CGSU/CAGPM

Duke University Medical Center

Durham, NC 27710

Participating sites:	
Institution	Principal Investigator
Duke University	Lori Orlando, MD, MHS
Medical College of Wisconsin	Joan Neuner, MD

Synopsis

Title of Study:	Coordinating Center for Implementing Familial Hypercholesterolemia Cascade Screening: A Feasibility Pilot
Protocol No:	Duke IRB # Pro00070388
Principal Investigator:	Lori Orlando, MD, MHS
Co-Investigators:	Geoffrey Ginsburg, MD, PhD Joan Neuner, MD
Coordinating Center:	Duke University, CAGPM Durham, NC
Participating sites:	Duke University Medical College of Wisconsin
Methodology:	Prospective, genomic
Number of subjects:	150 enrolled (200 recruited)

Glossary of Terms

ACMG	American College of Medical Genetics
APOB	Apolipoprotein B
FH	Familial Hypercholesterolemia
FHH	Family Health History
IGNITE	Implementing Genomic in Practice consortium
IRB	Institutional Review Board
KFV	Known familial variant
LDL	Low-density lipoprotein
LDLR	Low density lipoprotein receptor
MCW	Medical College of Wisconsin
PCSK9	Protein convertase subtilisin/kexin type 9

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1. Background and Rationale

Familial Hypercholesterolemia (FH) is an autosomal dominant disorder characterized by abnormally high levels of low-density lipoprotein (LDL).[1] Its high prevalence (at 1 in 500 it is the most common cause of inherited premature coronary heart disease) combined with its considerable morbidity (>50% of men and >30% of women develop cardiac disease by age 60) makes it a high priority condition.[2] Patients with FH have elevated levels of LDL starting in childhood and commonly have a known mutation affecting the LDL receptor (LDLR), apolipoprotein B (APOB), or proprotein convertase subtilsin/kexin type 9 (PCSK9) genes.[3] FH is often undiagnosed or untreated due to low screening rates within the general patient population and a lack of awareness within the treatment community. Indeed UK data suggests that almost 90% of individuals remain unidentified.[4] More recent reviews have provided similar estimates for the US.[5] Fortunately, a systematic DNA based screening has been shown to effectively increase identification of new cases. For example, using this methodology 2% of primary care patients with hyperlipidemia in Scotland were found to have disease causing mutations and more than 33,000 new cases have been identified in the Netherlands since 1994.[4,6] For these reasons the UK established guidelines for systematic screening in 2008; however uptake has been low.[7]

Cascade screening, defined as "a mechanism for identifying people at risk for a genetic condition by a process of systematic family tracing" can facilitate systematic screening and overcome many of the barriers limiting implementation of the UK guidelines.[1,8] It has most often and most successfully been applied to FH, cystic fibrosis, Duchenne muscular dystrophy, and alpha-1 anti-trypsin deficiency.[9] For example, in the Scottish study described above, not only were 2% of primary care patients with hyperlipidemia found to have FH, but 42% of their relatives were also found to have it.[4] In addition, data from Australia suggest that if relatives identified during a centralized cascade are treated in a lipid clinic, clinical utility is robust (based on proxy markers for cardiovascular risk).[10] This approach, as currently practiced, has also been demonstrated to be cost effective,[11] though efforts to gain greater traction have been limited by its high resource requirements.[12-14] Two countries with integrated healthcare systems, the UK and the Netherlands, have successfully tested cascade screening programs that primarily rely upon letters mailed to relatives, with the potential for augmentation by telephone calls to primary care providers when needed. In the U.S., the CASCADE FH study is testing DNA based screening among patients at specialized lipid centers, but otherwise there are no published US experiences of either population-based DNA screening or of cascade screening (PubMed search 3/27/15 with search terms 'cascade screening familial hypercholesterolemia', all 74 papers were hand reviewed).[5]

The rationale for this study is to identify individuals with FH utilizing tools of family health history study that we have already developed in order to minimize the burden to healthcare system. MeTree, a risk assessment web-service with clinical decision support for 30 medical conditions (including FH) has been integrated into the primary care workflow of 5 national healthcare systems: Medical College of Wisconsin (MCW), University of North Texas, Duke University, Essentia Rural Healthcare Institute, and David Grant Air Force Base as part of the "Implementation, Adoption, and Utility of Family History in Diverse Care Settings Study (Pro00047666)". The 'Implementing Genomics in Practice consortium' (IGNITE) funded "Implementation, Adoption, and Utility of Family History in Diverse Care Settings Study" provides a unique opportunity to evaluate a potential solution to cascade screening in the U.S. Automating the process by embedding risk assessment software within the workflow of primary care clinics, should allow screening of a large percentage of the U.S. population, quick identification of new cases, and streamlined notification of relatives with a minimum burden on the healthcare system. The study will be adapted to encompass FH cascade screening at two sites only, Duke University and MCW, and evaluate its impact as part of the ongoing real world clinical trial. The study sites are limited to two sites as the costs of training and protocol deployment are not warranted based on the small sample size. Further sites will be considered when we deploy a definitive funded protocol.

2. Trial Objectives

Specific Aim 1: To assess feasibility and uptake of molecular testing for FH in real world US primary care clinics

Since user-entered family history and lab data in MeTree can be used to identify individuals at risk for FH, the test population for this pilot will be those identified as at risk for FH after reviewing data in the MeTree database. As of 6/2/2016, 46 (3.9%) of those completing MeTree at the Duke and MCW clinical sites have been identified as at risk. This population will be contacted to undergo Next Generation sequencing for FH variants. Specifically, DNA from saliva samples will be extracted and enriched for all exons and the splice junctions of well described FH genes such as *LDLR*, *PCSK9*, and *APOB* using the IlluminaTruseq Custom Amplicon Kit consistent with MCW's current clinical laboratory practice. Variants will be evaluated for pathogenicity using MCW's standard clinical laboratory practice in accordance with current American College of Medical Genetics (ACMG) standards and consistent with previous studies.[11] If there are fewer than 50 IGNITE family history study participants meeting the inclusion criteria (section 3.2.1), additional individuals will be

recruited who meet the inclusion criteria (section 3.2.2) from among the primary care clinics and lipid clinics at the MCW.

Specific Aim 2: To discover the prevalence of genetic mutations among families with FH

A secondary benefit of this study is the ability to understand better the FH-related genomic mutations present within families identified through general population screening in the US. Previous studies have typically focused on the identification of mutations in known mutation hotspots such as lipid clinics in the UK, the Netherlands or on the eastern Seaboard of the US.[11] It is clear from experience in Milwaukee that the population of the upper Midwest has a different allele frequency than that included in the Framingham or other National Heart, Lung, and Blood Institute (NHLBI) sequencing cohorts available in the exome variant server. It is therefore likely that the mutation spectrum will be different in the IGNITE study sites. Furthermore, the inclusion of testing of all coding regions may identify further novel pathogenic variants.

Specific Aim 3: To assess feasibility and uptake of FH-related cascade screening among relatives of affected individuals

Participants sequenced in Specific Aim 1 who meet ACMG criteria for "FH pathogenic variants" will be encouraged to contact biological family members about cascade screening, and provided a "family letter" outlining the benefits of screening and what specific variant they should be screened for. This should minimize costs of cascade screening and expedite insurance coverage. To remove the insurance authorization burden as a hindrance to testing and assessing what the real uptake would be in the US if testing was covered, this "known familial variant" (KFV) testing will be offered at no charge to the patient/provider at a central MCW laboratory. FH KFV testing will be offered as a part of standard-of-care, and family members offered this test will be asked to provide consent for the study by signing the lab requisition form for the test. This will allow a direct assessment of the impact of cascade screening using a "family member letter" to reach relatives. In addition, it will provide an estimate of the number of identified cases as a direct result of cascade screening. The primary outcome will be uptake of testing by family members.

3. Patient Selection

Up to 200 patients will be screened and asked to participate in the study, to achieve a range of participants from 50 to 100 for probands and up to 50 family members.

3.1. Target Population

- FHH participants identified as being at risk for FH who have consented and are willing to be contacted for future studies (Duke & MCW participants)
- Any adult patient who meets the criteria for being at risk for FH and is likely to benefit from intervention (MCW participants only)
- Family members (biological relatives) of probands who have tested positive for FH pathogenic variants (adults only).

3.2. Inclusion Criteria

Participants will be eligible for inclusion in this study if the following criteria apply:

3.2.1. Existing FHH participants (probands)

Study participants of Duke Pro00047666 or MCW PRO00021035 who meet all three criteria:

a. Identified as being at risk for FH by meeting the following criteria:
   - Current or past LDL cholesterol ≥190 mg/dL ***OR*** current or past total cholesterol ≥310 mg/dL [≥8 mmol/L].

   AND one of the following:
   - a family history of FH or hypercholesterolemia

     OR
   - a personal history of early heart disease or a family history of early heart disease in a first degree relative (early heart disease defined as onset <55 for males and <65 for females)

b. Indicated in FHH study to be willing to be contacted for future studies
c. Have a phone access

3.2.2. New MCW participants (probands)

a. Be a patient of a FHH consented provider for study PRO00021035 at MCW who meet recruitment criteria for FHH parent study
b. Willingness to participate
c. Have a phone access
d. Identified as being at risk for FH by meeting the following criteria:

   1) Current or past LDL cholesterol ≥190 mg/dL ***OR*** current or past total cholesterol ≥310 mg/dL [≥8 mmol/L].

   AND one of the following:

   2) a family history of FH or hypercholesterolemia

   OR

3) a personal history of early heart disease or a family history of early heart disease in a first degree relative (early heart disease defined as onset <55 for males and <65 for females)

3.2.3. Family members of probands

a. Any adult biological relative of a study participant who has been genotyped and tested positive for having a pathogenic FH variant in question.
b. Willingness to participate
c. Have a phone access

3.3. Exclusion Criteria

A participant will **not** be eligible for inclusion in this study if any of the following criteria apply:

- Had a molecular genetic testing for FH previously
- Have a family member who had a molecular genetic testing for FH previously
- Do not meet inclusion criteria upon screening
- Unwilling to perform study-related activities

3.4. Inclusion of Minorities

Adult patients of all races and ethnic groups who are at risk for FH will be considered for study participation.

4. Registration Procedure

4.1. Institutional Registration

Registration at each study site/institution will be conducted according to the institution's established policies.

4.2. Informed Consent

After sending the recruitment letter with an information sheet that includes study activities to potential subjects (probands), the study team will do a phone screening to determine if subjects meet the inclusion criteria. Authorized study personnel will confirm that the patient is a potential candidate for study participation and confirm that the patient is eligible as defined in Sections 3.2 and 3.3 (Inclusion/Exclusion Criteria).

The study team will explain the scope of the study to each patient before obtaining informed consent by telephone. When obtaining informed consent on the phone, study personnel will:

First: Confirm that the patient is a potential candidate for study participation.

Next: Confirm that the patient is eligible as defined in Sections 3.2 and 3.3 (Inclusion/Exclusion Criteria).

Finally: Document in the Access database the date/time of the telephone consent, obtain all the information necessary for delivery of the consent form, a template of family letter, saliva DNA collection kit and the laboratory requisition form.

During the phone consent, the coordinator will ask if the subject anticipates any difficulty producing enough saliva and prefers an oral swab kit instead. Study team will ask the subject to sign a consent form that is mailed to him/her and return it to the study team. A template of family letter will also be sent so that the participant can review the content. Once the study team receives the signed consent, they will inform the MCW clinical diagnostic laboratory (DNL-Seq) to proceed with genetic testing. The study team will follow up in a week if they have not received the consent form or the kit. For non-responders to initial recruitment letter, the study site coordinator will follow up by phone, using a telephone script.

All adult biological relatives of study participants who test positive are considered candidates for cascade screening as part of this study and also as part of standard of care recommended by National Lipid Association. Probands who receive the test results will be asked to distribute the family letter to biological relatives, which is in keeping with standard clinical practice to account for deaths or unavailability of first degree relatives. The letter will ask family members to contact the clinical lab personnel in the DNL-Seq laboratory at MCW to get more information if they choose to get genetic testing through the study. The letter also includes information about FH test and elements of informed consent. The clinical lab personnel (not part of study team) will answer questions the family members may have about the genetic testing and also inform that certain de-identified information will be sent to the study team as part of research. The following information will be sent to the study team: number of sample collection kits sent out (total across all families), number of sample collection kits returned (total across all families), number of positive and negative results (total across all families), gender, age. If they agree to get the test done through the MCW laboratory and participate in the study by agreeing to share aforementioned information, they will receive a mail-in kit that includes a saliva DNA collection kit and a lab requisition form. After collecting saliva sample and signing the lab requisition form, they will return them to the MCW laboratory. The requisition form will explain which de-identified data will be used for the research study and be considered as an altered consent form.

Only adult family members will be asked to take the genetic testing.

5. Study Design

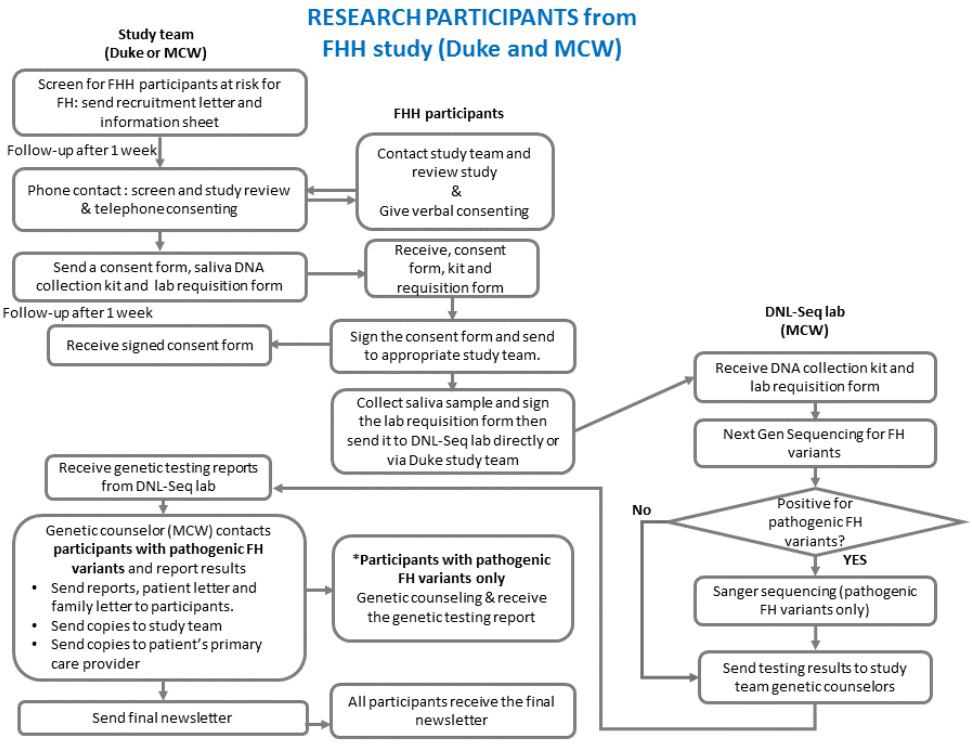
5.1. Expected Enrollment

The FHH tool, MeTree, will be utilized to identify potential subjects for this pilot study. 50 - 100 participants are expected to be recruited from the FHH study and engage up to 50 family members. The test population will be those who are identified as at risk upon review of the MeTree database (see section 3.2). FHH study participants-entered information in MeTree will be queried to select potential subjects who meet inclusion criteria for probands however MeTree's algorithm will **not** be used to identify them. To date 46 (3.9%) of those completing MeTree across the two clinical sites, Duke and MCW, have been identified as at risk for FH. New MCW patients who are not FHH participants will be also enrolled if fewer than 50 FHH participants are recruited.

5.2. Recruitment

Existing FHH participants at Duke or MCW who are identified as being at risk for FH (see section 3.2, Figure 1) will be contacted by email or letter which will ask them to undergo Next Generation sequencing for FH variants. The information sheet that includes all the elements of informed consent will be included in the recruitment letter so that potential subjects have a chance to learn more about FH genetic testing and the study activities before the phone screen/consenting.

Figure 1. A study activity schematic for FHH participants (both Duke and MCW patients)



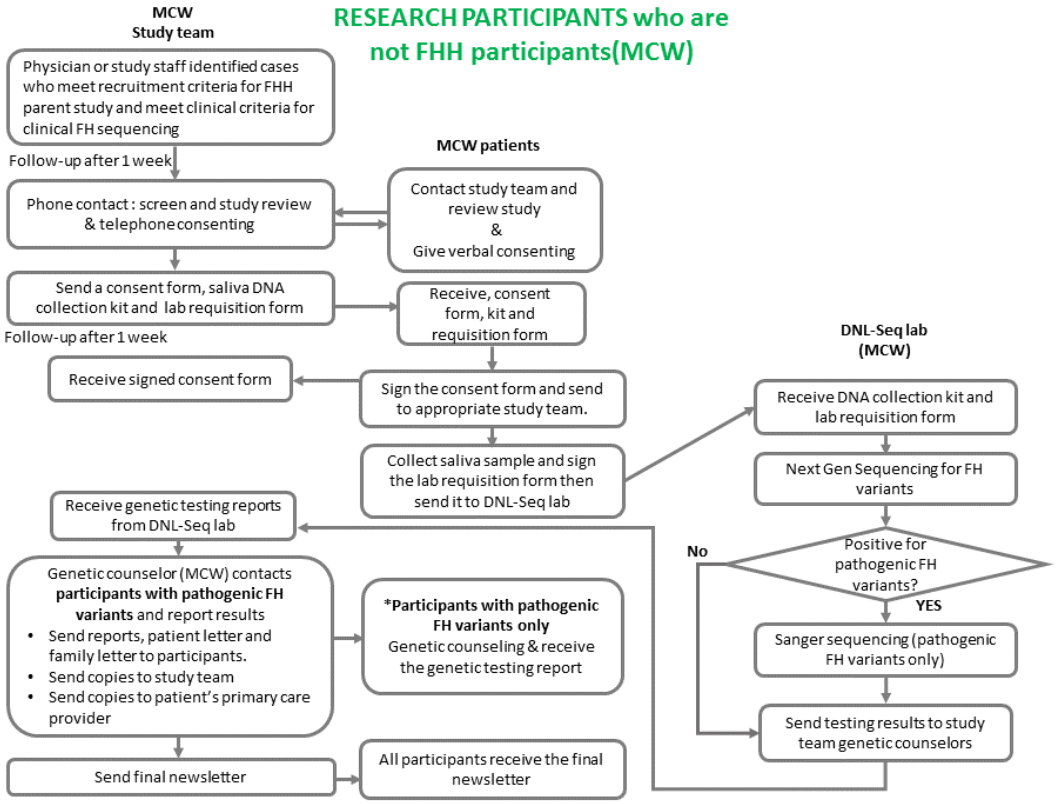
If there are fewer than 50 FHH participants recruited, then MCW will identify and recruit new patients who are identified as being at high risk for FH (section 3.2, Figure 2) by sending a recruitment letter.

This letter will include elements of informed consent, such as description of the study, its risks and benefits, further information about the saliva DNA collection kit, and genetic testing & analysis. Those who are interested will contact the site study coordinator who may briefly review the study again. If the patient is interested, the site study coordinator will conduct an informed consent by phone, record the date and time of consenting in the Access database, and collect information for requisition form (name of clinic, provider, clinic fax #, home address, phone #, *etc*.). During the phone consent, the coordinator will ask if the subject anticipates any difficulty producing enough saliva and prefers an oral swab kit instead. The site study coordinator will send the consent form to the subject so that subject can sign the consent form and return it to study team. The site study coordinator will also send the patient a saliva DNA collection kit or an oral swab kit and a requisition form together with the consent form. For non-responders to initial recruitment letter, the study site coordinator will follow up by phone, using telephone script for non-responders. 7 days after sending out the saliva DNA collection kit, the site study coordinator will conduct follow up calls up to 5 times to see if they received the kit, if they completed the requisition form and filled the saliva DNA

collection tube, and if yes, when the saliva DNA collection kit was returned. The site study doctor will be an ordering physician in the lab requisition form. Subjects will be asked to send the kit and the requisition form with the consent form using an enclosed envelope. Additionally, if the subject requests direct assistance with consenting and sample collection, the site coordinator will meet with the subject in a clinic or in private area and send the kit and requisition form to MCW's Developmental & Neurogenetics Laboratory Sequencing (DNL-Seq) on behalf of the subject. The MCW study team will obtain the requisition forms from the laboratory and record the date of receipt of the requisition form and saliva DNA collection kit in the Access database. Saliva DNA samples will be analyzed/genotyped by enriching for all exons and the splice junctions of the well described FH dominant genes such as *LDLR*, *PCSK9*, and *APOB* using the IlluminaTruseq Custom Amplicon Kit consistent with MCW current Clinical laboratory practice. Variants will be evaluated for pathogenicity using MCW standard clinical laboratory practice in accordance with current ACMG standards and consistent with previous studies.[11]

If the participant's NGS testing results reveal a pathogenic variant of FH genes in question, it will be confirmed by clinical Sanger sequencing and only confirmed results will be communicated with the participant by telephone from the MCW study team member who is either board certified genetic counselor, clinical geneticists, or research nurse. In addition, a letter containing these results will be mailed to the participant and mailed or faxed to the participant's provider/clinic if the participant indicates this in the requisition form. If the participant does not provide the information of his/her healthcare provider, the site study doctor will receive the test results and be available for consultation. While positive results are confirmed by Sanger sequencing, negative results are not verified by any additional clinical testing due to the limited budget in this feasibility study thus individual negative research results will not be shared with participants. However participants will be given a contact information to the genetic counselor in case they have questions about the testing.

Figure 2. A study activity schematic for new patients recruited at MCW clinic

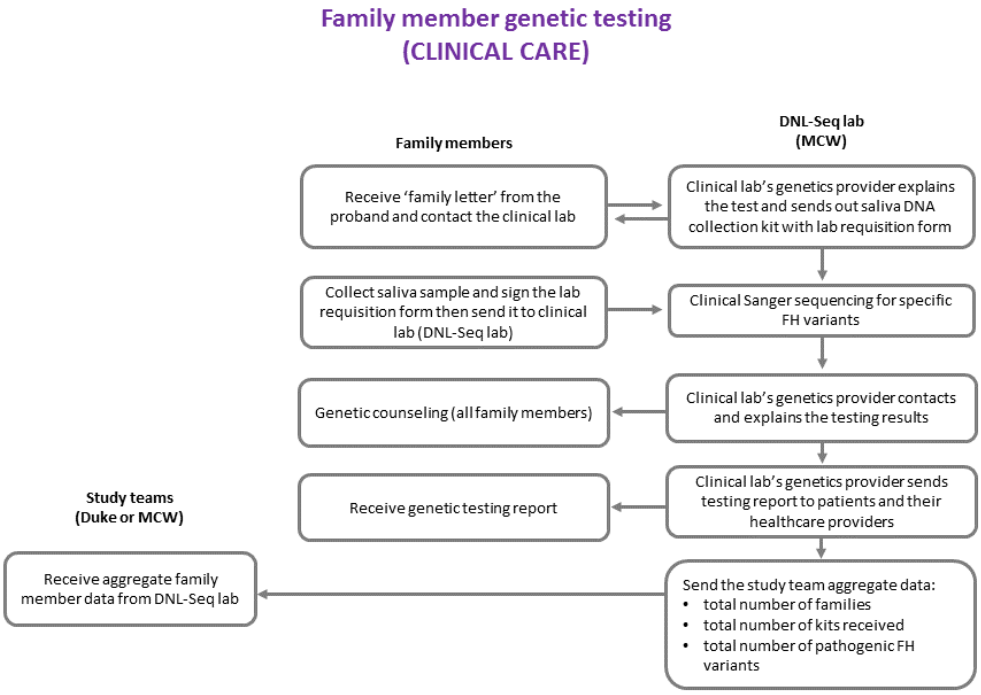


For participants with positive/diagnostic results, a 'family letter' will also be included with the positive test results (Figure 3). This letter outlines the benefits of screening and what specific FH variant they should be screened for. The participants will distribute the letters to their family members. Several kits will be sent to the participants with 'family letter' for distribution, if acceptable to them when discussed by telephone. The letter will inform family members that they may be at risk for FH and that they should be tested. They will also be informed that they can receive testing at any laboratory of their choosing through their insurance or at MCW's clinical lab at no charge. The only difference to the family members will be whether their use of this service and positive/negative status is reported in aggregate to the study team. If they undergo testing it will be performed under the supervision of an identified clinical provider (which the family member identifies, and to whom positive results will be returned) per current standard of care for clinical screening of family members. The family letter will also include a contact information to clinical laboratory personnel who will not be a genetics provider assigned to the research study. If family members are interested, they can contact a genetics provider affiliated with the clinical laboratory (DNL-Seq) at MCW who will be available by telephone and send the saliva DNA collection kit and requisition form. Family member's

testing will be done in routine clinical care and ordering physician will be Dr. Donald Basel in MCW. All test results (positive or negative) will be communicated back to family member by a genetics provider affiliated with the clinical laboratory and mailed or faxed to a clinical provider identified by the family member in the requisition form. If no clinical provider is listed on the requisition form, the results will be sent to the clinical lab director. The clinical lab will send the aggregate data to the study team that include: aggregate number of probands with family members being tested; aggregate number of family members being tested; aggregate number of family members who test positive for an FH variant.

Both proband and relatives will have direct access to the lab's clinical genetic counselor for questions regarding study findings and implications for health for self and family.

Figure 3. A study activity schematic for family member of the study subject whose test results are



positive

At the end of study, all study participants (probands) will be sent a newsletter that summarizes the test results and this will also notify them that the study has been completed. A newsletter will include the summary of study, which will include number of participants, demographic information, number of positive test results and negative results, and number of family members who returned the kits.

5.3. Schedule of Events

5.3.1. Monthly Schedule

1) Month 1-2

a) Identification of potential subjects (probands) from FHH participants at Duke or MCW who are identified as being at risk for FH from MeTree.

b) Recruitment letter and information sheet sent to potential subjects by email or letter.

c) Telephone screening and consenting for responders.

d) Sending a cover letter, consent forms, a template of family letter, a saliva DNA collection kit or an oral swab kit and requisition form by mail to participants.

e) Telephone contact for non-responders, consenting, and sending saliva DNA collection kit and requisition form.

f) Follow-up call to check if subjects have sent the consent form and saliva sample to the study team.

g) If there are fewer than 50 FHH participants recruited, then MCW site will identify and recruit new patients who are identified as being at high risk for FH: as described in b)-e)

2) Month 3

a) Analysis/Genotyping of DNA from subjects (probands) at MCW DNL-Seq lab.

3) Month 4

a) Telephone contact to relay positive/diagnostic test results to subjects by a genetic counselor or clinical research nurse in the study team (only for those who carry known pathogenic variants of FH genes)

b) Send test result report (positive test result only) to subjects with a 'family letter' and letter/fax to their providers.

c) For family members who contact the clinical lab, they will be sent saliva DNA collection kit or an oral swab kit and requisition form.

d) Family members send the sample and requisition form to MCW clinical laboratory.

4) Month 5-6

a) Genetic test for family members for specific FH variant at MCW lab (study team will not be involved in this process).

b) A healthcare professional in the MCW clinical laboratory will contact family members by phone to relay test results (positive or negative), followed by letter to family member. Also, letter and fax to their providers

c) A MCW clinical laboratory will send the aggregate data to the study team.

d) Study team will send the final newsletter to all participants (probands) to inform the completion of study

5.3.2. Study Specific Tests and Procedures

Genetic testing will be performed for genes most commonly associated with familial hypercholesterolemia such as *LDLR*, *PCSK9*, and *APOB*. All research and clinical genetic testing will be performed at the MCW's Developmental & Neurogenetics Laboratory-Sequencing (DNL-Seq), which is CLIA/CAP certified for both research and clinical genetic testing.

Saliva DNA collection kits or oral swab kits containing patient specimen will be sent to the laboratory for analysis. A combination of research Next Generation Sequencing (NGS) and clinical Sanger sequencing technologies will be used to cover the full coding regions of our genes of interest plus ~20 bases of non-coding DNA flanking each exon. As required, genomic DNA will be extracted from the patient specimen. For NGS, patient DNA corresponding to these regions is captured using an optimized set of DNA amplification primers from the IlluminaTruseq Custom Amplicon Kit (Illumina, San Diego, CA, USA). Captured libraries will be individually indexed and sequenced using the Illumina MiSeq instrument (Illumina, San Diego, CA, USA). Regions with insufficient coverage by NGS may be covered by Sanger sequencing. Variants will be evaluated for pathogenicity using MCW standard clinical laboratory practice in accordance with current ACMG standards and consistent with previous studies.[11] All pathogenic variants identified by the research NGS sequencing will be confirmed by clinical Sanger sequencing. For Sanger sequencing, Polymerase Chain Reaction (PCR) is used to amplify targeted regions. After purification of the PCR products, cycle sequencing is carried out in both the forward and reverse direction using the ABI BigDye Terminator v.3.1 kit (Applied Biosystems, Foster City, CA, USA). PCR products are resolved by electrophoresis on an ABI 3730xl capillary sequencer and data is analyzed using Mutation Surveyor v5.0 software (SoftGenics, State College, PA, USA). Only known pathogenic variants will be reported. Any other variants such as 'likely pathogenic', 'variants of uncertain significance (VUSs)', 'likely benign' variants or 'benign' variants will not be reported to participants.

For family member testing, the medically accepted standard Sanger sequencing will be used to confirm the presence or absence of the known FH variant gene according to the CLIA certified laboratories' standard operating procedures.

6. Adverse Events

An adverse event (AE) is the development of an unfavorable or unintended sign, symptom, disease or the deterioration of a pre-existing condition that occurs while a patient is enrolled in a clinical trial, whether the event is considered related or unrelated to the study interventions. An adverse event is any adverse change from the subject's baseline condition, including any clinical or lab test abnormality that occurs during the course of research after intervention has started.

6.1. Adverse Event Reporting

This study will not track AE related to patients' clinical care. However if a patient reports AE related to the study activities, the study team will notify PI and report it to IRB according to the procedure stated as follows: Information for the reported adverse event must be recorded in the Access database which will be used as electronic case report forms.

6.2. Serious Adverse Events (SAE) Reporting

SAEs that are reported by patients or their family member will be reported. Study site personnel will immediately alert the site PI as well as the coordinating center PI of any serious adverse event (defined below) experienced by a subject that is related to the study or results in death. In addition, serious adverse events related to study must be reported to regulatory authorities according to the definitions and timelines specified in the local laws and regulations. As this protocol involves standard-of-care routine genetic testing, we do not anticipate any SAEs for this pilot study.

A Serious Adverse Event is any untoward medical occurrence that:

- Results in death
- Is life-threatening (defined as an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or causes prolongation of existing hospitalization
- Results in significant or persistent disability or incapacity (defined as a short or long term, temporary, chronic or permanent disruption of the patient's ability to carry out normal life functions)
- Is a congenital anomaly or birth defects
- Results in the development of drug dependency or drug abuse or
- Is an important medical event (defined as a medical event that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (e.g., medical or

surgical) to prevent one of the other serious outcomes listed in the above definition. Examples of such events include, but are not limited to intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization).

6.3. Non-Serious Adverse Events

All other events that do not meet the SAE criteria.

7. Data Reporting/Regulatory Considerations

7.1. Data Entry

Participants who agree to participate in the study by telephone consent will have their information documented in a central Access database set up by MCW that will be shared and accessible by the coordinators from the two clinical sites. The database will be stored in HIPAA compliant manners. The database will be updated on consistent basis to track participant progress, including but not limited to the following:

- Date/time of telephone consent
- date of mailing of consent form, saliva DNA collection kit, and requisition form
- date of signed consent form by a study team
- date of signed requisition form, and date of receipt by MCW lab
- genotyping results; if positive, variant name
- if positive, number of saliva DNA collection kits provided for family members
- the number of cascade screening family members who return the saliva DNA collection kits to the clinical laboratory
- the number of cascade screening family members that tested positive for one of the FH variants

7.2. Data Storage and Confidentiality

Every effort will be made to ensure subject confidentiality. Each subject will be assigned a unique study ID. Only key research personnel will have access to the list of study IDs and patient names for the purposes outlined in Section 5 Study Design, including following up on recruitment requests, return of samples, and notification of positive genotyping results. Subject identifiers will not be made available to any other institutions, hospitals, insurers, or agencies. The results of studies emerging from this work may be published but individual subjects will not be identifiable in these publications.

It is the PI's responsibility to ensure the subjects' privacy. However, in compliance with federal guidelines, the investigator will permit a representative from the coordinating center, Duke Clinical Genomics Study Unit (CGSU)- Center for Applied Genomics and Precision Medicine (CAGPM), to review that portion of the subject's medical record that is directly related to the study, if needed. This will include all relevant study documentation including medical histories to verify eligibility and laboratory test results to verify transcription accuracy.

7.3. Records Retention

The Investigator must retain source documents, and case histories designed to record all observations and other data pertinent to the investigation (e.g. requisition forms) for the maximum period required by applicable regulations and guidelines, or Institution procedures.

If a change in the PI occurs, the records shall be transferred to a mutually agreed upon designee (e.g., another Investigator, IRB).

8. Statistical Considerations

8.1. Sample Size/Power Calculation

As this is a pilot to determine the feasibility of identifying patients at risk for FH through a self-reporting mechanism such as MeTree, and the potential for implementing FH cascade screening in a US based population, there is no requirement for a pre-determined sample size.

8.2. Analysis Plan

There is no rigorous analysis plan as this is a pilot study to determine the feasibility of implementing FH cascade screening in a US based population. The study team will record the total number of participants tested, the number of those who test positive for one of the variants listed, and the number of family members of those participants who test positive who accept the screening offer.

9. Protection of Human Subjects

9.1. Ethical Considerations

This study will be conducted in compliance with the protocol, Good Clinical Practice guidelines established by the International Conference on Harmonization, and the ethical standards set forth in the Declaration of Helsinki 2004 (available at: http://www.wma.net/e/policy/b3.htm).

9.2. Institutional Review

Prior to patient accrual, this protocol must be approved in writing by the DUHS IRB. A signed and dated statement that the protocol has been approved by IRB will be kept in the study binder in the Principal Investigator's staff office. The term of study approval will not exceed one year. A progress report will be submitted annually to the IRB and re-approval obtained to continue the study. The IRB will also approve any significant changes to the protocol as well as a change of PI. Records of all study review and approval documents will be kept on file by the PI and/or his staff. Adverse events will be reported to the IRB per established policy. The IRB will receive notification of the completion of the study and final report within 3 months of study completion or termination. The PI will maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents. Initial approval letters from each site's IRB will be obtained and submitted to Duke IRB prior to the initiation of the study. During the study conduct sites IRB continuing review letters will be obtained and submitted to Duke IRB annually.

9.3. Monitoring Plan of Participating Sites

The follow documents will be monitored by each participating site to ensure proper documentation:

- any source document pertaining to the telephone consent documentation
- requisition forms for saliva DNA collection kits completion

9.4. Protocol Amendments

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Prior to starting the study, the protocol will be approved by each institution's Institutional Review Board (IRB) or Independent Ethics Committee (IEC). Amendments to the protocol may be made only with consent of the lead site and the principal investigator and are subject to IRB approval prior to instituting.

9.5. Informed Consent Process

The PI or designee will fully explain the purpose and potential risks and benefits of the study to the subject by telephone prior to enrollment and address any questions posed by the subject in accordance with federal guidelines. The investigator or designee will explain to each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits, and any discomfort the study may entail. Each subject will be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or his/her relationship with the treating physician. After a subject verbally consents to the study, the PI or designee will mail the IRB approved

information sheet to the subject with the saliva DNA collection kit and requisition form. The subject will be provided with the PI and site coordinator's telephone number for questions prior to reading the information sheet, signing the requisition form, filling the saliva DNA collection kit, ensuring completeness of the requisition form and returning the entire package to the MCW study team.

The information sheet contains the appropriate statements regarding privacy and confidentiality of protected health information (PHI) as well as information on withdrawal from the study. The PI will report to the IRB any changes in the research protocol and all unanticipated problems involving risks to human subjects and others.

9.6. Protection of Privacy

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. Following this discussion, they will be asked to consent on the phone. The use and disclosure of protected health information will be limited to the individuals described in the information sheet.

It is the responsibility of the research staff to ensure that protocol subjects receive, and understand the study before enrolling the patient onto this trial. Personnel obtain verbal acknowledgment before the subject participates in this study, and review the returned saliva DNA collection kit and signed requisition form prior to any genotyping.

10. Risk/Benefit Assessment

10.1. Risks of Providing Saliva Samples

Risks associated with providing saliva samples are dry mouth and uncomfortable feelings associated with it however it will be only temporary.

10.2. Potential Risk of Emotional Reactions to the Genetic Test Results

Participants from FHH have already self-reported as being at risk for FH and the genetic tests will only add details of FH variants. Participants may have emotional reactions to learning that they do - or do not - carry a gene change for FH. Participants who feel distressed will be directed to the site study doctor and she may refer them to a genetic counselor or other provider for further assistance as necessary.

Cascade screening family members: Awareness of an inherited disorder may be new information for the family members. This new information may result in elevated concerns about health for self and

for other relatives, especially children in the family. Relatives will have direct access to a clinical genetic counselor to help address these concerns. The genetic testing will be provided as standard of care as recommended by the National Lipid Association guidelines. The test results (positive or negative) will be communicated back to family member by a medical professional in MCW laboratory (DNL-Seq) and mailed or faxed to a clinical provider identified by the family member in the requisition form.

10.3. Potential Risk of Loss of Confidentiality

Participant information and data will be stored on a secured server accessible by the PI and study staff. Hard copies of study data will be stored in a locked file drawer in a locked office. Participants may refuse to return the samples (saliva DNA collection kit) if they are uncomfortable with the genetic testing.

Per CLIA and CAP guidelines, DNA samples will be maintained in the clinical lab, according to clinical lab protocols, for not less than 3 years, and will not be available for research. CLIA requires laboratories to establish and follow written policies and procedures that ensure positive identification and optimum integrity of patient specimens from the time of collection or receipt in the laboratory through completion of testing and reporting of test results (42 CFR §493.1232). Depending on sample stability, technology, space, and cost, tested specimens for molecular genetic tests for heritable conditions should be retained as long as possible after the completion of testing and reporting of results. At a minimum, tested patient specimens that are stable should be retained until the next proficiency testing or the next alternative performance assessment to allow for identification of problems in patient testing and for corrective action to be taken. Tested specimens also might be needed for testing of current or future family members and for more definitive diagnosis as technology and knowledge evolve.

10.4. Alternative

The alternative for potential participants is not participating in the study.

10.5. Benefits

If a participant is enrolled and is found to be at risk for hyperlipidemia, his/her family members may benefit by being identified as being at risk for this genetic condition through the cascade screening.

10.6. Costs to the Subject

There will be no costs to the participant.

10.7. Compensation

There will be no compensation to the participant.

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