STUDY PROTOCOL

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Protocol for Family Pedigree Study on Cholesterol Efflux in the Dallas Heart Study

"The Genetic, Protein, and Lipid Basis of Variation in Cholesterol Efflux"

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OBJECTIVE

To create a family pedigree and biobank repository of blood and DNA from participants from the Dallas Heart Study with extreme low or high cholesterol efflux.

BACKGROUND

Low high-density lipoprotein cholesterol (HDL-C) is a major risk factor for atherosclerotic cardiovascular disease (ASCVD), the leading cause of death. However, strategies to improve atheroprotection by raising HDL-C levels have failed to improve outcomes. Cholesterol efflux from the macrophage to the circulation is the first critical step of reverse cholesterol transport, a key atheroprotective pathway. We directly measured the cholesterol efflux capacity of plasma in over 3,000 participants in the population-based Dallas Heart Study and were the first to show that cholesterol efflux at baseline has a graded, inverse association with incident ASCVD events and improves risk prediction of ASCVD beyond cardio-metabolic factors and lipid measures. Our findings established that measurement of *cholesterol efflux as a direct reflection of the anti-atherosclerotic pathway is clinically relevant*. Intriguingly, we found only weak correlations between cholesterol efflux and HDL-C, which may explain the failure of strategies targeting HDL-C levels to promote atheroprotection. The mechanisms that underlie variation in cholesterol efflux are unknown. In this study will try to identify genetic, protein, and lipid factors that govern variation in cholesterol efflux at a population level.

STUDY DESIGN

To establish the heritability of extreme cholesterol efflux with a prospective family pedigree cohort, participants and their relatives (parents, siblings, adult children, grandparents, aunts/uncles, cousins) of extreme low or high cholesterol efflux will be recruited by a study coordinator under my direction (the original consent for participation in the Dallas Heart Study also includes separate consent to be contacted for future studies, of which 99% assented to be contacted). The Dallas Heart Study maintains an active contact list of all participants and has successfully enrolled participants in multiple prospective family studies.

At enrollment, which will occur at UTSouthwestern Medical Center or at proband's home if the participant is not able or readily willing to come to UTSW for a study visit, Dr. Rohatgi or a study coordinator will perform a survey to collect information including: demographics health history, lifestyle measures, and medications. Blood will then be collected on-site by venipuncture and plasma, serum, and cells will be stored at -80° Celcius. All efflux measurements will be completed in the PI's laboratory. Plasma stores at -80° celcius will be depleted of apolipoprotein B lipoproteins using polyethylene glycol precipitation. Efflux will be determined as previously described from J774 macrophages to apolipoprotein B-depleted plasma. All efflux assays will be performed in duplicate in blinded fashion. Results will be normalized to the measured efflux by a pooled reference sample evaluated on every plate. All samples will be run in duplicate and the average value will be reported. In our own prior studies, intra-plate CV was 3.3% and inter-plate CV was 7.4%. Efflux measurements on each participant and their family members will be run on the same plate and repeated in each quadrant to minimize potential sources of analytical variability. The PI's laboratory is well-versed in high-throughput assessment of cholesterol efflux, having performed measurements in over 3000 participants in the Dallas Heart Study and

selected as a core laboratory to measure cholesterol efflux by an external investigator for over 1500 participants.

The expected outcome is the determination of degree of heritability of the extreme low or high cholesterol efflux phenotype, specific for sex and ethnicity. This will establish for the first time to what degree inherited verses environmental factors associate with variation in cholesterol efflux.

ELIGIBILTY CRITERIA

There are around 500 male and female participants who enrolled in the initial Dallas Heart Study (DHS1), who provided blood and had risk factor assessment are eligible. Those with existing cholesterol efflux measurements from our prior study will be included. Specific inclusion for this study will include those below or above the sex- and ethnicity-specific 10^{th} and 90^{th}% of cholesterol efflux. Family members of the DHS participants may also be invited to participate. All ethnic and racial categories will be included. Participants or their legal guardian must be able to give informed written consent prior to participating. This study includes no prospective direct benefit to individual subjects.

Exclusion criteria: Pregnant women or those with existing HIV, malignancy, or autoimmune disease will be excluded from the study.

RECRUITMENT

Family pedigree studies have been successfully completed from the Dallas Heart Study. Participants enrolled in the Dallas Heart Study (DHS1) who provided consent to be re-contacted for future studies and meet the inclusion/exclusion criteria will be recruited by phone, hard mail, and email. Two-five family members of these participants may also be included. Recruitment and study procedures will take place at UTSW in a research clinic. All participants who agree to participate will give informed written consent. A study staff member will explain the study and review the consent with each participant. A copy of each signed form will be kept in the participant's study file. Each participant will also receive signed copies. The total needed to complete the study would be around 180 participants.

TRAINING

All study staff have completed the appropriate training.

SOURCES OF RESEARCH MATERIAL

1) Blood samples for storage of plasma and serum, extraction of DNA, and isolation of monocytes and lymphocytes. The amount of blood drawn will be at most 75ml. Blood samples obtained will be for research purposes only, 2) demographic information, 3) family history and pedigree and 4) other self-reported pertinent information recorded on questionnaire.

POLICIES OF THE REPOSITORY

1) *Collection of samples* - Subjects will be identified on the collection container subject ID and/or accession number. When samples are distributed to the laboratory the only identifiers will be subject ID and/or accession number.
2) *Storage of samples* – Samples will be stored indefinitely in the Rohatgi Lab (K4.120) on South Campus or in the Human Genetics Core Laboratory, NA2-118 at UT Southwestern Medical Center. The primary contact will be Anand Rohatgi 214/645-7500, anand.rohatgi@utsouthwestern.edu. Storage containers will be labeled with the study subject ID number and/or accession number. These numbers can only be traced to subjects via a password protected, secure computer database in the Core Laboratory or via the subject's chart kept in a locked records cabinet in study coordinator's office.
3) *Linkage of sample to the subject* - When samples are distributed to the laboratory the only identifiers will be subject ID and/or accession number. All subsequent laboratory procedures will refer to the subject ID and/or accession number. Linkage of the subject's PHI (name, etc.) to subject IDs will be limited to the study staff.
4) *Use of samples* – Samples will be used for the study of cholesterol efflux. Plasma and serum aliquots will be stored for testing of biomarkers. DNA will be extracted from blood samples. Single nucleotide polymorphism (SNP) and sequence analysis will be performed on the DNA samples. Monocytes and lymphocytes will be isolated and transformed for immortalized cell lines. Samples will not be used by investigators outside of UT Southwestern except as part of an academic collaboration or a commercial agreement approved by the Office of Contracts management. Samples may be shared as part of such agreement, but in all cases, copies of these materials will be kept at UT Southwestern. Identifying information will not be shared with investigators outside of UT Southwestern.
5) *Use of samples by investigators not affiliated with UT Southwestern Medical Center* – A current record of Multiple Assurance for an IRB at another medical center will be maintained if data and specimens are sent from the repository at UT Southwestern to another medical center. All samples will be de-identified prior to being shared with any investigator and if an investigator at another medical center receives data and specimens from investigators at UT Southwestern, documentation will be maintained that a) the recipient of the samples from the UT Southwestern repository acknowledges that the conditions for use of the research material are governed by the IRB at UT Southwestern in accordance with 45 CFR 46, b) the recipient agrees to comply fully with all such conditions and to report promptly to the repository at UT Southwestern any proposed changes in the research project and any unanticipated problems involving risks to subjects or others, c) the recipient remains subject to applicable State or local laws or regulations and institutional policies which provide additional protections for human subjects, and d) the research material may be utilized only in accordance with the conditions stipulated by the IRB at UT Southwestern. Any additional use of this material requires prior review and approval by the IRB at UT Southwestern and, where appropriate, by an IRB at the recipient site.
6) *Approval of sample use* – Samples will be available to investigators and their collaborators after approval by the principal investigator. All research utilizing these samples will require approval by the IRB at UT Southwestern.
7) *Computer security* – All data pertaining to subjects' identities will be kept in a password protected file and will not be connected to the internet.
8) *Reports of DNA tests results* - The results of DNA tests are not intended to be used to influence decisions about a subject's medical care. The results of the genetic research tests will not be given to the study participants or their physicians. Results will not be given to private physicians, medical insurance providers, life insurance providers, or employers.
9) *Publication of private information* – Any information that could be linked to a subject will be published only with the subject's consent.
10) *Certificate of Confidentiality* – A Certificate of Confidentiality will be obtained.

11) *Release of subject's sample to a third party for purposes other than research* – Samples will not be released to a third party for purposes other than research.
12) *Future contact with subjects* – Subjects may be contacted to participate in future research, to invite a subject's family members to participate in research, or to obtain health information.

REMOVAL FROM STUDY
A subject may request to discontinue study participation. The subject will not be asked for further information or samples. The identity of the subject will be removed from the research records. However, neither the data generated by the subject's samples nor any samples will be destroyed because they cannot be linked to the subject in any way.

POSSIBLE RISKS TO SUBJECTS
There is minimal risk to subjects participating in this study. Risks from blood draws include, 1) discomfort, bleeding, and/or bruising, 2) dizziness or feeling faint, 3) infection may develop on rare occasions at the site where blood was collected. Blood will be drawn by trained, experienced personnel. There is also a risk of psychological, social, or legal harm to subjects in the event of loss of confidentiality. All research staff have completed appropriate Human Subjects and HIPAA training. Results of DNA tests will not be released for purposes other than research and a certificate of confidentiality will be obtained.

ADVERSE EVENTS
The study coordinator will be responsible for reporting any adverse events.

POSSIBLE BENEFITS
There is not prospect of direct benefit to the individual subject. Findings may benefit others in the treatment and prevention of atherosclerotic heart disease.

COSTS TO SUBJECTS
There are no costs to the subjects to participate in this research. After successful completion of the study visit, a participant will receive a $35 gift card and additional amount of $35 to cover for their transportation expenses.

BIOSTATISTICS
NA

OPTIONAL SUBSTUDY

Title – Understanding the Effect of Food Challenge on Lipid Metabolism and Cholesterol Efflux Capacity

Background-

Elevated plasma lipids and decreased high density cholesterol levels are associated with increased risk of atherosclerotic cardiovascular diseases. Plasma lipids are usually measured after 9hrs of fast even though most of the time in human life is spent in post prandial state (PP). It is known that lipid containing lipoprotein particles change in PP state and effect of such particle remodeling on cholesterol efflux is unknown. Our preliminary findings from current study show clear difference in cholesterol efflux pattern in fasting vs post prandial state. Therefore we plan to test this phenomenon in a controlled intervention.

Aim- The purpose of this optional sub study is to evaluate the effect of lipoprotein remodeling in post prandial state on cholesterol efflux.

After completion of the primary visit, we will determine eligibility of study participants to participate in the metabolic remodeling sub study based on their cholesterol efflux profiles. A total of 100 participants will be recruited from two groups - 10^{th} and 90^{th}% of cholesterol efflux (high and low). CEFS study participants who provided consent to be re-contacted for future studies and meet the inclusion-exclusion criteria will be recruited by phone, hard mail or email. All participants who agree to participate will give informed written consent. A study staff member will explain the study and review the consent with each participant. A copy of each signed form will be kept in the participant's study file. Each participant will also receive signed copies.

At enrollment, which will occur at UT Southwestern Medical Center, up to 50ml of fasting blood sample will be collected by venipuncture after 9hr overnight fast to isolate plasma, serum and cells. A standardized meal replacement shake, Ensure Plus (8oz) consisting of 13 g protein, 52 g carbohydrate and 11 g fat will be provided to the study participant and up to 25ml of post prandial blood sample will be obtained 2hrs after ingestion of the shake. Both, fasting and post prandial samples will be analyzed to measure the cholesterol efflux capacity, plasma lipids etc. During the visit, study staff will also perform a survey to collect demographics, health history, lifestyle measures and medications of the study participant.

After successful completion of the study visit, a participant will receive a $100 gift card and a parking voucher to cover parking expenses. There are no funds available to pay for transportation to and from UTSW, lost time away from work and other activities, lost wages or child care expenses.