Jackson Heart Study Manuscript Proposal

Manuscript Proposal Outline (Upload)

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1. **Proposal Title:** Genetic and Lifestyle factors that Modify Association of *PNPLA3* on Non-alcoholic Hepatic Steatosis Among the Jackson Heart Study

2. **Lead Author:** Hannah Scott

3. **Overview**

Provide a brief overview of the proposal including the nature of the problem to be addressed, scientific relevance, objectives/aims, research question/hypotheses, and methods/analytical plan (**<250 words**):

Non-alcoholic Fatty Liver Disease (NAFLD) is vastly approaching the number one most common liver disease not only in the United States which affects one-third of the population. Severe NAFLD subtype include non-alcoholic steatohepatitis (NASH), cirrhosis, liver failure and hepatocellular carcinoma. The rise in prevalence of NAFLD is largely due to the rise in obesity and insulin resistance that contributes to increase of lipid accumulation. NAFLD is a multifactorial phenotype, it is influenced by many genes acting singly, and interacting with other genes and with environmental factors such as psychosocial factors, nutrition, physical activity etc. We hypothesize that the association of *PNPLA3* gene with liver fat is modified by *ADIPOQ* and *ADIPOR1* genes in a sample of African Americans in the Jackson Heart Study. The goal of our project is to examine the joint effects of polymorphisms in the *PNPLA3* and *ADIPOQ* and *ADIPOR1* genes on NAFLD. This is a follow-up to previous study in the Jackson Heart Study that showed rs738409 SNP in *PNPLA3* to be significantly associated with hepatic steatosis.

Background/Rationale

Please include discussion on relevance of African Americans to the proposed topic (**<1000 words**).

The accumulation of fat in the cytoplasm of liver cells (or hepatocytes) in individuals with insignificant levels of alcohol consumption is referred to as non-alcoholic fatty liver disease (NAFLD).[1] Typically, this fat accumulation is characterized by the development of hepatic lesions very similar to those attributable to alcohol consumption, hence the name. If it proceeds unchecked, could result in injury of hepatocytes, inflammation and fibrosis - to cirrhosis, liver failure and hepatocellular carcinoma. Fortunately, as with alcoholic liver disease, it has been reported that a large proportion of patients with risk factors for NAFLD develop fatty liver and only a minority develop the advanced disease subtypes.[2]

The prevalence of NAFLD in the general population is reported to be between 2 to 24% in most countries where data is available but it is rising in tandem with the rising prevalence of obesity and insulin resistance.[3] The pathophysiology of NAFLD is centrally related to insulin resistance[4] and as such has a multifactorial etiology i.e. caused by a confluence of environmental and genetic factors. Factors such as depression[5, 6] and diet[7-10] have been reported to play a major role. Further, reports from the literature surmise that, like inflammatory states, anxiety and depression provide the additional requisite stimulus for histological progression in environmentally or genetically susceptible individuals.

Family studies and candidate gene analysis have observed that NALFD is genetically determined.[2, 11, 12] Most recently genome-wide association studies have identified several common variants associated with hepatic steatosis.[13] The most common of these variants is the *Palatin-like phospholipase domain containing 3 gene variant (PNPLA3)* I148M.[11, 14] According to Pingitore et al.[15], the *PNPLA3* gene encodes a transmembrane

polypeptide chain exhibiting triglyceride hydrolase activity. Research in animal models and human hepatocytes have demonstrated that the activity of PNPLA3 is regulated by glucose and insulin primarily via pathways involving sterol regulatory element binding protein-1c.[16, 17] Several other genes that have been reported to be associated with fatty liver and replicated across various populations include *TM6SF2*[18, 19]; *NCAN*, *PPP1R3B*, *GCKR* and *LYPLAL1*[13]; *COL13A1* and *EFCAB4B.*[20] However, most research work often focus on the study of either environmental or genetic factors independently and seldom consider the joint effects of these factors (i.e. how these factors could modulate each other). In the same manner, potential interactions between genes is rarely studied despite substantial evidence that etiology of multifactorial phenotypes such as NAFLD's is complex.[2]

For this study, we hypothesized that the association of *PNPLA3* gene with liver fat is modified by *ADIPOQ* and *ADIPOR1* genes in a sample of African Americans in the Jackson Heart Study. This research question is motivated by our previous work on these genes[12, 21]

4. **Research Hypothesis:** We hypothesize that the association of *PNPLA3* gene with liver fat is moderated by genetic factors (*ADIPOQ* and *ADIPOR1* genes) and psychosocial factors (depressive symptoms) in a sample of African Americans in the Jackson Heart Study.

5. **Inclusions/Exclusions**

We propose to analyze all JHS participants with imputed 1000Genomes data on *PNPLA3*, *ADIPOQ* and *ADIPOR1*, and who provided responses to the CESD questionnaire on depressive symptoms. We will also focus on participants with CT scan data on liver attenuation.

6. **Statistical Analysis Plan and Methods**

Include power calculations, if necessary.

Tests of Hardy-Weinberg equilibrium, genotypic association and associated summary statistics will be computed using SNPGWA. We will model the G allele at the rs738409 SNP of the *PNPLA3* gene and evaluate how its effect on NAFLD is moderated by genotypes at SNPs with clinical function (as determined from NCBI SNP data base: https://www.ncbi.nlm.nih.gov/SNP/) at the *ADIPOQ* and *ADIPOR1* genes. SNPs at the *ADIPOQ* and *ADIPOR1* genes will be expressed as counts of the minor allele. Depressive symptoms (present/absent) will be defined according to the Center for Epidemiological Studies Depression (CES-D) score ≥16.

Model:

$$Y = \beta_0 + \beta_c COVARIATES + \beta_{rs738409} RS738409 + \beta_G SNP + \beta_{rs738409*G} RS738409 * SNP$$

Where Y is liver fat phenotype, β_0 is the intercept; β_c is the vector of covariates (age, age^2, sex, number of drinks, principal components); $\beta_{rs738409}$ and β_G SNP effects and their interaction effects. $\beta_{rs738409*G}$ represents how much $\beta_{rs738409}$ and β_G SNP change per unit-increase in minor alleles at rs738409 and selected SNPs at *ADIPOQ* or *ADIPOR1* genes. For depressive symptoms, β_G SNP will be replaced by β_EE where E represents presence or absence of depressive symptoms.

We will use multiple linear regression model to test for the slope of rs738409 while controlling for covariates and other SNPs at the two selected genes or depressive symptoms. To transform liver attenuation to be normally, we will employ inverse normal transformation as proposed previously. We propose to control for age and age*age, sex, number of drinks, adiposity, population structure.

Meta-analysis: We propose to conduct meta-analysis in all GOLD consortium cohorts (African Americans, Europeans and Hispanics). This proposal is part of the GOLD Functional project, in which JHS is a participant.

7. **References (maximum 15)**

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