Protocol for l_mrna02_ex08_1_0716:

**Systems Approach to Biomarker Research in Cardiovascular Disease in the NHLBI’s Framingham Heart Study: The SABRe CVD Initiative**

The NHLBI’s Framingham Health Study (FHS), one of the world’s preeminent observational study settings, is devoted to increasing scientific knowledge by establishing a public resource of data and samples. For nearly 60 years, the people of Framingham have generously volunteered their time to this high-profile study and remain deeply committed to its success. The Systems Approach to Biomarkers Research in Cardiovascular Disease Initiative (SABRe CVD Initiative) will generate extensive biomarker data from 7000 FHS participants using multiple high throughput platforms including immunoassays, proteomics, metabolomics/lipomics, and gene expression and microRNA profiling. SABRe will generate thousands of new biomarkers from each participant, with data deposited with the NCBI and linked to the extensive phenotype and genotype data already in an NCBI data repository (as part of the NHLBI SHARe Genome-wide Association Project). All data from the SABRe, SHARe, and the nearly 60 years of phenotypic data from the FHS will be integrated into a massive resource that will be accessible to the outside scientific community in a manner consistent with the informed consent preferences of the Framingham participants.

**SABRe CVD Projects**

- **Project 1:** This project proposes to conduct a metabolomic and proteomic-based (Mx & Px) approach with the objective of identifying a novel set of markers of atherosclerotic CVD and metabolic risk factors in the FHS biosamples that will prove to be robust predictors of disease risk in this and other populations.
- **Project 2:** Immunoassays of 180 circulating protein biomarkers of atherosclerosis and metabolic syndrome in 7400 FHS participants.
- **Project 3:** Gene expression profiling of WBC derived RNA to characterize the genomic signatures of atherosclerosis and metabolic syndrome in 6000-7000 FHS participants (lab work to take place at NHLBI Core Microarray Lab).
- **Project 4:** MicroRNA profiling of WBC derived RNA to characterize microRNA regulation of gene expression and the relations of microRNA to clinical traits and diseases. microRNA profiling will be conducted in 6000-7000 FHS participants.

The SABRe Initiative is a state-of-the-art research enterprise to advance personalized medicine through biomarker discovery and validation. This project holds great promise for identifying mechanisms of disease and promoting the development of new diagnostics and therapeutics for diseases of high impact.

The specific aims for SABRe CVD are as follows:

1. **To identify the biomarker signatures of atherosclerosis as determined by:** a) aortic and coronary calcification on CT (data available in 3500 people), b) aortic plaque burden by MRI (n=2000), c) carotid intimal-medial thickness by ultrasound (n=3500), d) clinical atherosclerotic CVD (n=500), and e) the dynamic balance between arterial calcification and bone demineralization (n=3500).

2. **To identify the biomarker signatures of metabolic risk factors related to cardiovascular risk:** a) systolic and diastolic blood pressure (n=7000), b) body mass index (n=7000) and visceral adiposity by CT (n=3500), c) dyslipidemia (lipid levels, n=7000), and d) impaired fasting glucose, diabetes, and insulin resistance (glucose and insulin levels, n=7000).

3. **To identify genomic convergence** (convergence of signals from genetic variation and gene expression) with SABRe biomarker levels and clinical traits and diseases.
Technical Sources of Variability in microRNA concentrations: the Framingham Offspring CVD case-control study

Several technical sources contribute to variation in microRNA concentration (crossing threshold, Ct) values. Sources include (i) RNA isolation batch, (ii) RNA quality, (iii) RNA concentration, and (iv) 260/280 ratio. Isolation batch is a 27-level categorical variable in this study. Each of the other variables is continuous. We adjusted for the categorical isolation batch and continuous quality, concentration and 260/280 ratio in all the association analyses. The original spreadsheet for the 3 continuous technical variables contains zero or negative data. We treated these non-positive data as missing and did imputation based on simple linear regression models.