SUPPLEMENTAL MATERIAL

Appendix 1. Details of Blood Specimen Procurement and Processing

Baseline Laboratory Samples

Approximately 20mL of fasting blood was drawn from each subject in 3 tubes for laboratory and genetic analyses. The tubes were processed, refrigerated and sent overnight mail, with freezer packs, to the core laboratory (Clinical Reference Laboratory, Lenexa, KS) on a daily basis. Blood was analyzed for the following: hemoglobinA1c, glucose and insulin levels, high sensitivity C-reactive protein, ApoA1, 25(OH) Vitamin D, intact parathyroid hormone, phosphate, calcium, troponin-T, and pro-brain natriuetic peptide (pro-BNP). Patients' lipid phenotypes were determined by characterizing multiple lipidomic variables using the Atherotech VAP test (Atlanta, GA) to assess lipids (cholesterol, triglycerides and FFA), multiple lipoproteins (multiple HDL, LDL, IDL, VLDL, and Lp(a) species), and fatty acyl species in the triglyceride fraction (palmitate, palmitoleic, stearate, oleate, linoleate, linolenate, arachidonate, eicosapentaenoate, and docosahexaenoate). A 1ml sample of whole blood was frozen at -80ºC for future analyses.

For genetic analyses, genomic DNA was isolated and purified from whole blood using Qiagen QIAamp DNA Blood Midi Kit (if volume <2 mL) or Maxi Kit (if volume >2 mL) (Quiagen, Germantown, MD, USA).[1] DNA samples were divided equally into 2 fractions: one aliquot was transported monthly to the Applied Genomics Core Laboratory at Washington University in St. Louis; the second aliquot was held at Saint Luke's Mid America Heart Institute for long-term storage at -80°C. For the 7% of patients who provided saliva samples for genetic

analyses, genomic DNA was purified using the Oragene®•DNA sample collection kit (DNA Genotek Inc., Ottawa, Ontario, Canada)[2] at the Applied Genomics Core Laboratory.

After genomic DNA was purified from all available samples (blood and saliva), whole genome amplification was performed on 20 nanograms of DNA from each sample at one time (to optimize uniformity of amplification) using illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare, Piscataway, NJ, USA). Genomic and whole genome amplified DNA were plated separately (i.e. genomic DNA onto one set of plates and whole genome amplified DNA onto other sets of plates) onto duplicate sets of 96-well plates for future genotyping.

Follow-up Laboratory Samples

For patients who agreed to an in-home visit by trained medical personnel at 1- and 6-months, a urine sample and ~18mL of blood divided into 3 tubes were obtained for longitudinal laboratory analyses. These tubes were then sent to the core laboratory for processing. In patients who agreed to blood collection but for which an in-home visit was not possible for technical reasons, kits were mailed to the patients who were asked to take these kits to their physician. The treating physician was asked to complete the clinical measurements of weight, waist circumference, blood pressure, and pulse, and then mail the labs for processing.

References:

1. Qiagen. Sample & assay technologies: Qiaamp system. http://www.qiagen.com/products/genomicdnastabilizationpurification/qiaampsystem/default.aspx. Accessed on July 13, 2010.
2. Genotek D. DNA testing - DNA from saliva - oragene-DNA. http://www.dnagenotek.com/DNA_Genotek_Product_Oragene_DNA_A_Overview.html. Accessed on June 8, 2010.