SUPPLEMENTAL METHODS

Comparison of proteomic measurements across platforms in the Atherosclerosis Risk in Communities (ARIC) Study

Authors: Mary R. Rooney, Jingsha Chen, Christie M. Ballantyne, Ron C. Hoogeveen, Olive Tang, Morgan E. Grams, Adrienne Tin, Chiadi E. Ndumele, Faiez Zannad, David J. Couper, Weihong Tang, Elizabeth Selvin, Josef Coresh

Quick Thaw/Refreeze Protocol

Plasma samples (2 ml vials) were thawed at room temperature (20-22°C or 68 -74°F) for 25 minutes and were mixed by inverting 12 times to disrupt any concentration gradients formed during thawing. Plasma samples underwent centrifugation (5 minutes at 10,000 x g at room temperature) and were aliquotted as needed for laboratory analysis. Remaining thawed plasma samples were immediately refrozen at -80°C.

Additional Information on the Targeted Immunoassays

Luminex and ELISA assays were performed in duplicates (i.e. each sample was measured in duplicate). For the Luminex and ELISA assays, samples were run in a randomized and blinded fashion with calibrators (i.e. standard curves) and QC control materials included on each run. Batch size was n=37 samples, meaning that each plate included n=37 samples, calibrators and QC controls. All samples, calibrators and QC controls were run in duplicate on each plate. The Roche GDF-15 was measured in single analysis as is typical for this clinical assay.

Multiple configurations of the Luminex Assay were used for ST2, IL-6, TNF-α, IL-10, TIMP-1. Where there are multiple configurations of the same Luminex assay, we report correlations for each aptamer to each individual Luminex result. Results based on the multiple configurations of the same assay were not averaged.

Other Relevant Measurements for the Targeted Comparison

Serum creatinine was measured using the Jaffe method (soon after visit 5), and cystatin-C using the Gentian cystatin-C reagent (analyzed in 2012-13 using previously frozen samples). Both creatinine and cystatin-C were used to determine estimated glomerular filtration rate (eGFR)¹³ Coronary heart disease (CHD), heart failure (HF), and stroke were defined using standard methods in ARIC.¹⁴⁻¹⁶

Details on Duplicate Values Across Highly-Multiplexed Platforms

We provide 417 protein comparisons (366 unique proteins) for the Olink and SomaScan platforms. Thirty-one proteins have more than one aptamer measured on SomaScan, i.e., measuring >1 SOMAmer ID for each entrezgenesymbol. Eleven proteins were measured on two different Olink panels and have 2 OlinkIDs for each entrezgenesymbol, e.g. C-C motif chemokine 2 (MCP1) was measured on Olink's CVD III panel OID00484 and on the Inflammation panel OID00576. Pairwise comparisons were performed, e.g., DLK1 has 3 comparisons for 1 Olink antibody and 3 SomaScan aptamers and the Olink value did not differ.