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

Running head: Comparison of proteomic platforms

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ABSTRACT

Background: The plasma proteome can be quantified using different types of highly-multiplexed technologies, including aptamer-based and proximity-extension immunoassay methods. There has been limited characterization of how these protein measurements correlate across platforms and to absolute measures from targeted immunoassays.

Methods: We assessed the comparability of 1) highly-multiplexed aptamer-based (SomaScan v4; Somalogic) and proximity-extension immunoassay (OLINK Proseek® v5003; Olink) methods in 427 Atherosclerosis Risk in Communities (ARIC) Study participants (Visit 5, 2011-2013), and 2) 18 of the SomaScan protein measurements against targeted immunoassays in 110 participants (55 CVD cases, 55 controls). We calculated Spearman's correlations (r) between the different measurements and compared associations with case-control status.

Results: There were 417 protein comparisons (366 unique proteins) between the SomaScan and Olink platforms. The average correlation was $r=0.46$ (range: -0.21 to 0.97; 79 (19%) with $r\geq 0.8$). For the comparison of SomaScan and targeted immunoassays, 6 of 18 assays (GDF15, ST2, MMP1, adiponectin, leptin, resistin) had good correlations ($r\geq 0.8$), 2 had modest correlations ($0.5\leq r<0.8$; osteopontin, IL6), and 10 were poorly correlated ($r<0.5$; TIMP1, MMP3, MMP7, MCP1, IL10, VCAM1, ICAM1, IL18, TNF- α , visfatin) overall. Correlations for SomaScan and targeted immunoassays were similar according to case status.

Conclusions: There is variation in the quantitative measurements for many proteins across aptamer-based and proximity-extension immunoassays (~1/2 showing good or modest correlation and ~1/2 poor correlation) and also for correlations of these highly-multiplexed technologies with targeted immunoassays. Design and interpretation of protein quantification studies should be informed by the variation across measurement techniques for each protein.

Keywords: proteins, immunoassay, multiplex immunoassays, epidemiology studies, cardiac disease

INTRODUCTION

The advent of highly-multiplexed technology to simultaneously measure thousands of proteins has fueled a recent boom in proteomics research. Methods for measuring the human plasma proteome include an aptamer-based(1) approach and a proximity-extension immunoassay(2) approach. These highly-multiplexed approaches provide the relative concentrations of proteins, using different methodologies. The aptamer approach, for example, can be impacted by features such as three-dimensional epitope shape modification or masking in multi-molecule complexes. The extent to which protein measurements correlate across platforms is relatively uncharacterized.(3, 4) Characterizing the comparability across highly-multiplexed proteomics platforms is crucial for synthesizing findings that utilize different platforms to measure the human proteome. Few studies have examined correlations of relative protein measurements across platforms and with absolute measures from targeted immunoassays used in research and clinical settings.(3, 5-8)

Using data from the Atherosclerosis Risk in Communities (ARIC) Study, we firstly assessed the comparability of highly-multiplexed aptamer-based (SomaScan by SomaLogic) and proximity-extension immunoassay (Olink) methods in 427 participants. We also correlated relative abundance on both platforms, including cystatin-C, to estimated glomerular filtration rate (eGFR) and to absolute measures of plasma cystatin-C (clinical immunoassay). Secondly, we tested the comparability of 18 aptamer-based protein measurements against targeted immunoassays in 110 participants (55 cardiovascular disease (CVD) cases and 55 controls). The study design allowed us to conduct an exploratory study of how differences might translate to associations with CVD. Additionally, among 29 participants, we explored correlations of 16 proteins that were measured on all platforms (SomaScan, Olink, targeted immunoassays).

METHODS

Study Design

The ARIC Study is a community-based cohort study which began in 1987-1989 when the 15,792 participants were middle-aged. The participants were recruited from four US communities: Forsyth County, North Carolina; Jackson, Mississippi; suburban Minneapolis, Minnesota; Washington County, Maryland. Visit 5 occurred in 2011-2013 and was attended by 6,538 participants. At visit 5, participants underwent phlebotomy. At each ARIC site, the plasma underwent standardized processing and storage in -80°C freezers until the specimens were shipped on dry ice to the ARIC central laboratory(7, 9). Not previously (never) thawed ARIC Visit 5 samples were thawed using a standardized quick thaw/refreeze protocol, aliquoted and shipped on dry-ice to Somalogic and Olink for analysis (See Supplemental Methods). Samples had one freeze-thaw cycle when arriving at the site of lab analysis (Somalogic and Olink) and were analyzed in single analysis in a randomized and blinded fashion at each laboratory site.

Aptamer-based proteomic platform

The SomaScan Version 4 platform uses multiplexed modified DNA-based aptamer technology (Somalogic; Boulder, Colorado, USA).(1) The relative concentrations of 5,284 aptamers were quantified on plates at Somalogic using processes previously described with relative fluorescence intensity calibrated using standards on each plate and normalized for plate variation(1, 10-12) and overall concentration of all proteins in a sample using adaptive normalization by maximum likelihood (ANML). Briefly, short single-stranded DNA with modified nucleotides, known as Slow Off-rate Modified Aptamers (SOMAmers), were used as protein-binding reagents. These SOMAmers have sequences that can be quantified using DNA detection technology.

Using blind duplicates of the specimens from 187 (4%) ARIC Study visit 5 participants with available SOMAmer data, we derived coefficients of variation using a method by Bland and Altman(13) ($CV_{BA} = e^{\sqrt{\text{mean}(\text{variance}(\ln(X)))}} - 1$). We excluded 329 SOMAmers with a $CV_{BA} > 50\%$,

a variance < 0.01 on the log scale, or binding to mouse Fc-fusion, contaminants, or non-proteins, which resulted in 4,955 SOMAmers (4,712 unique proteins) for potential comparison to Olink proteins. The median inter-assay CV_{BA} for the SOMAmers was 6.6%. The median split sample reliability coefficient (intraclass correlation coefficient) was 0.96. We transformed Somalogic normalized values using the following equation [$\log_2(\text{SomaScan protein abundance}) + \log_2(1/\text{dilution})$] to account for dilutions (1:5, 1:200, 1:20000).

Immunoassay proteomic platform

The Olink Proseek® v.5003 platform (Olink; Uppsala, Sweden) uses a multiplex immunoassay approach based on proximity-extension assay technology.⁽²⁾ Briefly, pairs of oligonucleotide-labeled antibodies were added to the plasma samples and could bind to the target protein present. DNA hybridization occurred when the pair of oligonucleotide-labeled antibodies were in close proximity. This sequence was quantified using a standard qPCR technique. Five Olink panels (CVD II, CVD III, inflammation, organ damage, cardio-metabolic) were assayed.

The relative concentrations of the 460 proteins (92 proteins per panel; 12 proteins were measured on 2 panels) were measured within each plasma sample. Olink data was represented as normalized protein expression (NPX) values which were expressed on the log₂ scale. Using 29 blind duplicates in the ARIC Study, the median inter-assay CV_{BA} for the Olink proteins was 3.4% and the median split sample reliability coefficient was 0.86. Relative protein concentrations below the limit of detection (LOD) were imputed to the LOD. Specimens underwent 1:2025 dilution for the cardiometabolic panel and the CVD III panel underwent 1:100 dilution. To account for dilutions, we added $\log_2(1/\text{dilution})$ to the Olink NPX values that were already expressed on the log₂ scale.

Targeted Immunoassays

Immunoassays were performed in a CLIA/CAP accredited laboratory (with prior experience in LDT and high complexity testing) according to manufacturers' protocols. The Human Magnetic Luminex Assay (R&D Systems; Minneapolis, Minnesota, USA) was used to measure 14 analytes: growth/differentiation factor 15 (GDF-15), interleukin-1 receptor-like 1 (ST2), osteopontin, interleukin-6 (IL-6), interstitial collagenase (MMP-1), metalloproteinase inhibitor 1 (TIMP-1), stromelysin-1 (MMP-3), matrilysin (MMP-7), C-C motif chemokine 2 (MCP-1), interleukin-10 (IL-10), vascular cell adhesion protein 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), interleukin-18 (IL-18), tumor necrosis factor (TNF- α). Four adipokines were measured with ELISAs (adiponectin, leptin, resistin by R&D Systems; visfatin by Invitrogen; Waltham, Massachusetts, USA).

Multiple configurations of the Luminex Assay were used for ST2, IL-6, TNF- α , IL-10 and TIMP-1. Luminex assays were performed according to manufacturer's protocol and intra- and inter-assay CVs are listed in **Supplemental Table 1**. Assay CVs for the adipokines are shown in **Supplemental Table 2**. GDF-15 was also measured using the clinical Elecsys immunoassay (Roche Diagnostics; Mannheim, Germany) on an automated Cobas e411 immunoanalyzer. The assay has a limit of detection (LOD) of 400 pg/mL and an inter-assay CV of 4.8% in our cohort. Details on the targeted immunoassays and other relevant measurements are provided in the **Supplemental Methods**.

We \log_2 -transformed these immunoassay measurements for consistency with the protein measures from the SomaScan and Olink platforms.

Study Design for Comparison of Highly-Multiplexed Platforms

The study design for the comparison of highly-multiplexed platforms is depicted in **Figure 1**. The SomaScan and Olink platforms were used to analyze previously stored plasma samples obtained at ARIC visit 5. Visit 5 specimens were measured on the SomaScan platform

in 2018 while Olink was measured in 2019. The relative abundance of 5,284 proteins were measured at Somalogic using previously stored plasma from ARIC visit 5 participants (n>5000). Stored plasma samples for 500 ARIC participants (250 cases, 250 controls) were tested on the Olink platform. Eligible cases included those without prevalent HF at visit 5 but who developed HF subsequently. Eligible controls were those without prevalent HF at visit 5 who did not develop HF over the same time period and matched based on age and sex. Incident HF was defined using the Framingham Heart Study HF definition(14) and/or as participants who were hospitalized at least once with a HF diagnosis without a prior HF diagnosis.

Study Design for Targeted Comparison

We used data from a pilot study conducted using stored plasma samples of ARIC visit 5 (2011-2013) participants. Participants with prevalent CVD (defined by CHD, stroke, or HF) at visit 5 were not included in this pilot study. Participants with incident CVD by 31 December 2017 were eligible to be cases. Controls included those without incident CVD, who did not die within 5 years after visit 5. The pilot study included 58 cases and 58 controls. Cases were balanced by age (≥ 73 or < 73 years, median), sex, race, and eGFR (≥ 60 or < 60 mL/min/1.73m²). Controls were frequency matched to the age (± 10 years), sex, race and eGFR groupings of cases. There were 3 participants (2 cases, 1 control) whose results did not meet SomaScan QC standards. Therefore, to maintain the matched nature of the study, our final analytic sample size for our targeted comparison included 110 participants (55 cases, 55 controls).

Statistical Analysis

Statistical analysis was performed using R version 4.1.2.

Analysis for Comparison of Highly-Multiplexed Platforms

Olink and SomaScan protein values were presented on the \log_2 scale. For proteins with multiple measurements on the same platform, we report pairwise comparisons for all measurements, e.g., Delta Like Non-Canonical Notch Ligand 1 (DLK1) has 3 comparisons for 1 Olink immunoassay and 3 SomaScan aptamers (Supplemental Methods). We graphed Spearman's correlation coefficients (and reported p-values) for the 417 protein pairs found in both Olink and SomaScan with vs without excluding outliers (>3 standardized residuals from linear regression). After excluding outliers, we summarized the correlations using histograms and summary statistics (mean, median, percentiles). We reported the intercept, slope from unadjusted linear regression (x =SomaScan, y =Olink) and provided summary statistics (mean, SD, SD/mean) for each Olink and SomaScan protein value after accounting for dilutions. We correlated the proteins on each platform with clinical measures of kidney function—eGFR and cystatin-C are strongly related to the plasma proteome(15)—to try to determine which assay is more biologically relevant to kidney function when the assays are poorly correlated. In an exploratory analysis, we examined how cross-platform correlations for the proteins varied across the dilution bins.

Analysis for Comparison between SomaScan and Targeted Immunoassays

We assessed the comparability of 18 plasma analyte pairs measured using aptamer-based (SomaScan) versus targeted immunoassay methods. We generated Spearman's correlation coefficients. Where multiple immunoassay configurations (e.g. multiplex-1, Roche) were used, we report correlations for each configuration compared to Soma and inter-immunoassay correlations. We used logistic regression to obtain age, sex, and race-adjusted odds ratios and 95% CI's for incident CVD (i.e., case definition) per \log_2 (analyte).

RESULTS

Comparisons of the Highly-Multiplexed Platforms

On the SomaScan platform, we had measurements for 4,955 SOMAmers (4,712 unique proteins) in 5,327 ARIC Study participants. On the Olink platform, we measured abundances of 460 proteins (448 unique) in 500 individuals. There were 427 participants with both SomaScan and Olink measurements and there were 417 protein assay comparisons (366 unique proteins) with overlap between the SomaScan and Olink platforms, after excluding proteins with $CV_{BA} > 50\%$ or negative protein values. In the 427 ARIC participants, the mean age was 78 years (SD=5), 44% were women, and 24% self-identified their race as Black (**Table 1**).

The mean correlation of the proteins overlapping on the SomaScan and Olink platforms was 0.46 (median: 0.53, range: -0.21 to 0.97, interquartile range: 0.13 to 0.76). The distribution of the protein correlations was bimodal, with good to excellent correlations for about half of the protein pairs (19% $r \geq 0.8$, 34% $0.5 \leq r < 0.8$) and poor correlations for the other half (47% $r < 0.5$, including 51 $r < 0$) (**Figure 2**). One of the modes was at a correlation near 0. There were 295 correlations with $p\text{-values} < 1.2 \times 10^{-4}$ ($< 0.05/417$). Protein correlations were similar without removing outliers (**Supplemental Figure 1**). Individual Spearman's correlations (p -values) with and without removal of outliers are provided in **Supplemental Table 3**. For each analyte, the intercept and slope from linear regression and the number of outliers removed (outliers per protein comparison: mean 4.4, median 4, range: 0-13) are also reported in Supplemental Table 3. For proteins with >1 measurement on platforms, the average intra-platform protein correlation was $r=0.32$ (range: -0.25 to 0.98) on SomaScan (**Supplemental Table 4**) and was $r=0.91$ (range: 0.85 to 0.98) on Olink (**Supplemental Table 5**).

Average correlations for the overlapping proteins measured on Olink and SomaScan according to dilution bins on the two platforms are shown in **Supplemental Figure 2**. Cross-platform correlations were lowest for proteins measured in the most concentrated samples (i.e. Olink no dilution, SomaScan 1:5 dilution), which included the majority of proteins on each

platform (64% SomaScan, 56% Olink; mean correlation of 0.37 in Olink panels without dilution vs 0.56 in Olink panels with dilutions). No other clear patterns emerged according to dilution bins.

Both platforms had proteins that had good to excellent negative correlations with eGFR (**Figure 3**; SomaScan had 19 proteins with $r < -0.5$, Olink had 44 proteins with $r < -0.5$) while most other proteins were poorly correlated ($|r| < 0.5$) with eGFR. The proteins with good to excellent negative correlations with eGFR on both platforms tended to be more negatively correlated with Olink than with the SomaScan quantification. Of note, SomaScan and Olink measurements of cystatin-C (CST3) were strongly correlated ($r = 0.71$) and were highly inversely correlated with eGFR on both platforms (SomaScan-eGFR: $r = -0.87$, Olink-eGFR: $r = -0.79$) and with plasma cystatin-C (measured by traditional immunoassays, SomaScan-cystatin-C: $r = 0.89$, Olink-eGFR: $r = 0.81$). Proteins that were not correlated with eGFR on SomaScan often had a similarly poor correlation for Olink quantification but a number of the proteins showed a markedly more negative correlation with Olink than SomaScan (Figure 3).

Comparisons of Highly-Multiplexed Platforms versus Targeted Immunoassay Methods

There were 110 participants (mean age 74 years, 49% were women, 44% self-identified their race as Black) in the targeted comparison of the 18 analytes. Characteristics of the 110 participants included in the targeted comparison study are shown in **Table 1** and are reported overall and by case status. Given the 1:1 matching, participants were similar in terms of age, sex, race, and eGFR.

Assays with excellent overall correlation ($r \geq 0.8$) between SomaScan and targeted immunoassays included GDF15, ST2, MMP1, adiponectin, leptin, resistin (**Table 2**). Assays with modest overall correlation ($0.5 \leq r < 0.8$) included osteopontin and IL6. Assays with poor overall correlation ($r < 0.5$) included TIMP1, MMP3, MMP7, MCP1, IL10, VCAM1, ICAM1, IL18, TNF- α , and visfatin. Inter-immunoassay correlations were high except for the modest inter-

immunoassay correlation for TNF- α ($r=0.59$) and poor inter-immunoassay correlations for TIMP1 ($r=0.18$) and IL10 ($r=0.17$). Correlations tended to be similar when stratified by case status, except for TIMP1 (**Table 2**).

GDF-15 (per log2) measured using SomaScan [OR 2.81 (95%CI: 1.38-6.16)] and both the immunoassays [Multiplex-1 OR 2.75 (1.54-5.35); Roche OR 2.51 (1.44-4.72)] were strongly associated with incident CVD (**Table 2**). Higher levels of osteopontin measured using the SomaScan [OR_{per log2(osteopontin)} 3.24 (1.23-10.02)] and the Multiplex-1 immunoassay configuration [1.69 (1.05-2.97)] were associated with elevated odds of future CVD. Higher levels of TIMP1, MCP1, and IL18 from one of the targeted assays were associated with incident CVD, while measurements based on the SomaScan were not statistically significantly associated with incident CVD. Higher levels of resistin measured using the SomaScan [OR_{per log2(resistin)} 3.41 (95%CI: 1.47-8.91)] and the targeted immunoassay [3.14 (1.66-6.52)] were associated with incident CVD.

There were 29 participants with measurements for 16 analytes (TNF, MMP1, Leptin, GDF15, MMP7, VCAM1, resistin, ST2, IL6, osteopontin, MCP1, IL18, TIMP1, MMP3, IL10, ICAM1) that overlapped across all platforms. We did not provide correlations for TNF because TNF concentrations for all 29 participants were below the Olink LOD. In this small subset, there was substantial variation in pairwise correlations of the protein measurements across platforms (**Supplemental Table 6**). Pairwise correlations across the platforms/configurations were generally good or modest for MMP1, leptin, GDF15, MMP7, VCAM1, resistin, ST2, IL6, and osteopontin. For the remaining proteins (MCP1, IL18, TIMP1, MMP3, IL10, ICAM1), Olink tended to have higher correlations with the targeted immunoassay, however correlations for these proteins were largely poor or modest, except for Olink IL10 versus hs-Multiplex ($r=0.76$) and Olink MMP3 vs Multiplex2 ($r=0.87$).

Discussion

In this study, we assessed the comparability of two proteomic platforms using aptamer (SomaScan version 4) and proximity-extension immunoassay (Olink Proseek® v.5003) based methods. We also evaluated the comparability of 18 targeted protein measurements using aptamer-based methods against targeted immunoassays, and in 29 participants examined correlations across all platforms. While many proteins were highly correlated across platforms, a substantial number were not correlated at all. Heterogeneous correlations between proteins assays can result from the assays measuring different features (epitopes, folding, modifications) of the same protein, the same features differently, or features outside the target protein (mis-identification, interference). Understanding assay agreement and heterogeneity is important for scientists interpreting protein measures and biological associations in studies that utilize proteomic platforms.

Previous studies comparing highly-multiplexed proteomics platforms (aptamer-technology vs immunoassay) have similarly reported a substantial range in the correlations for the overlapping proteins. One study reported on 871 overlapping plasma proteins measured on the aptamer-based (SomaScan Version 4) and immunoassay (Olink) from 485 participants.⁽⁴⁾ The distribution of Spearman's correlation coefficients [median $r=0.38$ (range: -0.61 to 0.96)] was similar to our study (**Figure 2**). They reported that 64% of identified protein quantitative trait loci (pQTLs) were concordant across the platforms, which support that the majority of overlapping, measurable proteins are measuring the intended protein targets. However, pQTLs may not directly translate to changes in circulating protein abundance. Another study reported that 106 of 163 pQTLs identified using the Somalogic platform were also identified on the Olink platform.⁽¹⁶⁾ This research, together with our findings, suggest many of the overlapping proteins measured on popular multiplex assays may be capturing the intended biologic targets; however, quantitative agreement can vary substantially for many other proteins.

Most other comparison studies of proteomic platforms have been based on smaller sample sizes and/or older panels which measured fewer proteins (**Supplemental Table 7**).^{(3, 6,}

16-18) One of the prior comparison studies reported a similar distribution in SomaScan (version 1.1k) vs Olink protein correlations (median 0.36, range: -0.58 to 0.93) to our study (Figure 2) for 425 overlapping protein measurements among 10 MI patients (48 plasma samples due to repeated measurements).(3) There were 249 correlations of SomaScan aptamers vs Olink reported in the prior comparison that overlapped with our study, and the overlapping correlations were quite comparable to our findings (**Supplemental Figure 3**, $r=0.59$, $p<0.001$). Potential reasons for the differences in correlations between studies may relate to the different SomaScan versions (version 4 versus 1.1k), sample size ($n=425$ versus $n=10$) and populations (community-based study versus MI patients).

Our findings inform thoughtful application and interpretation of assays in the rapidly evolving and expanding field of proteomics. Researchers should be cognizant of potentially divergent findings across platforms, specifically those using multiplex immunoassays vs aptamer-based methods (~1/2 proteins had good or modest correlations and ~1/2 poor correlations). Assay comparisons provide information about agreement but validity requires knowing the biologically relevant aspect of the protein. Immunoassays often disagree with each other so disagreement with a proteomic platform does not definitively imply which is superior. It is also important to consider assay characteristics as factors that could influence comparisons across platforms. For example, the SomaScan platform reported values below the LOD, while protein values below the LOD on Olink were imputed to the LOD (average % below the LOD in ARIC was 1% on SomaScan and 10% on Olink). Olink values for 3 proteins (IFN-gamma, IL2, ILTA4H) in ARIC were all below the LOD and could not be correlated with SomaScan measurements. Correlations were very poor when the majority of values on one platform were below the LOD (e.g. IL13, IL2). Additionally, the SomaScan version 4 has ~5000 aptamers available while fewer proteins were measured on the Olink panels. Our findings suggest that ~1/2 of the overlapping proteins from the different platforms are highly (19%) or moderately (34%)

correlated but the other ~1/2 had poor correlations ($r < 0.5$) and may be capturing different information.

Limitations include, first, the long-term storage of the plasma specimens and possible protein degradation. However, we have previously demonstrated in ARIC the high reliability of protein markers over time using the SomaScan platform.⁽⁷⁾ Second, for both Olink and SomaScan, relative protein abundance (not absolute concentrations) was examined and may not be on the same scale. Third, power was limited, given the case-control designs, to examine associations with incident events. However, we still found that all measures of GDF-15, a known cardiac biomarker⁽¹⁹⁾, were predictive of incident CVD. Finally, extrapolation of the percentage of protein assay agreement to the full and growing coverage (~7,000 assays in SomaScan and ~3,000 assays in Olink latest platforms) should be done with caution but we should expect substantial variation between assays for different proteins.

Strengths of this analysis include the large sample size and rich assortment of phenotypic measurements. We were able to compare analytes across multiple assays (including targeted immunoassays), and to compare these analytes to eGFR—a biologically relevant consideration for many proteins—in efforts to identify which assay was perhaps of greater biologic relevance. Many proteins shared inverse correlations with eGFR, however, a fraction of the Somalogic proteins had much weaker correlations with eGFR than the correlations found for those proteins measured on Olink (Figure 3, Supplemental Table 3). Redundant protein quantification across (and possibly within) platforms could increase our knowledge of disease associations.

In this study of 427 participants with 417 overlapping protein comparisons (366 unique proteins) measured using two different highly-multiplexed proteomic platforms, we found that correlations between platforms varied substantially. Future research is needed to characterize the complex underlying traits or pathways contributing to discordant findings of specific proteins across platforms. Overall, researchers should consider the specific performance characteristics

of each assay for each protein in planning and interpreting studies that involve proteomic measurements. Continued efforts to standardize untargeted (and targeted) assays with detailed comparisons of when and how they align is important. Future studies should also examine how well untargeted platforms allow for assessment of change in proteins over time.

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Data Availability: The data generated in this study may be made available upon reasonable request. Consistent with a prespecified policy for access of ARIC data, requests may be submitted to the ARIC steering committees for review. The analytic methods and study materials may be made available to other researchers for purposes of reproducing the results or replicating the procedure upon reasonable request.

References

1. Gold L, Ayers D, Bertino J, Bock C, Bock A, Brody EN, Carter J, et al. Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS One* 2010;5:e15004.
2. Assarsson E, Lundberg M, Holmquist G, Björkstén J, Bucht Thorsen S, Ekman D, Eriksson A, et al. Homogenous 96-Plex PEA Immunoassay Exhibiting High Sensitivity, Specificity, and Excellent Scalability. *PloS One* 2014;9:e95192.
3. Raffield LM, Dang H, Pratte KA, Jacobson S, Gillenwater LA, Ampleford E, Barjaktarevic I, et al. Comparison of Proteomic Assessment Methods in Multiple Cohort Studies. *Proteomics* 2020;20:e1900278.
4. Pietzner M, Wheeler E, Carrasco-Zanini J, Kerrison ND, Oerton E, Koprulu M, Luan J, et al. Synergistic insights into human health from aptamer- and antibody-based proteomic profiling. *Nat Commun* 2021;12:6822.
5. Liu RX, Thiessen-Philbrook HR, Vasan RS, Coresh J, Ganz P, Bonventre JV, Kimmel PL, et al. Comparison of proteomic methods in evaluating biomarker-AKI associations in cardiac surgery patients. *Transl Res* 2021;238:49-62.
6. Kukova LZ, Mansour SG, Coca SG, de Fontnouvelle CA, Thiessen-Philbrook HR, Shlipak MG, El-Khoury JM, et al. Comparison of Urine and Plasma Biomarker Concentrations Measured by Aptamer-Based versus Immunoassay Methods in Cardiac Surgery Patients. *J Appl Lab Med* 2019;4:331-42.
7. Tin A, Yu B, Ma J, Masushita K, Daya N, Hooegeven RC, Ballantyne CM, et al. Reproducibility and Variability of Protein Analytes Measured Using a Multiplexed Modified Aptamer Assay. *J Appl Lab Med* 2019;4:30-9.
8. Lopez-Silva C, Surapaneni A, Coresh J, Reiser J, Parikh CR, Obeid W, Grams ME, et al. Comparison of Aptamer-Based and Antibody-Based Assays for Protein Quantification in Chronic Kidney Disease. *Clin J Am Soc Nephrol* 2022:CJN.11700921.
9. The ARIC Investigators. 2012. Manual of Operations Number 7: Blood – Urine Collection and Processing.
10. Walker KA, Chen J, Zhang J, Fornage M, Yang Y, Zhou L, Grams ME, et al. Large-scale plasma proteomic analysis identifies proteins and pathways associated with dementia risk. *Nat Aging* 2021;1:473-89.
11. Candia J, Cheung F, Kotliarov Y, Fantoni G, Sellers B, Griesman T, Huang J, et al. Assessment of Variability in the SOMAscan Assay. *Sci Rep* 2017;7:14248.
12. Williams SA, Kivimaki M, Langenberg C, Hingorani AD, Casas JP, Bouchard C, Jonasson C, et al. Plasma protein patterns as comprehensive indicators of health. *Nat Med* 2019;25:1851-7.
13. Bland JM, Altman DG. Measurement error proportional to the mean. *BMJ* 1996;313:106.
14. McKee PA, Castelli WP, McNamara PM, Kannel WB. The natural history of congestive heart failure: the Framingham study. *N Engl J Med* 1971;285:1441-6.
15. Yang J, Brody EN, Murthy AC, Mehler RE, Weiss SJ, DeLisle RK, Ostroff R, et al. Impact of Kidney Function on the Blood Proteome and on Protein Cardiovascular Risk Biomarkers in Patients With Stable Coronary Heart Disease. *J Am Heart Assoc* 2020;9:e016463.
16. Sun BB, Maranville JC, Peters JE, Stacey D, Staley JR, Blackshaw J, Burgess S, et al. Genomic atlas of the human plasma proteome. *Nature* 2018;558:73-9.
17. Petrera A, von Toerne C, Behler J, Huth C, Thorand B, Hilgendorff A, Hauck SM. Multiplatform Approach for Plasma Proteomics: Complementarity of Olink Proximity Extension Assay Technology to Mass Spectrometry-Based Protein Profiling. *J Proteome Res* 2021;20:751-62.
18. Graumann J, Finkernagel F, Reinartz S, Stief T, Brödje D, Renz H, Jansen JM, et al. Multi-platform Affinity Proteomics Identify Proteins Linked to Metastasis and Immune Suppression in Ovarian Cancer Plasma. *Front Oncol* 2019;9:1150-.
19. Wollert KC, Kempf T, Wallentin L. Growth Differentiation Factor 15 as a Biomarker in Cardiovascular Disease. *Clin Chem* 2017;63:140-51.

Table 1. Participant characteristics of the highly multiplexed platform and targeted protein assay comparisons: The ARIC Study (2011-2013)

	Highly Multiplexed Platform Comparison (SomaScan vs Olink)	Targeted Assay Comparison (SomaScan vs Targeted Immunoassays)*		
		Overall	Cases	Controls
N	427	110	55	55
Age, years	77.9 (5.4)	74.2 (4.5)	74.8 (5.3)	73.5 (3.6)
Female	187 (43.8%)	54 (49.1%)	27 (49.1%)	27 (49.1%)
Black	101 (23.7%)	48 (43.6%)	24 (43.6%)	24 (43.6%)
Body mass index, kg/m ²	29.1 (5.6)	29.9 (5.5)	30.9 (6.4)	28.9 (4.3)
eGFR	67.9 (18.3)	64.5 (21.3)	61.7 (23.6)	67.4 (18.5)
Diabetes	150 (36.0%)	39 (36.1%)	23 (42.6%)	16 (29.6%)
Fasting glucose, mg/dL	115.5 (35.3)	120.4 (45.2)	125.9 (53.0)	115.0 (35.6)
HbA1c, %	6.1 (1.1)	6.2 (1.2)	6.5 (1.5)	5.9 (0.6)
Hypertension	336 (79.8%)	87 (79.1%)	48 (87.3%)	39 (70.9%)
Systolic BP, mmHg	133 (20.1)	132.4 (15.9)	134.4 (17.7)	130.4 (13.7)
Ejection fraction, %	63.4 (8.8)	64.5 (6.9)	63.1 (8.3)	66.0 (4.8)
Incident CHD	62 (14.5%)	11 (10.0%)	11 (20.0%)	0 (0%)
Incident heart failure	224 (52.5%)	29 (26.4%)	29 (52.7%)	0 (0%)
Incident stroke	22 (5.2%)	15 (13.6%)	15 (27.3%)	0 (0%)

Data are presented as mean (standard deviation) or N (%).

*In the targeted assay comparison, baseline participant characteristics were similar according to cardiovascular disease case status (p-values>0.05), except for ejection fraction (p-value=0.02).

Table 2. Spearman's correlations for 18 targeted analytes measured using the SomaScan platform (aptamer-based) and targeted immunoassay methods and OR's (95% CIs) for incident cardiovascular disease (CVD): The ARIC Study (2011-2017)

Protein	Platform	Overall		Control		Case		OR for incident CVD (95% CI)
		Correlation with SomaScan	Inter-immuno assay Correlation	Correlation with SomaScan	Inter-immuno assay Correlation	Correlation with SomaScan	Inter-immuno assay Correlation	
GDF15	SomaScan	-	-	-	-	-	-	2.81 (1.38-6.16)
	MP1	0.83	0.88	0.84	0.81	0.81	0.90	2.75 (1.54-5.35)
	Roche	0.92		0.92		0.90		2.51 (1.44-4.72)
ST2	SomaScan	-	-	-	-	-	-	1.09 (0.51-2.35)
	ELISA	0.91	0.84	0.89	0.73	0.92	0.91	1.31 (0.64-2.79)
	MP1	0.88		0.82		0.92		1.67 (0.77-3.88)
Osteopontin	SomaScan	-	-	-	-	-	-	3.24 (1.23-10.02)
	MP1	0.56	-	0.53	-	0.56	-	1.69 (1.05-2.97)
IL6	SomaScan	-	-	-	-	-	-	2.72 (0.98-8.40)
	hsELISA	0.69	0.91 (vs hsMP) 0.81 (vs MP1) 0.76 (vs hsELISA)	0.66	0.91 (vs hsMP) 0.82 (vs MP1) 0.78 (vs hsELISA)	0.73	0.92 (vs hsMP) 0.80 (vs MP1) 0.75 (vs hsELISA)	1.41 (0.96-2.11)
	hsMP	0.63		0.62		0.63		1.38 (0.93-2.10)
	MP1	0.50		0.55		0.46		1.52 (0.76-3.13)
MMP1	SomaScan	-		-		-		-
TIMP1	MP2	0.87	-	0.89	-	0.86	-	1.14 (0.76-1.72)
	SomaScan	-	-	-	-	-	-	2.60 (0.74-12.56)
	ELISA	0.30	0.18	0.01	0.06	0.57	0.26	3.20 (1.21-9.52)
MP1	0.11	0.22		-0.04		2.38 (0.21-28.17)		
MMP3	SomaScan	-	-	-	-	-	-	1.90 (0.46-8.25)
	MP2	-0.16	-	-0.23	-	-0.10	-	1.61 (0.95-2.81)
MMP7	SomaScan	-	-	-	-	-	-	1.76 (0.84-3.88)
	MP2	0.49	-	0.24	-	0.61	-	1.34 (0.97-1.88)
MCP1	SomaScan	-	-	-	-	-	-	1.05 (0.47-2.33)
	MP1	0.46	-	0.26	-	0.53	-	2.35 (1.13-6.08)
IL10	SomaScan	-	-	-	-	-	-	3.72 (1.11-16.69)
	hsMP	0.03	0.17	-0.02	0.17	0.04	0.17	1.05 (0.84-1.32)
	MP1	-0.17		-0.21		-0.19		1.07 (0.72-1.59)

VCAM1	SomaScan	-	-	-	-	-	-	3.46 (0.94-13.95)
	MP1	0.36	-	0.31	-	0.37	-	1.36 (0.68-2.73)
ICAM	SomaScan	-	-	-	-	-	-	1.26 (0.60-2.68)
	MP1	-0.02	-	-0.02	-	-0.04	-	1.46 (0.90-2.44)
IL18	SomaScan	-	-	-	-	-	-	2.11 (0.98-4.79)
	MP1	0.14	-	-0.01	-	0.19	-	3.01 (1.24-7.86)
TNF- α	SomaScan	-	-	-	-	-	-	2.06 (0.59-8.82)
	hsMP	0.16	0.59	0.32	0.72	0.08	0.50	1.54 (0.78-3.15)
	MP1	0.14		0.21		0.07		1.75 (0.62-5.16)
Adiponectin	SomaScan	-	-	-	-	-	-	0.70 (0.39-1.22)
	ELISA	0.91	-	0.91	-	0.91	-	0.88 (0.56-1.37)
Leptin	SomaScan	-	-	-	-	-	-	1.02 (0.69-1.51)
	ELISA	0.95	-	0.97	-	0.95	-	1.06 (0.84-1.37)
Resistin	SomaScan	-	-	-	-	-	-	3.41 (1.47-8.91)
	ELISA	0.86	-	0.82	-	0.88	-	3.14 (1.66-6.52)
Visfatin	SomaScan	-	-	-	-	-	-	0.34 (0.08-1.13)
	ELISA	-0.05	-	-0.02	-	-0.08	-	1.12 (0.86-1.49)

Figure 1. Study design for the proteomic comparisons of highly multiplexed aptamer-based (SomaScan) and proximity-extension immunoassay methods (Olink Proteomics) and the study design for the targeted comparisons of SomaScan and immunoassays (research and clinical use). For the comparison of highly multiplexed platforms, there were 417 SomaScan versus Olink protein assay comparisons in total (366 unique proteins). In exploratory analyses, there were 29 participants with 16 protein measurements (TNF, MMP1, Leptin, GDF15, MMP7, VCAM1, resistin, ST2, IL6, osteopontin, MCP1, IL18, TIMP1, MMP3, IL10, ICAM1) that overlapped across the SomaScan, Olink, and targeted immunoassay platforms (Olink TNF was excluded in this subset of n=29 due 100% of Olink values being below the limit of detection).



Visit 5 (2011-2013)
Participants aged 66-90 years

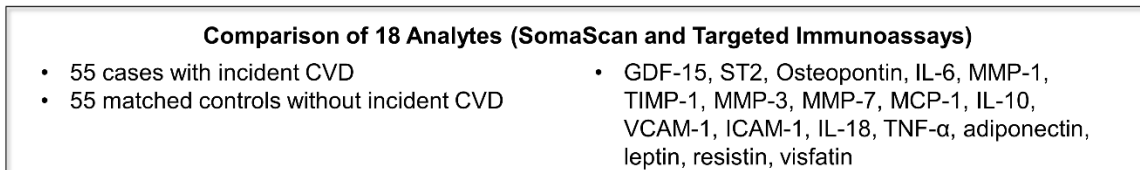
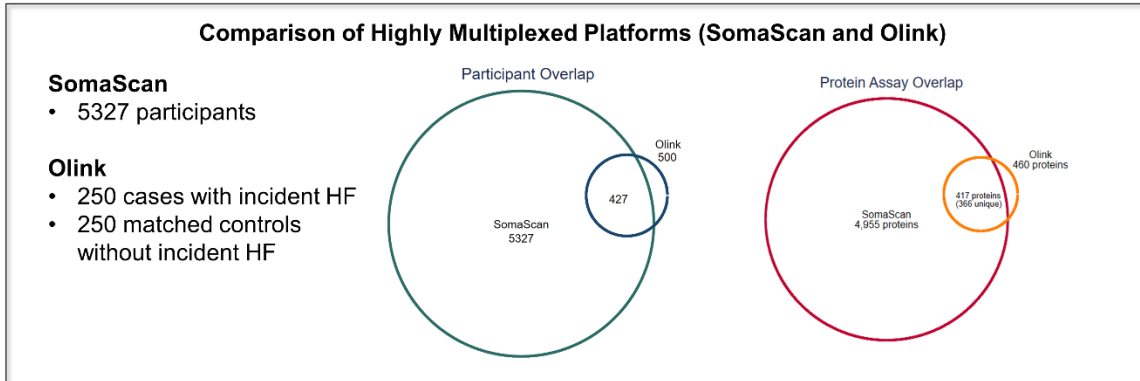


Figure 2. Histogram of Spearman's correlations between the 417 overlapping protein assay comparisons for the Olink and SomaScan platforms with outliers removed. Outliers for each protein defined by protein abundance >3 standardized residuals based on simple linear regression with SomaScan protein (x-variable) and Olink protein (y-variable).

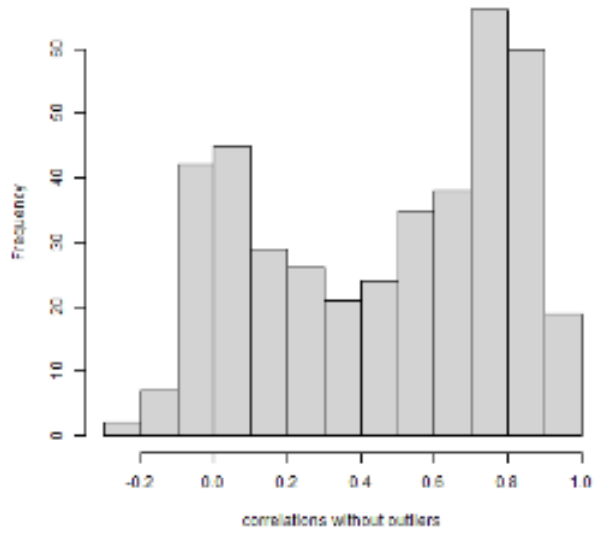


Figure 3. Scatterplot of the correlations of the 417 overlapping proteins measured on the Olink and SomaScan platforms against eGFR. Spearman's correlations of the proteins measured using SomaScan vs eGFR (x-axis) and Spearman's correlations of the proteins measured using Olink vs eGFR (y-axis). Red line is the identity line.

Names of the labeled proteins: ADM, adrenomedullin; AMBP, alpha-1-microglobulin; BAMBI, BMP and activin membrane-bound inhibitor homolog; CAPG, macrophage-capping protein; CD4, T-cell surface glycoprotein CD4; CD5, T-cell surface glycoprotein CD5; CD46, membrane cofactor protein; CD93, complement component C1q receptor; COL18A1, endostatin; CSF1, macrophage colony-stimulating factor 1; CST3, cystatin-C; DCN, decorin; DNER, delta and Notch-like epidermal growth factor-related receptor; EFEMP1, EGF-containing fibulin-like extracellular matrix protein 1; EGFR, epidermal growth factor receptor; EPHB4, ephrin type-B receptor 4; FABP4, fatty acid-binding protein, adipocyte; FAS, tumor necrosis factor receptor superfamily member 6; Gal9, galectin-9; GDF15, growth/differentiation factor 15; IGFBP6, insulin-like growth factor-binding protein 6; IL10RB, interleukin-10 receptor subunit beta; IL15RA, interleukin-15 receptor subunit alpha; IL16, interleukin-16; IL18BP, interleukin-18-binding protein; KLK6, kallikrein-6; LTBR, tumor necrosis factor receptor superfamily member 3; MFAP5, microfibrillar-associated protein 5; PAPP, pappalysin-1; PDL1, Basement membrane-specific heparan sulfate proteoglycan core protein; PGF, placenta growth factor; PLC, basement membrane-specific heparan sulfate proteoglycan core protein; PROC, vitamin K-dependent protein C; RARRES2, retinoic acid receptor responder protein 2; SLAMF1, signaling lymphocytic activation molecule; SPON2, spondin-2; TIMP1, metalloproteinase inhibitor 1; TF, serotransferrin; TFF3, trefoil factor 3; TNC, tenascin; TNFR1, tumor necrosis factor receptor superfamily member 1A; TNFR2, tumor necrosis factor receptor superfamily member 1B; TNFRSF9, tumor necrosis factor receptor superfamily member 9; TNFRSF10A, tumor necrosis factor receptor superfamily member 10A; TNFRSF14, tumor necrosis factor receptor superfamily member 14; TRAILR2, tumor necrosis factor receptor superfamily member 10B; UMOD, uromodulin; UPAR, urokinase plasminogen activator surface receptor; VEGFA, vascular endothelial growth factor A; VSIG2, V-set and immunoglobulin domain-containing protein 2.

