Introduction
Factors that lead to atherosclerosis can exert systemic effects, including impaired kidney function, potentially creating distinct urine protein signatures prognostic of cardiovascular disease (CVD) risk. Urine possesses several attractive features for biomarker discovery and assessment of disease, including CVD, as it is readily available, can be collected noninvasively, and enables monitoring a wide range of physiological processes.

The aims of this project were as follows:
- To determine the number of proteins detectable in urine
- To explore the relationship between the urinary proteome and risk of secondary CVD events in individuals with stable coronary heart disease (CHD)
- To identify proteins in urine that may reveal new biological pathways and systems underlying CVD risk
- To compare the prognostic performance of protein biomarkers in urine and in plasma in predicting CVD events

Methods Overview
- 24 h urine samples were assayed for proteins from 818 participants in the observational Heart & Soul cohort of outpatients with stable CHD, collected at study baseline (Beatty et al. J Am Heart Assoc: 4(7): e001646).
- Follow-up > 11 y.
- Kidney function ranged from normal to moderately impaired.
- A total of 4316 proteins were measured using SOMAScan®, a high-throughput assay that uses modified aptamers as binding reagents.
- A protein-based normalization was performed.
- Urine proteins were analyzed for their association with the CVD outcome (defined as myocardial infarction, stroke, heart failure or death) and CVD risk prediction models were constructed using the LASSO method and backwards selection.

SOMAscan® Proteomics

Proteins are quantified using Slow Off-rate Modified Aptamer (SOMAmer®) reagents, which are chemically modified single strands of DNA that bind to individual proteins as follows:

1. Immobilized SOMAmer reagents are mixed with plasma or urine.
2. SOMAmer reagents form highly specific and stable binding to their target proteins, and after a series of washes that disrupt non-specific interactions, SOMAmers are released from their target-protein complexes in a denaturing buffer.
3. Released SOMAmers are hybridized to their complementary sequences on a microarray chip, and quantified by fluorescence. The resulting signal intensity is a direct reflection of the protein levels in the original sample.
Urine Dilution Curves
Examples of linear & non-linear titration

■ Each sample titration starts at a relative fluorescence unit (RFU) level proportional to the SOMAmer protein content.
■ Some samples will saturate the SOMAmer and titrate into the linear range while others will titrate into the background.
■ Of the 4316 proteins assayed in urine, 2315 had acceptable linearity across 2+ logs of dilution space, constituting our urine proteome for analyses.

CVD Prognostic Markers in Urine
A total of 608 proteins were prognostic for the composite CVD outcome in a univariable analysis (p < 0.05, Bonferroni corrected).

Pathway Analysis for Top 100 SOMAmer Reagents

<table>
<thead>
<tr>
<th>Rank</th>
<th>Pathway</th>
<th>Count</th>
<th>%</th>
<th>P-Value</th>
<th>Fold Enrichment</th>
<th>Bonferroni</th>
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<tbody>
<tr>
<td>1</td>
<td>Complement and coagulation cascades</td>
<td>19</td>
<td>20.43</td>
<td>5.34E-14</td>
<td>9.14</td>
<td>3.26E-12</td>
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<td>2</td>
<td>Acute inflammatory response</td>
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<td>9.96E-08</td>
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<td>3</td>
<td>Response to wounding</td>
<td>26</td>
<td>27.96</td>
<td>4.43E-08</td>
<td>3.31</td>
<td>5.04E-05</td>
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<td>4</td>
<td>Coagulation</td>
<td>12</td>
<td>12.90</td>
<td>4.68E-08</td>
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<tr>
<td>5</td>
<td>Blood coagulation</td>
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<td>5.81E-08</td>
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<td>Regulation of body fluid levels</td>
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<td>3.95E-07</td>
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<td>Activation of plasma proteins involved in acute inflammatory response</td>
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<td>2.90E-06</td>
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<td>Inflammatory response</td>
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<td>5.40E-06</td>
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A urine 7-protein multivariate risk model generated to predict 4 y CVD events performed significantly better than Framingham secondary risk model and similarly to a 9-protein risk model in plasma (Ganz et al., JAMA: 315(23):2532; 2016).

**Conclusion**

A 4 y CVD risk prediction model based on urinary proteins performs similarly to a model based on plasma, suggesting the potential clinical utility of urine as a matrix for early CVD detection.