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Detection of low-abundance serum proteins associated with cardiovascular diseases for prognostic purposes

Classic risk factors of cardiovascular diseases have limitations as predictive and prognostic tools

As the leading cause of death in the United States and worldwide, cardiovascular disease (CVD) includes a family of diseases that affect the heart and blood vessels[1,2]. The primary origin of CVD is most often atherosclerotic in nature, in which fatty plaque deposits line arteries and obstruct circulation of blood[2]. The etiology of CVD is multifactorial and is shaped by the interaction of genes and environment and further influenced by age and gender[3].

Since its initiation in 1948, the Framingham Heart Study has identified measurable elements or characteristics that are associated with the occurrence or prediction of CVD, collectively termed risk factors[4]. Classic risk factors associated with CVD include conditions like hypertension, hyperlipidemia, and diabetes, yet many individuals with CVD do not present with any of the classic risk factors. There are, therefore, limitations to using risk factors alone as prognostic tools for CVD[5,6]. There is thus a substantial need to identify biomarkers that complement existing clinically relevant indicators[7].

Circulating protein biomarkers offer a reliable tool for prediction of cardiovascular diseases

Circulating proteins offer a promising source for CVD biomarkers as proteins associated with atherosclerosis and myocardial fibrosis are released in blood[8,9]. To this end, multi-protein biomarker panels have been employed to study whether prediction of risk for CVD can be improved

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using discovery and known target-based proteomic assays with mass spectrometry[10,11]. However, mass spectrometry-based approaches suffer from bias towards more abundant proteins. This is a particularly important obstacle considering that the human blood proteome is estimated to consist of thousands of protein molecules, many of which are pathologically relevant to CVD but are present in very low abundance (nanomolar range or less)[7]. Similarly, multiplexing of antibody-based assays for capture and detection of targets is limited by cross-reactivity[12].

The SomaScan® Assay overcomes the limitations of poor detection resolution and non-specificity by using SOMAmer® reagents, short oligonucleotides with amino acid-like side chains, which can bind to proteins with high sensitivity and specificity[13] over an 10-log dynamic range. The SomaScan Assay is thus an unbiased multiplex approach for detecting novel blood biomarkers that can shed light on causal disease pathways and help stratify individuals for prognostic and predictive purposes. There are more than 25 publications in which the SomaScan Assay was used to probe cardiovascular disease to date-a few key manuscripts are highlighted in the proceeding section.

Using the SomaScan Assay for accurate identification of proteins associated with CVD risk and outcome

One of the first applications of SOMAmer-based detection of proteins associated with CVD using the SomaScan Assay was led by Drs. Robert Gerszten, Steven Carr, and colleagues at Harvard University. In a publication resulting from the collaboration, Ngo et al. describe the identification of biomarkers for early myocardial injury in 15 individuals undergoing septal ablation treatment for abnormal thickness of heart muscle, or hypertrophic cardiomyopathy[14]. The ablation treatment regimen produces a localized injury (infarct) of the thickened portion of the heart and is therefore useful as a model for studying circulating proteins resulting from heart injury.

Of the 1,129 proteins that were measured by Ngo et al. using the SomaScan Assay, 217 were changed in response to the planned heart injury. Seventy-nine (79) of these were validated in a second cohort as having positive association with myocardial infarction. Both novel and previously characterized biomarkers associations with CVD were identified from this approach.

In a similar study, Gerszten and colleagues used a further expanded version of the assay platform to measure 4,783 distinct protein analytes[15]. The latter study by Jacob et al. identified and validated 29 proteins from the planned MI study that were also elevated in patients with spontaneous myocardial infarction. They also discovered biomarkers that had been previously reported to function in cardiac mechanotransduction and hypertension.

The SomaScan Assay has also been used to corroborate and expand on genomic studies of CVD via the identification of gene locus-protein associations. Howsen et al. identified 15 novel gene variants that are associated with coronary artery disease[16]. Two of these variants were associated with altered plasma protein abundance, including a protein with roles in regulating cholesterol metabolism[16]. Similarly, 120 gene locus-protein associations were identified in a different study involving over 2000 participants[17]. One of the gene variants identified reduced plasma levels of a ApoE linked to circulating cholesterol and this was experimentally validated in cultured human liver cells[17].

For risk stratification in stable coronary heart disease, SomaLogic derived and validated a 9-protein model using a subset of the 1129 proteins measured, to predict 4 year cardiovascular outcomes with superior performance to the best combination of risk factors and lab measurements (Ganz et al.[18])

Most recently, a proteomics study using the SomaScan Assay was used in conjunction with the Framingham Heart Study Offspring to identify biomarkers for atrial fibrillation in 1,885 participants. This study identified 8 proteins associated with AF, none of which had been previously associated with risk for AF[20].

Future potential of the SomaScan assay

Blood biomarkers for CVD risk can also be studied in other conditions that predispose individuals to CVD as was the case for a previous study of Duchenne Muscular Dystrophy (DMD), in which elevated levels of the protein ST2 were identified in DMD patients that suffered from cardiomyopathy[21]. Such studies would particularly benefit from the SomaScan Platform for time course monitoring of protein concentration changes, which can provide critical information regarding strategies for intervention within appropriate time frames.

While the body of work applying the SomaScan Assay to cardiovascular disease research is currently impressive and growing, there is much that we can continue to learn-especially now that the menu of analytes has grown to 7,000 unique protein measurements.

The SomaScan Platform has already been used to study health status in a huge range of areas from cancer to aging to CVD, some of the most interesting results have led to diagnostic, prognostic and predictive tests, currently available as SomaSignal™ tests. These multi-protein tests are not only available to pharmaceutical and academic researchers to yield new insights in heath information but are now also available to the general health care practitioner as a laboratory developed test, heralding a new era of health and wellness management.

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