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Detection of low-abundance serum proteins associated with prediabetes for predictive and prognostic purposes

There is mounting interest in identifying multiple biomarkers during the prediabetic or early diabetic stage to predict and prevent full-blown diabetic onset.

Diabetes mellitus refers to a family of metabolic disorders that are characterized by elevated blood glucose concentrations, or hyperglycemia[1,2]. The International Diabetes Federation estimated that in 2015 there were 415 million diabetes cases worldwide in the 20–79 year old age group and predicted that number to increase to 640 million by 2040[1]. There are two major types of diabetes[3]. Whereas Type 1 diabetes mellitus (T1DM) is an autoimmune disorder resulting from an immune response against pancreatic insulin producing beta-cells of the self, Type 2 diabetes mellitus (T2DM) results from diminished secretion of insulin by the pancreas and insulin resistance in skeletal muscle and the liver[2,3]. T2DM, previously called adult-onset diabetes, accounts for >90% of diabetes cases and is expected to grow rapidly[4].

Predicting disease onset via clinical biomarkers

For T2DM, there is an intermediate stage between healthy and elevated levels of blood glucose, termed prediabetes, that is also expected to rise substantially over the next 20 years[5]. Progression from prediabetes to diabetes can be significantly reduced by interventions including diet and exercise. Diagnosing prediabetes early could therefore help curb the rise of T2DM.

Methods to precisely diagnose prediabetes and diabetes by identifying associated biomarkers have been proposed to prevent or delay advanced stages of the disease; however, currently known biomarkers like the glycated proteins hemoglobin (HbA1c) and albumin are limited by poor sensitivity of detection and inaccuracy under certain clinical contexts[4,5]. Additionally, the American Diabetes Association and the World Health Organization differ in their recommended healthy thresholds for fasting plasma glucose concentrations and HbA1c testing requirements. As such, there is mounting interest in identifying multiple biomarkers during the prediabetic or early diabetic stage to predict and prevent full-blown diabetic onset.

Known biomarkers and associated genetic loci are insufficient for predicting diabetes risk

While multiple genetic loci associated with T2DM have been identified, the use of genetic information to predict future cases of diabetic onset has not been significantly successful to date. A study published in 2008 by Meigs et al. included 2776 participants of the Framingham Offspring Study, who were genotyped for 18 single nucleotide polymorphisms (SNPs) known to be associated with diabetes[6]. The authors found that the 18 risk SNPs provided only a modest increase in risk discrimination over common clinical risk factors and speculated that phenotypic factors could be more reliable determinants of risk of diabetes than known genetic factors. Furthermore, a meta-analysis of 34 published reports of T2DM risk prediction found no substantial advantage of adding genetic and known circulating biomarkers to traditional risk factors[7]. While changes in circulating levels of small RNA molecules called microRNAs (miRNAs) have correlated with diabetic risk, there is no consensus on which miRNAs are specific and relevant to diabetic risk, and technical challenges remain with blood sampling to detect low abundance miRNAs[8].

Serum protein biomarkers, particularly autoantibodies against insulin and islet cells, are often used for assessing T1DM[9]. However, autoantibodies in T1DM are often generated in later stages of disease, and not all autoantibodies are associated with progression of diabetes. There has thus been interest in identifying novel biomarkers at early diabetic stages that are accurate and relatively easy to detect[4,5,9].

Applying the SomaScan® Assay to accurately identify serum proteins associated with diabetic risk and outcome

The SomaScan Assay is ideally suited for detecting low abundance protein analytes associated with diabetes. This assay uses modified DNA aptamers, short oligonucleotides that fold into peptide-like structures, to bind thousands of different proteins with high affinity and selectivity[9]. Over the last two years, several groundbreaking studies have made headway towards identifying and characterizing serum protein biomarkers for T2DM and prediabetes using the SomaScan Assay.

Diabetes is associated with chronic kidney disease (CKD) and cardiovascular diseases (CVD) in a majority of cases[3,5]. However, not all individuals with diabetes present with CKD, and CKD-protected individuals were shown to have a different plasma proteomic signature compared to their CKD-prone diabetic cohorts in a recent study employing the SomaScan Assay[12]. Out of 1,129 plasma proteins investigated, 302 proteins were significantly altered in CKD-protected patients, and many of

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the downregulated proteins identified are known to play a role in renal cell damage. Lending support to the author's hypothesis that increased glucose metabolic activity and mitochondrial biogenesis can halt progression of CKD, four glycolytic enzymes and one mitochondrial biogenesis enzyme were upregulated in CKD-protected individuals over subjects with CKD.

In a study including 100 human subjects that underwent an oral glucose tolerance test (OGTT, the gold standard in T2DM diagnosis), the SomaScan Assay provided insights into fundamental biological functions of pancreatic beta-cells[10]. Beta-cell function was assayed by multiple measures that considered the ratio of glucose levels in blood to the concentration of insulin secreted. Subsequently, changes were assessed in 1,129 serum proteins before and during OGTT. This approach identified 22 proteins associated either positively or negatively with beta-cell function, and the top candidate protein was validated in subsequent in-vitro experiments to modulate insulin secretion[10].

In a larger study involving 413 pre-diabetic participants over a span of ten years, the SomaScan Assay was applied to predict disease risk using a 375-protein model, wherein the participants were divided into a derivation cohort to model protein candidates associated with diabetic outcomes and a blinded validation cohort that tested the derivation model **(Table 1)**[11]. 23% of participants developed diabetes during the course of the study. The predictive model generated using SomaScan data accurately predicted progression from prediabetes **(Figure 1)** and outperformed an OGTT model on the same participants at predicting disease risk[11].

In a different study identifying biochemical changes in individuals with insulin-induced hypoglycemia, different protein signatures were obtained in 10 individuals with T2DM and seven healthy controls[13]. The SomaScan Assay revealed that T2DM subjects infused with insulin had changes in concentration for 13 different proteins, many of which were proinflammatory in nature and are implicated in hypoglycemia-induced cardiovascular complications.

Figure 1. Output for predictive model of diabetes progression derived by Williams et al[11].

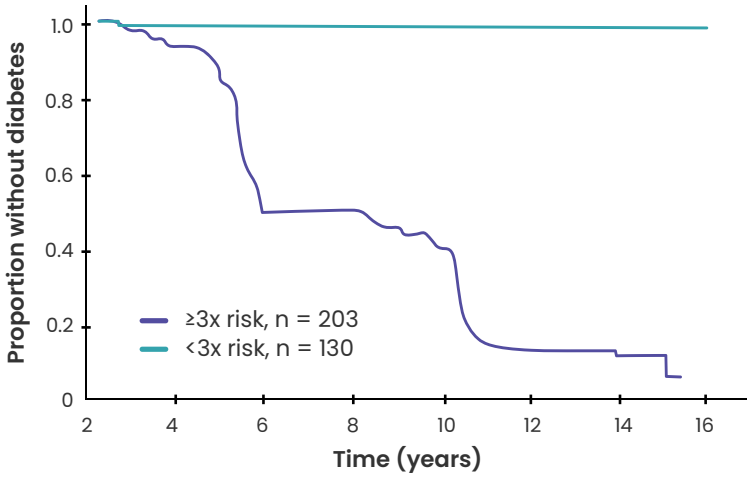

Table 1. Performance metrics for diabetes-related models developed using SomaScan data. Adapted from Williams et al[11]

Model output (truth standard)	Action	Source*	Participants (n)	Metric	Result
Body fat: % (DEXA)	Derivation: best subject characteristics	Fenland (70%)+	8030	r^2	0.74
	Derivation: 219-protein model	Fenland (70%)	8030	r^2	0.92
	Validation: 219-protein model	Fenland (15%)	1721	r^2	0.92
Lean body mass: kg (DEXA)	Derivation: best subject characteristics	Fenland (70%)	8030	r^2	0.74
	Derivation: 115-protein model	Fenland (70%)	8030	r^2	0.83
	Validation: 115-protein model	Fenland (15%)	1721	r^2	0.82
Weekly physical activity: kJ kg-1 d-1 (actigraphy and individually calibrated heart rate)	Derivation: 65-protein model	Fenland (70%)	8187	r^2	0.36
	Validation: 65-protein model	Fenland (15%)	1754	r^2	0.38
Future metabolic health risks					
Conversion from pre-diabetes to diabetes within 10 years, above or below 3× risk	Derivation: OGTT fasting and 2-h glucose	Whitehall II (80%)^	330	Accuracy	61%
	Derivation: 375-protein model	Whitehall II (80%)	330	Sensitivity improvement over OGTT	30%
	Validation: 375-protein model	Whitehall II (20%)	83	Accuracy	67%
				Sensitivity improvement over OGTT	6%

*% source refers to the fraction of the total samples from the source study that were used
+Fenland study, no. 10.22025/2017.10.101.00001
^Whitehall study, no. MR/ R024227/1

Potential applications for the SomaScan Assay in diabetes research

Previous studies have demonstrated the power of the SomaScan Assay in identifying proteomic signatures relevant to pancreatic and renal cell function in diabetic subjects, as well as the ability of models based on SomaScan data to predict diabetes progression. Further studies involving individuals with familial histories of diabetes could help identify the factors linking genetic risk variants with disease onset. Additionally, research using the SomaScan Assay could help identify risk factors or players involved in subsequent stages of diabetic progression. Once definitive factors are identified, the SomaScan Assay could further be used to monitor patient response to treatment or intervention strategies in clinical trials.

The studies described in this document make a clear case for pursuing circulating protein biomarkers as prognostic and diagnostic indicators for diabetes. With the ability to measure 7,000 circulating proteins simultaneously, the SomaScan Assay is the ideal tool for measuring proteomic changes relating to diabetes and other metabolic diseases. RUO tests for diabetes-related indicators (glucose tolerance, lean body fat, and visceral fat) based on SomaScan Technology are now available from SomaLogic, Inc.

To learn how you can get started using the SomaScan Assay for diabetes research, visit SomaLogic.com/discovery.

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Founded in 2000, SomaLogic, a global leader in proteomics, pioneered the SomaScan Platform with unparalleled coverage. Unlike any other technology, the SomaScan Assay enables users to take up to 11,000 protein measurements from just 55 µL of various body fluids like plasma, serum, CSF, and urine.

The proprietary SomaScan Assay measures proteins with high specificity, high throughput, and high reproducibility, which enables the possibility of faster, more precise drug discovery. Our A.I. and machine learning-powered bioinformatics algorithms, operated in tandem with the company's database of more than 750,000 protein samples, helped to create a growing suite of SomaSignal® Tests. These tests provide additional insights into the current health status of patients and the future risk of conditions and diseases. Custom and disease-specific panels are also available for a more targeted approach.

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