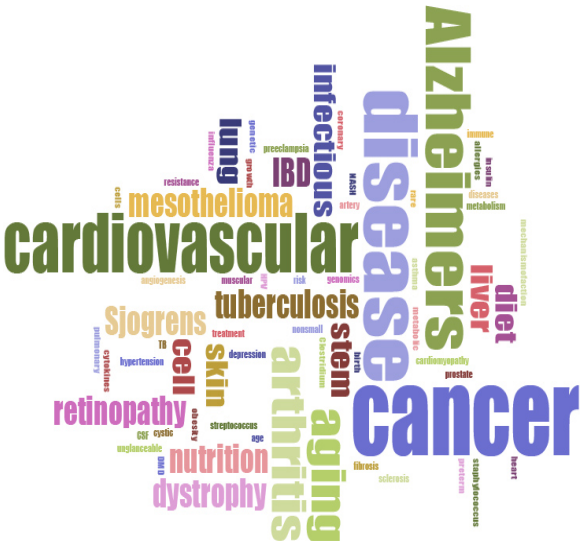
SomaLogic

Annotated SomaLogic and Third-party Publications



This partial list of peer-reviewed publications includes manuscripts describing applications of the technology (below) as well as basic research on the technology itself (starting on p. 41).

Note: Links are provided to the manuscript and if article access requires a subscription, it is noted in each entry below.

I. SOMAscan® Assay/SOMAmer® Reagent Applications

Basic, preclinical and clinical research

Sun, BB *et al.* (2018) "Genomic atlas of the human plasma proteome." *Nature* **558**: 73-79. **(Subscription required)**
https://www.nature.com/articles/s41586-018-0175-2

Over the past decade, genome-wide association studies (GWAS) have identified thousands of DNA variants that are linked to complex traits and diseases but have not explained exactly why they are important. The vast majority of DNA differences flagged by GWAS lie in regions of the genome with no known function and have small effect sizes. This surprising finding makes establishing causal relationships or determining disease risk extremely difficult, even for conditions with a strong hereditary component such as obesity or cancer.

In the largest study of its kind to-date, an international team led by researchers from the University of Cambridge and Merck used the SOMAscan® assay to measure the levels of 2,994 plasma proteins and compared those levels with 10.6 million DNA variants across 3,301 healthy individuals of European heritage. They identified 1,927 genetic variants that impact the levels of 1,478 plasma proteins, of which ~90% had not been previously reported. Many of the variants act in "trans" (i.e., they lie far from the gene whose activity is altered, typically on different chromosomes). *Trans* associations are particularly interesting because they can highlight biological connections that are difficult to predict otherwise.

The authors cross-referenced their findings with known disease-associated GWAS variants to identify proteins that might cause disease. Some disease-associated proteins are targets of existing drugs, which suggests possible therapeutics for new indications. Connecting protein perturbations to disease endpoints also allows identification of new drug targets and potential safety concerns for drugs under development. These results also suggest that monitoring protein levels over time may be sufficient for medical or health management.

Kim, CH *et al.* (2018) "Stability and reproducibility of proteomic profiles measured with an aptamer-based platform." *Sci Rep* **8**(1): 8382.
https://www.ncbi.nlm.nih.gov/pubmed/29849057

The goal of this study led by researchers at Harvard Medical School was to examine the stability and reproducibility of the SOMAscan assay on blood samples obtained under "less-than-ideal" conditions. They performed three different pilot studies: one that assessed SOMAscan reproducibility using split sample sets, another that compared samples processed 0, 24 or 48 hours after collection, and a third that compared samples collected at baseline and a year later. The levels of 1305 proteins were measured in blood plasma taken from 14 male and female locally recruited volunteers, from 16 female participants of the original Nurses' Health Study (NHS) and from 40 female participants in the NHS II (NHS and NHS II were prospective studies that looked at risk factors for chronic disease). Overall, the SOMAscan reproducibility was excellent with coefficients of variation <20% for 99% of proteins and <10% for 83% of proteins. Although the number of samples investigated was small, the results help demonstrate that the SOMAscan assay may be at least partially unaffected by the kinds of collection and processing conditions typically seen with archived blood samples from population-based studies.

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Jin P *et al.* (2018) "Plasma from some cancer patients inhibits adenoviral Ad5f35 vector transduction of dendritic cells." *Cytotherapy* **20**(5): 728-739. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/29655599

Cancer immunotherapy involves isolating a person's immune cells, training them to attack cancer cells, and then reintroducing them into the patient. The immune cells are usually cultured using the patient's own serum or plasma (as opposed to serum pooled from healthy subjects) as it reduces the chances of exposure to an infectious disease. Researchers from the NIH were manufacturing dendritic cells to produce a protein called HER2/neu and noted highly variable expression when they cultured cells in media containing the patient's own plasma. To try to understand the source of the inconsistencies, they used the SOMAscan assay to measure the levels of 1322 proteins in 8 plasma samples that resulted in low HER2/neu expression and 12 that resulted in high HER2/neu expression. SOMAscan analysis revealed 29 proteins that were differentially expressed between the two groups, and the levels of 14 proteins were sufficient to predict which plasma samples gave high or low HER2/neu expression.

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Spitali, P *et al.* (2018) "Tracking disease progression non-invasively in Duchenne and Becker muscular dystrophies." *J Cachexia Sarcopenia Muscle, epub ahead of print*.
https://www.ncbi.nlm.nih.gov/pubmed/29682908

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are muscle wasting diseases caused by mutations in the gene that produces the protein dystrophin. BMD symptoms are typically milder and worsen at a slower rate than DMD, but both diseases are fatal. Muscle biopsies are often used to assess disease progression and therapeutic efficacy but are costly and highly invasive. In this study, investigators in the Netherlands, the UK and Sweden used the SOMAscan assay to compare blood serum proteins in DMD patients, BMD patients and healthy controls. They identified 10 proteins that could discriminate between the three groups. The researchers also conducted a longitudinal analysis of DMD patients, measuring the levels of over 4000 proteins in 14 patients for an average of 4 years, and identified 427 proteins that changed significantly as the disease progressed. These proteins warrant further investigation as candidate blood biomarkers for monitoring muscular changes in DMD patients using less invasive methods than tissue biopsies.

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Hess, AL *et al.* (2018) "Analysis of circulating angiopoietin-like protein 3 and genetic variants in lipid metabolism and liver health: the DiOGenes study." *Genes Nutr* **13**: 7.
https://www.ncbi.nlm.nih.gov/pubmed/29619113

Approximately one quarter of U.S. adults have metabolic syndrome, a set of conditions (obesity, high blood pressure, high cholesterol/triglycerides, high blood sugar) that increases their risk of type 2 diabetes, stroke, heart attack, non-alcoholic fatty liver disease. Angiopoietin-like 3 (ANGPTL3) is a protein produced in the liver that may be involved with development of metabolic syndrome. This study led by Danish researchers used the SOMAscan assay to measure plasma levels of ANGPTL3 and other protein markers of liver health in participants of a European weight loss study. While they did not find a clear link between circulating levels of ANGPTL3 and lipid metabolism during weight loss, there did seem to be a link between ANGPTL3 and certain markers of liver function, and they identified two gene regions that associate with changes in ANGPTL3 during dietary intervention.

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Simats, A *et al.* (2018) "Characterization of the rat cerebrospinal fluid proteome following acute cerebral ischemia using an aptamer-based proteomic technology." *Sci Rep* **8**(1): 7899.
https://www.ncbi.nlm.nih.gov/pubmed/29784938

The goal of this study from researchers at the Universitat Autònoma de Barcelona in Spain was to understand what happens to molecules in the brain immediately following a stroke. They used the SOMAscan assay to measure protein levels in the cerebrospinal fluid taken from rats before and 30 minutes after a surgically-induced stroke. After discounting any proteins found altered in sham surgical controls, they identified 716 proteins that were significantly changed, most of which were involved in inflammatory response and neuronal death processes. Five proteins that were among the top hits (CKB, CaMK2B, CaMK2D, CaMK2A and CMPK) were examined further in circulating blood samples taken from human patients before and <6 hours after suffering an ischemic stroke. The levels of CKB and CMPK were significantly higher in stroke patients than in controls and the levels of CaMK2B and CMPK were significantly higher in stroke patients who had worse functional outcomes. This SOMAscan study in a rodent model successfully identified three proteins for further study as potential biomarkers of strokes in humans.

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Depner CM *et al.* (2018) "Mistimed food intake and sleep alters 24-hour time-of-day patterns of the human plasma proteome." *Proc Natl Acad Sci U S A, epub ahead of print.* **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/29784788

The body's internal workings are tuned to natural rhythms that rise and fall over the course of a day. When those timings are disrupted (e.g. jet lag, shift work), health problems can occur. To gain a better understanding of what happens when biological clocks are disrupted, a team lead by researchers at the University of Colorado Anschutz medical campus investigated how plasma proteins fluctuate over the course of a 24-hour day. They used the SOMAscan assay to measure the levels of 1129 proteins in blood samples collected from six healthy male volunteers on a "circadian aligned" schedule (eat during the day and sleep at night) compared to a "circadian misaligned" schedule (sleep during the day and eat at night). The switch from a day to a night shift significantly changed the patterns and/or levels of 127 proteins, including many associated with the immune system function, energy metabolism and cancer. These findings may help explain why night shift workers are more prone to metabolic diseases and

emphasizes the importance of timing when conducting diagnostic blood testing and administering medications.

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Lam, MPY *et al.* (2018) "Harnessing the power of proteomics to assess drug safety and guide clinical trials." *Circulation* **137**(10): 1011-1014. **(Subscription required)**
http://circ.ahajournals.org/content/137/10/1011?iss=10

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Williams, SA *et al.* (2018) "Improving assessment of drug safety through proteomics: early detection and mechanistic characterization of the unforeseen harmful effects of Torcetrapib." *Circulation* **137**(10): 999-1010.
http://circ.ahajournals.org/content/137/10/999.long

This article from Pfizer, the University of California, San Francisco, the Karolinska Institute and SomaLogic demonstrates how monitoring blood-based protein changes in response to experimental therapies could one day improve the efficiency and safety of drug development. Researchers used the SOMAscan assay to measure the levels of 1129 proteins in samples from ILLUMINATE, Pfizer's phase 3 clinical trial of torcetrapib, a drug candidate for treating heart disease. Torcetrapib raises levels of 'good' cholesterol and lowers levels of 'bad' cholesterol and was expected to be a blockbuster drug that reduced the risk of serious cardiovascular events such as heart failure and stroke. Instead, an increase in deaths and heart problems was seen in trial subjects taking torcetrapib, and ILLUMINATE was abruptly terminated. This happened in 2006 after Pfizer had invested 15 years and nearly a billion dollars in torcetrapib development.

The new study used a previously validated nine-protein cardiovascular risk score (Ganz, P *et al.* (2016) *JAMA* 315(23): 2532-2541.) to successfully predict the harmful effects of torcetrapib after three months of treatment—much earlier than the point at which ILLUMINATE was stopped (~18 months). The work also provides new insights into how torcetrapib acts in the body and possible clues to its toxicity. Analysis of the 200 proteins that changed significantly compared to matched controls revealed that torcetrapib had widespread and unanticipated effects on immunity and inflammation. In addition, changes in eight proteins were linked to synthesis or function of aldosterone, a steroid hormone involved in regulating blood pressure. These results help explain the hypertensive side effects seen early in torcetrapib's development.

Torcetrapib is a cholesteryl ester transferase (CETP) inhibitor, a drug class that is of considerable interest to the pharmaceutical industry. In addition to Pfizer, both Eli Lilly and Roche had CETP inhibitors that were dropped late in development due to lack of efficacy. However, Merck recently announced that their drug candidate anacetrapib successfully completed the longest CETP trial to date. This article shows how profiling proteins could provide early warning of off-target effects and help speed drug development. It also suggests that these same proteins can be used to monitor the ongoing effectiveness of drug treatment in individuals and populations. In their accompanying editorial, Lam and Ge state that "With continual development and lowering costs of proteomics technologies, future trials will likely be routinely embedded with longitudinal proteomics profiling to enhance participant safety and inform drug assessment."

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McGarrah, RW *et al.* (2018) "Integrative omics: harnessing the proteome to maximize the potential of the genome." *Circulation* **137**(11): 1173-1175. **(Subscription required)**
http://circ.ahajournals.org/content/137/11/1173.long

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Benson, MD *et al.* (2018) "Genetic architecture of the cardiovascular risk proteome." *Circulation* **137**(11): 1158-1172. **(Subscription required)**
http://circ.ahajournals.org/content/137/11/1158.long

Changes in protein levels reflect the functional consequences of gene variants and can help establish hereditary causes of disease. Previously, investigators at the Beth Israel Deaconess Medical Center used the SOMAscan assay to identify 156 plasma proteins that associated with clinical risk factors for developing cardiovascular disease (CVD)—age, sex, cholesterol, blood pressure, diabetes, and smoking (ref: Ngo, D *et al.* (2016) "Aptamer-based proteomic profiling reveals novel candidate biomarkers and pathways in cardiovascular disease." *Circulation* **134**(4): 270-285.). In this study, the researchers integrated genomic data into their SOMAscan-based proteomic profiling and found a number of new connections between gene variants and circulating proteins that are important in CVD. One of the DNA-protein associations led to the discovery that the gene for protein phosphatase 1 (PPM1G) regulates the levels of apolipoprotein E, a cholesterol transporter. This is the first time that PPM1G has been linked to lipid metabolism. The authors have made their gene variant-protein association data publicly available, which should hasten the discovery of additional insights into CVD biology, potential biomarkers and putative drug targets. The accompanying editorial by McGarrah and Shah explains how proteomic information helps expand beyond the static 'snapshot' of CVD provided by genetic studies.

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Parolo, S *et al.* (2018) "Combined use of protein biomarkers and network analysis unveils deregulated regulatory circuits in Duchenne muscular dystrophy." *PLoS One* **13**(3): e0194225.
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0194225

Duchenne muscular dystrophy (DMD) is a disease characterized by progressive muscular atrophy and death, usually by young adulthood. Although the genetic cause of DMD (mutations in the gene that produces the protein dystrophin) has been known since the mid-80s, it is still poorly understood how loss of dystrophin drives DMD progression.

In this study, Italian researchers re-analyzed previously published SOMAscan assay data (ref: Hathout, Y et al. (2015) "Large-scale serum protein biomarker discovery in Duchenne muscular dystrophy." *Proc Natl Acad Sci U S A* **112**(23): 7153-7158.) to identify 52 proteins that were significantly different in the DMD patients, of which 27 had been identified in the previous study. The researchers found that only six of the proteins were needed to diagnose DMD with 100% accuracy. Using previously developed computational methods to see how proteins relate to one another, they identified several biological processes and functions that are dysregulated in DMD. In addition to demonstrating how rich SOMAscan data are (and how they can be continuously mined for new insights), this study expands our molecular understanding of DMD, which is essential for developing effective treatments.

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Wang, X *et al.* (2018) "Chemotherapy-induced differential cell cycle arrest in B-cell lymphomas affects their sensitivity to Wee1 inhibition." *Haematologica* **103**(3): 466-476.
http://www.haematologica.org/content/103/3/466.long

A single cell divides to create two new genetically identical daughter cells in a controlled series of events called the "cell cycle." There are four stages to the cell cycle and progression from one stage to the next is governed by three checkpoints, where the process can arrest to ensure that problems such as DNA damage are fixed before being passed on to the daughter cells. If the damage isn't repaired the cell will die, so figuring out a way to bypass the checkpoints could be a strategy for killing tumor cells containing chemotherapy-induced DNA lesions.

The focused goal of this study conducted by investigators at the University of Colorado Anschutz Medical Campus was to understand how inhibition of a particular checkpoint protein called Wee1 affects the actions of the chemotherapeutic agents cytarabine (Ara-C) and doxorubicin (DOX) on B cell lymphomas. They used the SOMAscan assay to look at differences in protein levels between B cells that were left untreated or treated with Ara-C. Surprisingly, only 3 of the 1310 proteins in the SOMAscan assay changed significantly, including 2 proteins (cyclin A2/B1) that are known to control progression through the cell cycle. Addition of a Wee1 checkpoint inhibitor promoted cell death, but only for cells arrested in particular stages of the cell cycle, and the stage of arrest could be shifted by adding DOX. Their results suggest that a combination of Ara-C, DOX and Wee1 inhibitor could be effective for treating specific types of B cell cancers.

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Zhang, W *et al.* (2018) "Proteomic analysis reveals distinctive protein profiles involved in CD8(+) T cell-mediated murine autoimmune cholangitis." *Cell Mol Immunol*., *epub ahead of print.* **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/29375127

Primary biliary cholangitis (PBC) is an autoimmune disease in which the bile ducts of the liver are slowly destroyed. This destruction causes bile and other toxins to build up, leading to further damage and eventual liver failure. An international team led by investigators at the University of California, Davis have developed a mouse model of PBC, and in this study, they used the SOMAscan assay to look at blood proteins of the mice at different ages. They found significant differences in the serum protein profiles of diseased mice compared to healthy mice and identified a number of proteins that warrant further investigation for their potential importance in progression of human PBC.

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Christensson, A *et al.* (2018) "The Impact of the Glomerular Filtration Rate on the Human Plasma Proteome." *Proteomics Clin Appl* **12**(3): e1700067. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/29281176

Approximately 30 million adults in the US have chronic kidney disease (CKD), a devastating illness in which the kidneys gradually lose their ability to filter blood. The standard indicator of kidney decline is a diminished "glomerular filtration rate (GFR)," as assessed by the time it takes a kidney to clear a compound injected into the subject. However, GFR is costly and time-consuming to measure and is not

very accurate at discerning mild kidney impairment. More sensitive methods are needed that enable early detection of CKD when therapeutic intervention is still possible.

The goal of this study from researchers at Skåne University Hospital and SomaLogic was to see whether blood proteins could provide and accurate picture of kidney function. They used the SOMAscan assay to measure the levels of 2893 proteins in plasma taken from 364 people with a wide range of GFRs. The protein that was most significantly negatively correlated with GFR (levels increased as GFR decreased) was the well-established kidney biomarker cystatin C. Many other proteins were negatively or positively correlated with GFR and warrant further investigation as potential biomarkers. Such proteins may also reveal the underlying biological mechanisms that lead to kidney failure and possible treatment strategies.

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Wasiak, S *et al.* (2018) "Benefit of Apabetalone on Plasma Proteins in Renal Disease." *Kidney International Reports* **3**(3): 711-721.
https://www.sciencedirect.com/science/article/pii/S2468024917304576

Bromodomain and Extra-Terminal domain (BET) proteins, which help turn specific genes on and off, are important drug targets for a wide range of conditions including cancer, neurological disorders and obesity. Apabetalone, a BET inhibitor under development to treat cardiovascular disease (ref: Wasiak, S *et al.* (2017) "Downregulation of the complement cascade in vitro, in mice and in patients with cardiovascular disease by the BET protein inhibitor apabetalone (RVX-208)." *J Cardiovasc Transl Res,* **10**(4): 337-347.), can reduce renal inflammation in patients with severely impaired kidney function. To better understand the effects of apabetalone, a team led by investigators at Resverlogix Corp. compared the levels of circulating proteins in healthy patients and those with chronic kidney disease (CKD). Blood samples from eight people with CKD and eight matched controls were collected before and after taking apabetalone and analyzed using the SOMAscan assay. The levels of 169 proteins differed significantly in CKD patients compared to controls. Many of the identified proteins are well-established markers of kidney function but some are entirely new. Within 12 hours, a single dose of apabetalone significantly lowered the levels of proteins that contribute to inflammation, atherosclerosis and fibrosis. These results suggest that apabetalone may be useful for treating not just CKD, but multiple diseases in which the BET proteins play a role.

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Zaghlool, SB *et al.* (2018) "Deep molecular phenotypes link complex disorders and physiological insult to CpG methylation." *Hum Mol Genet* **218**(3): 347 e341-347 e314.
https://www.ncbi.nlm.nih.gov/pubmed/29325019

Adding a methyl group to DNA is a way to change gene expression without altering the DNA sequence itself. The most common sites for DNA methylation are on cytosines that are followed by guanines (CpG). CpG methylation occurs during normal embryonic development and aging but may also be altered by environmental stress, lifestyle and disease. Previously, an international team led by researchers at the Weill Cornell Medical College in Qatar identified 20 CpG sites that associated with obesity-, diabetes- and smoking-related blood metabolites. In this study, the investigators used a multi-omics approach to replicate and expand upon their earlier findings. They conducted blood, urinary, and salivary metabolomics, lipidomics, glycomics and SOMAscan proteomics analysis on 359 samples from a multi-ethnic cohort, and identified 138 associations between CpG sites and biomarkers of obesity, diabetes

and smoking. Their preliminary results lead to new hypotheses for future studies to help understand the causal relationships between DNA methylation sites and disease phenotypes.

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Thrush, AB *et al.* (2018) "Diet-resistant obesity is characterized by a distinct plasma proteomic signature and impaired muscle fiber metabolism." *Int J Obes (Lond)* **42**(3): 353-362.
https://www.ncbi.nlm.nih.gov/pubmed/29151592

The goal of this study was to see if there are physiological differences between obese individuals who successfully lose weight on diets and those who don't. An international team led by scientists at the University of Ottawa compared the muscle tissue, cells and circulating proteins of 20 women classified as either obese diet sensitive (ODS) or obese diet resistant (ODR) based on their weight loss after six weeks of meal replacement. They found distinct metabolic differences between ODS and ODR women under both fasting conditions and after eating a high fat meal. SOMAscan analysis identified a number of blood proteins whose levels differed significantly between the two groups, and that may help predict weight loss success.

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Hussein, AI *et al.* (2018) "Serum proteomic assessment of the progression of fracture healing." J Orthop Res **36**(4): 1153-1163**. (Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/28971515

The goal of this study was to see whether changes in protein levels could be used to monitor bone regeneration. Currently, this assessment is done using radiology and physical examination, which are subjective and hard to detect reliably, particularly in the early stages of the healing process. Researchers at the Boston University School of Medicine used the SOMAscan assay to monitor blood protein levels in mice as they recovered from a bone fracture. They identified 692 proteins that changed significantly over a seven-week time course of healing, most of which are known to function in repair processes such as coagulation, immune response, bone and blood vessel formation. This study shows the potential of plasma proteins to predict normal bone healing and could lead to a minimally invasive diagnostic for human fracture care.

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Vilar-Gomez, E *et al.* (2018) "Non-invasive assessment of non-alcoholic fatty liver disease: Clinical prediction rules and blood-based biomarkers." *J Hepatol* **68**(2): 305-315. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/29154965

Non-alcoholic fatty liver disease (NAFLD) is a leading cause of liver disease worldwide. Liver inflammation (NASH) and fibrosis are key determinants of NAFLD prognosis but require liver biopsy, which is not suitable for routine screening. This review discusses less invasive and more cost-effective methods, including the SOMAscan assay, for predicting NASH and advanced fibrosis in patients with NAFLD.

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Oller Moreno, S *et al.* (2018) "The differential plasma proteome of obese and overweight individuals undergoing a nutritional weight loss and maintenance intervention." *Proteomics Clin Appl* **12**(1). **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/28371297

Obesity is a global problem that affects all people of all ages and incomes. Worldwide, obesity rates have more than doubled since 1980. Although weight gain is preventable, no country has successfully reduced obesity rates in over 30 years.
Drug treatment for obesity has been only moderately successful, partly because the ability to lose weight and keep it off depends in part on each person's physiology and metabolism. Thus, approaches that are tailored to an individual's specific body chemistry are needed to help manage weight more effectively.

In this study, Nestlé researchers used mass spectrometry together with the SOMAscan assay to analyze samples from overweight or obese (but non-diabetic) individuals enrolled in a multi-center European dietary intervention study. Plasma proteins were measured before and after successful weight loss. Most of the proteins whose levels changed significantly are known, but the study also identified new proteins that if validated could serve as potential biomarkers for obesity and/or weight loss.

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Oak, P *et al.* (2017) "Attenuated PDGF signaling drives alveolar and microvascular defects in neonatal chronic lung disease." *EMBO Mol Med* **9**(11): 1504-1520.
https://www.ncbi.nlm.nih.gov/pubmed/28923828

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Forster, K *et al.* (2018) "Early Identification of Bronchopulmonary Dysplasia Using Novel Biomarkers by Proteomic Screening." *Am J Respir Crit Care Med* **197**(8): 1076-1080. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/29053024

Ventilation with supplemental oxygen can be a life-saving treatment for preterm infants who have difficulty breathing on their own, but it puts some at risk of developing neonatal chronic lung disease, also known as bronchopulmonary dysplasia (BPD). Two different studies led by investigators in Germany used the SOMAscan assay to better understand the molecular underpinnings of BPD. The paper from Oak *et al.* used the SOMAscan assay to look at plasma protein levels in preterm infants with gene variants linked to BPD. They found that impairment of platelet-derived growth factor (PDGF, a protein that regulates cell growth and division) leads to air sac and blood vessel defects seen in BPD. The researchers then found that treatment with supplemental PDGF improved lung function in a mouse model of the disease. The paper by Förster *et al.* used the SOMAscan assay to discover and validate a set of 12 blood proteins whose levels were significantly different in preterm infants who developed BPD. These studies represent the first steps towards developing tools for early diagnosis and treatment of preterm infants at risk for BPD.

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Barbour, C *et al.* (2017) "Molecular-based diagnosis of multiple sclerosis and its progressive stage." *Ann Neurol* **82**(5): 795-812. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/29059494

Neurological diseases such as multiple sclerosis (MS) are extremely hard to diagnose since there is no easy way to see inside the brain of a living person at a sufficient level of detail. Currently, there are no laboratory or physical tests that can definitively establish if a person has MS, and many common conditions (e.g. depression, migraine, fibromyalgia) resemble MS on brain scans and in clinical

symptoms. Misdiagnosis of MS is a frequent problem (by some estimates >20% misdiagnosis rate) that puts many patients at unnecessary risk.

Scientists at the NIH have developed a protein-based diagnostic test for MS that greatly outperformed the current gold standard. They used the SOMAscan assay to measure the levels of 1128 proteins in the cerebral spinal fluid (CSF) of 225 people from 6 different groups: healthy donors, those with different types of MS (relapsing-remitting, primary or secondary progressive), and those with inflammatory or non-inflammatory neurological disorders that mimic MS (e.g., meningitis or epilepsy). The 500 proteins with the highest signals were used to generate a computer algorithm that could distinguish MS from non-MS patients in a separate patient cohort with 90.6% accuracy. A different CSF protein-based classifier was able to differentiate patients with progressive vs. relapsing-remitting forms of MS with 89.4% accuracy. They were unable to create a classifier that could distinguish between different types of progressive MS based on CSF protein levels, which suggests that primary and secondary progressive MS may be biologically equivalent. Taken together, these results may help improve MS diagnosis, monitoring disease progression and predicting treatment efficacy.

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Carayol, J *et al.* (2017) "Protein quantitative trait locus study in obesity during weight-loss identifies a leptin regulator." *Nat Commun* **8**(1): 2084.
https://www.ncbi.nlm.nih.gov/pubmed/29234017

Genome-wide association (GWAS) studies have identified many common gene variants in obese individuals but have not explained how that genetic variability contributes to obesity. To help understand the functional consequences of obesity-related gene differences, researchers from the Nestlé institute of Health Science, Quartz Bio, University of Toulouse, Maastricht University Medical Centre and University of Copenhagen used the SOMAscan assay to examine how gene variants affected the levels of 1129 blood proteins in 494 obese individuals placed on an eight-week diet. They identified 192 proteins that associated with body mass index (BMI) prior to dietary intervention, a third of which were regulated by obesity-related gene variants. A particularly interesting finding was that lower levels of a protein called Fam46A led to higher levels of the protein leptin, a hormone produced by fat cells that helps control appetite. Very little is known about the biological function of Fam46A. This study is one of the first to demonstrate at a molecular level how gene variants can affect the levels of key proteins involved in weight management. These findings may also help researchers design tailored treatments for obesity and other complex metabolic conditions that have so far proven difficult to address.

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Skarke, C *et al.* (2017) "A Pilot Characterization of the Human Chronobiome." *Sci Rep* **7**(1): 17141.
https://www.ncbi.nlm.nih.gov/pubmed/29215023

Each person has an internal “clock,” and various physiological traits (metabolism, blood pressure, body temperature, etc.) rise and fall based on each individual’s unique daily rhythms. Previous studies have shown that most available drugs act on protein targets whose levels oscillate over the course of a day, so understanding a person’s “chronobiome” may help doctors not only give the right treatment but give it at the right time—a primary goal of precision medicine. In this pilot study, a team lead by researchers at the University of Pennsylvania Perelman School of Medicine tested the feasibility of detecting time-dependent signals in six healthy men over four months. Through the course of the study, the men reported what they ate and wore remote sensors as they went about their daily activities. Biological specimens collected over two 48-hour periods were analyzed using a “multi-omics” approach

(microbiome, metabolome, transcriptome and SOMAscan-measured proteome). Despite the small sample size, time-dependent patterns were visible, but more frequent sampling will be needed to confidently differentiate signal from noise—which the researchers hope to accomplish in upcoming studies.

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Romero, R *et al.* (2017) "The maternal plasma proteome changes as a function of gestational age in normal pregnancy: a longitudinal study." *Am J Obstet Gynecol* **217**(1): 67 e61-67 e21. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/28263753

The goal of this study led by researchers at Wayne State University School of Medicine was to characterize the maternal proteome to better understand the biological processes that are affected over the course of a normal pregnancy. Blood samples were collected at different times during the pregnancies of 43 mothers who delivered at term and analyzed using the SOMAscan assay. The levels of 1125 proteins were measured, 112 of which changed significantly as a function of gestational age. Nine of those proteins increased by more than five-fold over gestation, and are involved in processes such as growth regulation, embryogenesis, angiogenesis, immunoregulation and inflammation. This preliminary study helps establish a baseline for the early identification of deviations that signal an abnormal pregnancy, perhaps even early enough to prevent complications or mortality.

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Jacob, J *et al.* (2017) "Application of large scale aptamer-based proteomic profiling to "planned" myocardial infarctions." *Circulation* **137**(12): 1270-1277. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/29222138

In this study, researchers at the Novartis Institute for BioMedical Research, Beth Israel Deaconess Medical Center, and Brigham and Women's Hospital used the SOMAscan platform to measure the levels of ~5,000 proteins in blood samples taken from patients undergoing a "planned" heart attack, a medical procedure that can help reduce severely overgrown heart muscle (hypertrophic cardiomyopathy). They analyzed plasma taken before and at different time points after the procedure, looking for proteins whose levels changed significantly. Their results not only confirmed findings from a prior study that used an earlier, smaller version of the SOMAscan platform (ref: Ngo, D *et al.* (2016) "Aptamer-based proteomic profiling reveals novel candidate biomarkers and pathways in cardiovascular disease." *Circulation* **134**(4): 270-285.) but also identified nearly 150 new proteins, many of which had not been previously associated with heart damage. Twenty-nine of the proteins that were significantly increased within an hour after a planned heart attack were also elevated in patients who suffered "unplanned" heart attacks.

This article is the first published description of large-scale protein profiling at a level that has not previously been reported. The expanded SOMAscan assay platform provides opportunities for unbiased discovery of disease markers to improve diagnosis, predict future events, monitor responses to therapies and identify targets for drug development. Ongoing studies by these authors are applying this expanded SOMAscan platform to larger groups of patients.

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Spitsin, S *et al.* (2017) "Antiinflammatory effects of aprepitant coadministration with cART regimen containing ritonavir in HIV-infected adults." *JCI Insight* **2**(19): e95893.
https://www.ncbi.nlm.nih.gov/pubmed/28978797

HIV-infected individuals take antiretroviral therapies to help keep the virus at bay, but still suffer from systemic inflammation and immune dysfunction, which affects their quality of life and ability to survive. In this article, researchers at the Children's Hospital of Philadelphia Research Institute and the Perelman School of Medicine at the University of Pennsylvania describe a dose and time escalation clinical trial of the anti-inflammatory drug aprepitant on 12 HIV-positive patients. The goal was to see if co-administration of aprepitant with ritonavir (an antiretroviral medication) would safely reduce residual inflammation. The SOMAscan assay was used to assess the global effects of aprepitant and identified 176 plasma proteins whose levels changed after drug treatment. These included proteins involved in inflammation and immune regulation as well as blood, lipid and cholesterol metabolism, which warrant caution and further investigation.

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Curran, AM *et al.* (2017) "Sexual dimorphism, age, and fat mass are key phenotypic drivers of proteomic signatures." *J Proteome Res* **16**(11): 4122-4133. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/28950061

An international team led by investigators at University College Dublin Institute of Food and Health and the Nestlé Institute of Health Sciences used the SOMAscan assay to see if a person's sex, age and body fat mass are reflected in their blood protein profile. In healthy individuals (94 men and 102 women), they identified proteins that were significantly different depending on sex (141 proteins), age (51 proteins) and fat mass (112 proteins), respectively, and validated their results in a separate cohort. This study illustrates the need to consider these factors when developing protein markers for use in diagnosis or treatment strategies for many different diseases and conditions.

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Pannerec, A *et al.* (2017) "Vitamin B12 deficiency and impaired expression of amnionless during aging." *J Cachexia Sarcopenia Muscle* **9**(1): 41-52.
https://www.ncbi.nlm.nih.gov/pubmed/29159972

Scientists in Singapore and the Nestlé Institute of Health Sciences used SOMAscan in a study of the connection between vitamin B12 deficiency and aging. The researchers measured levels of methylmalonic acid (MMA), an indicator of vitamin B12 deficiency, in blood samples from 238 participants in a Singapore aging study. MMA levels were significantly higher in elderly participants compared to young controls, and even higher in elderly participants who were classified as physically frail. They then analyze blood protein changes in a rat model of aging where the animals had been fed a controlled diet that contained the recommended amounts of vitamin B12. They found that age-related vitamin B12 deficiency correlates with high levels of the protein amnionless (AMN) in blood serum. AMN is involved in B12 absorption and transport, and these studies suggest that it could be used as a biomarker for early detection of vitamin B12 deficiency.

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Coghlan, RF *et al.* (2017) "A degradation fragment of type X collagen is a real-time marker for bone growth velocity." *Sci Transl Med* **9**(419): eaan4669.
https://www.ncbi.nlm.nih.gov/pubmed/29212713

Bone growth is often used as a metric of healthy development, but bones grow slowly making it difficult to measure growth rates in real time. In this article, an international team led by scientists at the Shriners Hospitals for Children and the Oregon Health and Science University in Portland found that a protein fragment of type X collagen correlates with bone growth. Using a SOMAmer reagent that binds tightly to the fragment, they developed an assay to measure the protein in plasma, serum and dried blood spots. This assay may prove to be a useful tool to monitor fracture healing and treatment progress for growth disorders.

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Sullivan, KD *et al.* (2017) "Trisomy 21 causes changes in the circulating proteome indicative of chronic autoinflammation." *Sci Rep* **7**(1): 14818.
https://www.ncbi.nlm.nih.gov/pubmed/29093484

Down syndrome, or Trisomy 21, is caused by having three copies of chromosome 21 instead of two. Although the genetics of Down syndrome have been known for 60 years, it is still unclear how having the extra chromosome leads to various Down syndrome traits, including changes in common disease susceptibilities (e.g., Down syndrome individuals are more likely to develop Alzheimer's, leukemia and autoimmune disorders, but less likely to develop solid tumors and cardiovascular disease). Understanding the biology that underlies these differences could inform a wide range of medical conditions that affect not only Down syndrome individuals, but the entire population.

In the largest and most comprehensive study of its kind to date, investigators at the Crnic Institute for Down Syndrome, the Sie Center for Down Syndrome, the University of Colorado, and SomaLogic measured the levels of over 3500 proteins in the blood of 165 Down syndrome patients and compared them to 98 non-Down syndrome controls. They identified 299 proteins that differed significantly between the two groups. Surprisingly, most of these proteins are not encoded by genes located on chromosome 21, but are associated with immune system control. Down syndrome individuals appear to have something that resembles an autoinflammatory condition, with elevated levels of proteins that promote inflammation but deficiencies in proteins that help eliminate foreign pathogens. The findings provide a new framework for understanding the physiological mechanisms that drive the altered disease susceptibilities seen in individuals with Down syndrome, and suggest that individuals with Down syndrome could benefit from therapies that decrease or modulate immune responses.

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Candia, J *et al.* (2017) "Assessment of variability in the SOMAscan assay." *Sci Rep* **7**(1): 14248.
https://www.ncbi.nlm.nih.gov/pubmed/29079756

In this article, researchers at the NIH conducted a meta-analysis of SOMAscan assay performance in blood serum and plasma. They analyzed multiple runs that used an earlier version of the assay capable of measuring 1305 different protein analytes, assessing different procedures for data processing as well as assay variability within and between runs. The paper is accompanied by an interactive web-based tool.

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Scriba, TJ *et al.* (2017) "Sequential inflammatory processes define human progression from *M. tuberculosis* infection to tuberculosis disease." *PLoS Pathog* **13**(11): e1006687. https://www.ncbi.nlm.nih.gov/pubmed/29145483

An estimated 1.7 billion people—one quarter of the world's population—are infected with the bacterium that causes tuberculosis (TB), but only ~10% develop active pulmonary disease. In this article, scientists from the South African TB Vaccine Initiative, the University of Cape Town, the Center for Infectious Disease Research and SomaLogic looked for changes in various molecules in blood that together could predict the risk of TB progression from latent to active disease. The time between the initial blood collection and TB diagnosis ranged from 1 to 894 days, so the investigators could construct a timeline of changes that occurred as the disease evolved.

The blood analyses revealed that TB progression associated with sequential modifications of immunological processes. Some of these processes, such as type I/II interferon signaling and complement cascade, were elevated as early as 18 months before TB diagnosis.

Understanding the biology of progression from infection to active pulmonary TB opens the door to blood-based tests that may determine those who are at risk of developing active disease and who need early treatment. These findings could also help development of better vaccines and host-directed therapies to battle the TB epidemic.

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Habiel, DM *et al.* (2017) "Divergent roles for clusterin in lung injury and repair." *Sci Rep* **7**(1): 15444. https://www.ncbi.nlm.nih.gov/pubmed/29133960

Idiopathic pulmonary fibrosis (IPF) is characterized by lung scarring that prevents sufferers from taking deep breaths. There is no cure and patients typically die of respiratory failure within a few years after diagnosis. In this article, an international team of researchers led by investigators at Cedars-Sinai Medical Center and MedImmune demonstrate that the protein clusterin plays an important role in IPF. clusterin is produced by cells in response to stress and exists in different forms located either inside or outside cells. Using a combination of gene expression, flow cytometry, histology and the SOMAscan assay, the researchers found significantly higher levels of clusterin inside lung cells but significantly lower levels circulating in the blood of IPF patients compared to either chronic obstructive pulmonary disease (COPD) or healthy controls. Studies in human cells and in mice exposed to a toxic agent demonstrated that clusterin has different effects on cell regeneration and lung repair depending on where it's located. These results suggest possible therapeutic strategies for IPF that warrant further investigation.

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Wermuth, PJ *et al.* (2017) "Identification of novel systemic sclerosis biomarkers employing aptamer proteomic analysis." *Rheumatology (Oxford), epub ahead of print.* **(Subscription required)** https://www.ncbi.nlm.nih.gov/pubmed/29140474

Systemic sclerosis (SSc) is a chronic autoimmune disorder characterized by overproduction of collagen that leads to hardening of the skin and internal organs. There is an unmet need for clinically validated, non-invasive biomarkers to diagnose and manage SSc. This review illustrates the potential of the SOMAscan platform to discover SSc biomarkers and to improve management of rheumatic diseases.

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Lertudomphonwanit, C *et al.* (2017) "Large-scale proteomics identifies MMP-7 as a sentinel of epithelial injury and of biliary atresia." *Sci Transl Med* **9**(417): eaan8462. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/29167395

Biliary atresia (BA) is a rare disease that occurs in infants where their bile ducts become blocked. Bile accumulates in the liver and causes damage that can lead to liver failure. Early diagnosis is critical for successful treatment, but BA is often difficult to distinguish from newborn jaundice. In this article, an international team led by researchers at Cincinnati Children's Hospital Medical Center used the SOMAscan assay to compare protein levels in blood serum taken from infants with BA, normal age-matched controls, and those with prolonged jaundice (longer than 2 weeks). The data revealed significantly higher levels of matrix metalloproteinase-7 (MMP-7) in infants with BA. MMP-7 combined with γ-glutamyltranspeptidase, a marker of decreased bile flow, predicted BA with 95% accuracy in two independent cohorts. Studies in mice point to a role for MMP-7 in disease pathogenesis since MMP-7 concentrations increased when bile duct injury was induced in mice, and blocking MMP-7 function decreased tissue damage. Taken together, these results illustrate the potential of MMP-7 as a diagnostic biomarker of BA and perhaps even a new therapeutic target.

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Russell, T *et al.* (2017) "Potential of high-affinity, Slow Off-Rate Modified Aptamer (SOMAmer) reagents for *Mycobacterium tuberculosis* proteins as tools for infection models and diagnostic applications." *J Clin Microbiol* **55**(10): 3072-3088.
https://www.ncbi.nlm.nih.gov/pubmed/28794178

-and-

De Groote, MA *et al.* (2017) "Discovery and validation of a six-marker serum protein signature for the diagnosis of active pulmonary tuberculosis." *J Clin Microbiol* **55**(10): 3057-3071.
https://www.ncbi.nlm.nih.gov/pubmed/28794177

Tuberculosis (TB) is one of the top 10 causes of deaths worldwide. Because TB is spread through the air by people with active lung infections, early detection and treatment is important for disease containment. Examining and culturing lung sputum is the standard method for diagnosing TB, but there is a need for more rapid tests with greater accuracy. The World Health Organization (WHO) has defined non-sputum, point-of-care diagnostics for active TB screening as a high priority.

In this pair of articles, SomaLogic researchers assessed the ability of the SOMAscan assay to diagnose active pulmonary TB from blood serum. Russell *et al.* describes the generation of SOMAmer reagents to specific TB bacterial proteins and incorporation of those SOMAmers into the SOMAscan assay. Although the SOMAmers bound tightly to their intended targets, the test could not distinguish between TB patients and non-TB controls, probably because the bacterial proteins are not at high enough concentrations in circulating blood.

In contrast, De Groote *et al.* describe finding distinct differences in human host serum protein levels between TB and non-TB patients. Previous work produced a nine-protein model that predicted active TB with 80% sensitivity and 84% specificity (De Groote, MA *et al.* (2013) *PLoS One* 8(4): e61002.). This study used an expanded version of the SOMAscan assay to measure the levels of over 4,000 human proteins in

nearly 1,500 serum samples. The samples were from patients in seven different countries who were diagnosed with TB or had TB-like symptoms and who were either HIV negative or positive. While some of the proteins that distinguished TB from non-TB groups were the same as those identified previously, many were new. This prompted the investigators to create and validate a refined six-protein model with 90% sensitivity and 80% specificity. These results reached the performance criteria outlined by WHO for point-of-care TB screening and justify further diagnostic development.

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Anderson, J *et al*. (2017) "Interleukin 1 receptor-like 1 protein (ST2) is a potential biomarker for cardiomyopathy in Duchenne muscular dystrophy." *Pediatr Cardiol* **38**(8): 1606-1612. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/28821969

Duchenne muscular dystrophy (DMD) is a rare genetic disorder that causes progressive loss of muscle function until an early death, usually from heart muscle failure. Early detection of heart disease is critical for prolonging the lives of DMD patients but is difficult since most DMD patients do not display typical symptoms. To try to identify biomarkers of DMD cardiac disease, this study from the Children's Research Institute used the SOMAscan assay to measure the levels of 1125 proteins in blood serum from DMD patients with or without heart disease and compared them to those of healthy controls. Elevated levels of the inflammation protein ST2 were seen in DMD patients with cardiac dysfunction compared to DMD patients with normal cardiac function and controls. These results warrant further investigation to see if ST2 can be used to monitor heart disease progression in DMD patients and to enable early detection, which is essential for starting mitigation therapies.

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Ali A *et al.* (2017) "Efficacy of individualised diets in patients with irritable bowel syndrome: a randomised controlled trial." *BMJ Open Gastro* **4**(1): e000164.
http://bmjopengastro.bmj.com/content/4/1/e000164

Irritable bowel syndrome (IBS) is a chronic condition that affects the large intestine. Although IBS does not appear to harm the tissue, the symptoms can be painful, embarrassing or even disabling for sufferers. The exact cause of IBS is unknown and there is no cure, but a new study from investigators at Yale University provides evidence that avoiding foods that trigger inflammation can help alleviate IBS symptoms.

The researchers conducted a four-week dietary intervention on 58 adults with IBS. Each participant was tested for sensitivity to various foods, and then half were told to avoid foods that activated their white blood cells (intervention group), and the other half were told to avoid foods that did not activate their white blood cells (control group). IBS symptoms and quality of life were assessed prior to starting the diet, on the last day of the diet, and four weeks after ending the diet. All participants showed some improvement (likely a placebo effect), but those in the intervention group reported feeling significantly better than those in the control group. SOMAscan analysis of blood from six people with the best responses showed reduced levels of elastase, an enzyme that degrades various proteins and may affect gut permeability. This finding may provide new insight into the mechanisms by which diet—and subsequent inflammation—contributes to IBS.

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Wagner, BD *et al.* (2017) "Proteomic profiles associated with early echocardiogram evidence of pulmonary vascular disease in preterm infants." *Am J Respir Crit Care Med* **197**(3): 394-397. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/28650220

Many premature babies are diagnosed with pulmonary vascular disease (PVD), which is characterized by abnormal blood flow between the heart and lungs. In this study from the University of Colorado Denver, the SOMAscan assay was used to measure proteins in blood samples from 100 preterm infants, 44 of which had PVD. Researchers identified 18 proteins that a week after birth could distinguish babies who developed PVD from those who did not. Eight of these proteins had no previous association with PVD. Although preliminary, these results may provide insight into why some infants develop respiratory problems, and how these problems contribute to lung diseases in adulthood. The authors note that the SOMAscan is particularly well-suited for neonatal studies since only a small amount of blood (50uL serum) is needed to measure >1000 proteins.

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Wang, J *et al.* (2017) "Identification of unique proteomic signatures in allergic and non-allergic skin disease." *Clin Exp Allergy* **47**(11): 1456-1467.
https://www.ncbi.nlm.nih.gov/pubmed/28703865

Chronic inflammatory conditions such as psoriasis (PS), atopic dermatitis (eczema, AD) and contact dermatitis (CD) cause skin rashes that can be itchy, painful or embarrassing. Accurate diagnosis is needed for effective management but can be difficult without a skin biopsy. Investigators at MedImmune used the SOMAscan assay to measure the levels of 1129 proteins in blood serum taken from 12 patients with PS, 20 with AD, 10 with CD, 10 with both AD and CD, and 10 healthy controls. Overall, 66 proteins were significantly increased and 64 proteins were significantly decreased in at least one of the diseases, and PS, AD and CD could be distinguished from one another based on their unique protein signatures. These data need further validation, but measuring the circulating proteome may lead to a less invasive method for diagnosing inflammatory skin conditions and provide insights into disease pathogenesis and targeted treatments for severe cases.

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Aghaeepour, N *et al.* (2017) "An immune clock of human pregnancy." *Sci Immunol* **2**(15): eaan2946.
https://www.ncbi.nlm.nih.gov/pubmed/28864494

A successful pregnancy requires that the mother's immune system can still attack foreign invaders but leave the growing fetus alone. Researchers at Stanford University found that this trick requires a series of precisely timed immune adaptations throughout fetal development. They obtained blood samples collected at early, middle, late and post-pregnancy time points for 18 women who delivered at full-term and then analyzed them using mass cytometry (a technique that can determine which immune cells are present and how they respond to compounds that mimic bacteria and viruses). They fed these data into a computer algorithm to develop a model that predicts the immune system changes that occur over the course of a normal pregnancy, and then validated the resulting model on 10 additional women. Using the SOMAscan assay, they identified those proteins that likely have a critical role in modulating the function of certain types of white blood cells during pregnancy. The researchers next hope to compare blood samples from mothers who deliver too early to see if premature births can be predicted and prevented.

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Rhodes, CJ *et al.* (2017) "Plasma proteome analysis in patients with pulmonary arterial hypertension: an observational cohort study." *Lancet Respir Med* **5**(9): 717-726.
https://www.ncbi.nlm.nih.gov/pubmed/28624389

In this multicenter study, researchers from the UK, France and Germany used the SOMAscan assay to measure the levels of 1,129 proteins in blood samples from patients with pulmonary arterial hypertension (PAH). PAH is a rare, incurable disease where the small arteries in the lung progressively narrow, and the heart is forced to pump harder and harder until it eventually fails. The investigators identified nine proteins that differentiated PAH survivors from non-survivors and used this protein panel to develop a risk score that predicted patient survival better than existing clinical tests. The protein-based risk score was then validated on two separate patient cohorts, including one that contained 43 paired plasma samples taken when PAH was first diagnosed and after treatment. Increased risk scores at follow-up correlated with poorer survival rates and outperformed established measures. These results suggest that the nine-protein risk score could be used to monitor PAH progression, assess treatment efficacy and stratify patients in clinical trials. Further investigation of the proteins that make up the panel may provide insights into the causes of PAH and possible therapeutic targets.

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Belongie, KJ *et al.* (2017) “Identification of novel biomarkers to monitor β-cell function and enable early detection of type 2 diabetes risk.” *PLoS One* **12**(8): e0182932.
https://www.ncbi.nlm.nih.gov/pubmed/28846711

Progression to type 2 diabetes is caused by a decline in function of pancreatic beta cells that produce and secrete insulin. Current methods for assessing beta cell function lack accuracy and reproducibility, so it is difficult to predict whether a person with higher than normal blood sugar levels will progress to diabetes. This is important since as of 2015, diabetes was the seventh leading cause of death in the US and an estimated 84.1 million American adults were pre-diabetic.

In this article, an international team lead by investigators at Janssen Pharmaceuticals and the University of Glasgow conducted a retrospective study of blood samples from the RISC (Relationship between Insulin Sensitivity and Cardiovascular Disease) cohort, a well-characterized group of healthy, non-diabetic Europeans whose beta cell function and insulin sensitivity were tested at baseline and after three years. The researchers profiled plasma proteins and microRNAs in 40 RISC participants who showed the largest decline in beta cell function at follow up and compared them to 40 matched controls who showed no decline. The SOMAscan assay analysis showed several proteins whose levels were significantly different between the two groups. Some such as adiponectin, a hormone that regulates glucose, have known links to type 2 diabetes, but others were novel. Many of the proteins and RNAs were associated with a process that is important for pancreas formation during development. These results need to be validated, but could provide better indicators of beta cell function and new prevention therapies for type 2 diabetes.

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Erez, O *et al.* (2017) "The prediction of late-onset preeclampsia: Results from a longitudinal proteomics study." *PLoS One* **12**(7): e0181468.
https://www.ncbi.nlm.nih.gov/pubmed/28738067

Preeclampsia is a pregnancy condition characterized by maternal high blood pressure that can progress rapidly and result in serious or even fatal complications for mother and baby. A recent study found that in 2012, the medical costs of preeclampsia were $2.18 billion for the first year after delivery. To identify patients at risk of developing late-onset (after 34 weeks) preeclampsia, scientists at the NIH and Wayne State University used the SOMAscan assay to measure the levels of 1,125 proteins in plasma from women who had normal pregnancies and those who experienced late-onset preeclampsia. Over the course of gestation, 36 proteins differed significantly between the two groups. Of these, the best predictors were high levels of the immune protein matrix metalloproteinase 7 (MMP-7) early in pregnancy (8-16 weeks) and low levels of the placental growth factor (PIGF) later in pregnancy (after 22 weeks). In addition, decreased PIGF levels correlated with the more severe form of preeclampsia. These results suggest that monitoring protein levels during pregnancy can help identify mothers who will develop late-onset preeclampsia and those who are at greatest risk for complications. This information could help physicians manage and treat the disorder, improve the safety of mother and child and lower the associated health costs.

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Fitzgibbons, TP *et al.* (2017) "Activation of inflammatory and pro-thrombotic pathways in acute stress cardiomyopathy" *Frontiers in Cardiovascular Medicine* **4**: 49.
http://journal.frontiersin.org/article/10.3389/fcvm.2017.00049/full

Stress cardiomyopathy (SCM) is a temporary weakening of the heart that is triggered by intense emotional or physical stress (e.g., loss of a loved one, winning the lottery, asthma attack, etc.). Although it is not caused by clogged arteries, SCM symptoms mimic those of a heart attack (shortness of breath, chest pain), and patients are often subject to unnecessary heart catheterization. To identify markers of SCM, a group led by investigators at the University of Massachusetts medical school used the SOMAscan assay to measure the levels of 1,310 proteins in blood serum from patients with SCM, patients with acute myocardial infarction (AMI) and normal controls. They found that proteins involved in inflammation and coagulation were activated in SCM patients *vs.* normal controls. This finding was unexpected and may explain why SCM patients are at higher risk for future heart disease or heart failure. Four proteins were increased in SCM relative to AMI compared to normal controls. These results require further validation but may provide better, less invasive ways to distinguish SCM from heart attack patients.

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Sun, HH *et al.* (2017) "Diagnosis and prognosis-review of biomarkers for mesothelioma." *Ann Transl Med* **5**(11): 244.
https://www.ncbi.nlm.nih.gov/pubmed/28706912

Malignant pleural mesothelioma (MPM) is an aggressive lung cancer caused by previous asbestos exposure, usually decades before the disease is detected. This review discusses the most recent and promising markers of MPM—including a panel of 13 proteins discovered using the SOMAscan platform (Ostroff, RM *et al.* (2012) *PLoS One* **7**(10): e46091.)—and their potential for diagnosing and treating future MPM patients.

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Rossios, C *et al.* (2017) "Sputum transcriptomics reveal up-regulation of IL-1 receptor family members in severe asthma." *J Allergy Clin Immunol* **141**(2): 560-570. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/28528200

Most asthma can be managed using standard medications such as inhaled corticosteroids, but severe asthma often does not respond to traditional treatments. There are no universally accepted criteria to diagnose severe asthma, and the exact causes of airway inflammation likely vary between patients. This lack of understanding and 'one size fits all' approach impairs quality of care and, for many, the disease remains either poorly controlled or not controlled at all. In this study, scientists in the UK analyzed gene and protein expression in sputum samples from people with severe asthma (non-smokers and smokers), moderate asthma (non-smokers) or no asthma (non-smokers). SOMAscan analysis revealed several inflammatory factors and immune system proteins that differed significantly in severe asthma patients compared to those with mild asthma or healthy controls. These results should help elucidate various mechanisms that cause disease pathogenesis and guide targeted therapies.

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Wasiak, S *et al.* (2017) "Downregulation of the complement cascade in vitro, in mice and in patients with cardiovascular disease by the BET protein inhibitor apabetalone (RVX-208)." *J Cardiovasc Transl Res,* **10**(4): 337-347.
https://www.ncbi.nlm.nih.gov/pubmed/28567671

Apabetalone (RVX-208) is a first-in-class small molecule drug being developed by Resverlogix Corp. to treat cardiovascular disease (CVD). To better understand the biological pathways that are modulated by RVX-208, scientists at Resverlogix used the SOMAscan assay to measure blood proteins in plasma samples from patients with coronary artery disease who were given either placebo or RVX-208. They found that RVX-208 leads to a significant reduction in circulating levels of complement proteins and activators. The complement system is part of the body's innate immune response that promotes inflammation by helping antibodies and white blood cells kill microbes and clear damaged cells. Complement activity is tightly controlled since overstimulation is associated with chronic inflammation, susceptibility to infectious disease, metabolic syndrome and atherosclerosis. Reduced expression of complement proteins by RVX-208 did not appear to interfere with normal immune function as there was no increase in infections amongst those taking RVX-208. These results suggest that repressing the complement system may contribute to the decreased incidence of major adverse cardiac events seen in RVX-208 clinical trials and provide a general strategy for reducing CVD risk.

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DeBoer, EM *et al.* (2017) "Proteomic profiling identifies novel circulating markers associated with bronchiectasis in cystic fibrosis." *Proteomics Clin Appl* **11**(9-10). **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/28452194

Bronchiectasis is a condition where the lung airways thicken and become damaged due to inflammation. It is a hallmark of cystic fibrosis (CF) and is linked to disease progression and mortality. Current techniques for monitoring bronchiectasis are CT scanning (which involves repeated radiation exposure) and bronchoalveolar lavage (which is invasive). Thus, finding noninvasive biomarkers of bronchiectasis is highly desirable. Researchers at the University of Colorado Medical School used the SOMAscan assay to measure plasma protein levels in 26 children with CF. Twenty-two proteins showed significant correlation with the severity of bronchiectasis and structural lung injury as deduced from CT scans. Several were novel proteins that has not been previously linked to CF or bronchiectasis and with further validation may be a less harmful way to assess structural lung damage in children with CF.

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Wang, T *et al.* (2017) "GDF15 is a heart-derived hormone that regulates body growth." *EMBO Mol Med* **9**(8): 1150-1164.
https://www.ncbi.nlm.nih.gov/pubmed/28572090

Bodily organs communicate with each other by secreting hormones that help regulate metabolism and maintain whole body health. Little is known of heart-derived hormones, although heart disease is associated with Failure To Thrive (FTT), a condition where children do not grow normally. Researchers at the Children's Hospital of Philadelphia and the University of Pennsylvania of Perelman School of Medicine used the SOMAscan assay and RNA sequencing to identify plasma proteins whose levels were altered in a mouse model of human FTT. They identified growth differentiation factor 15 (GDF15) as a heart secreted factor that inhibits growth hormone signaling by the liver. They found elevated plasma concentrations of GDF15 in children with heart disease compared to age-matched healthy controls. Furthermore, those with heart disease and FTT had GDF15 levels that were 80% higher than those with heart disease and normal body weight.

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Howson, JMM *et al.* (2017) "Fifteen new risk loci for coronary artery disease highlight arterial-wall-specific mechanisms." *Nat Genet* **49**(7): 1113-1119.
https://www.ncbi.nlm.nih.gov/pubmed/28530674

An international team of scientists led by researchers at the University of Cambridge, the University of Pennsylvania and Stanford University conducted a large-scale study of genetic variants associated with coronary artery disease (CAD). They analyzed results from over 250,000 CAD patients and controls and identified 15 new regions of the genome that had not been previously linked to CAD. These regions contain genes that are involved in cellular adhesion, atherosclerosis, white blood cell migration, inflammation and smooth muscle cell differentiation. To identify the disease pathways and biological functions controlled by these regions, the researchers conducted protein profiling of 3,301 blood samples using the SOMAscan assay. One DNA variant correlated with expression of apoliproprotein L1, a major component of high density lipoprotein (HDL) particles. Another DNA variant correlated with levels of protein C, which helps maintain the permeability of blood vessel walls. These and previous results point to both traditional (cholesterol) and novel (arterial wall) mechanisms that lead to CAD susceptibility.

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Tasaki, S *et al.* (2017) "Multiomic disease signatures converge to cytotoxic CD8 T cells in primary Sjogren's syndrome." *Ann Rheum Dis* **76**(8): 1458-1466.
https://www.ncbi.nlm.nih.gov/pubmed/28522454

The goal of this research is to elucidate the pathology of Sjögren syndrome (SS), an autoimmune disease that attacks the tear and salivary glands. Previously, the researchers used the SOMAscan assay to profile serum proteins in samples from SS patients vs. healthy controls (Nishikawa, A *et al.* (2016) *Arthritis Res Ther* 18(1): 106). In this study, they profiled RNA transcripts of the same blood samples and integrated the two data sets. Their 'multiomic' approach identified SS-associated pathways and linked them to different white blood cell types. These results should aid development of targeted therapies and biomarkers of disease progression.

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Westwood, S *et al.* (2017) "The influence of insulin resistance on cerebrospinal fluid and plasma biomarkers of Alzheimer's pathology." *Alzheimers Res Ther* **9**(1): 31.
https://www.ncbi.nlm.nih.gov/pubmed/28441961

Insulin resistance (IR) is a pathological condition in which the body fails to respond to insulin. Previous research demonstrated that IR may contribute to mental decline and an increased risk of developing Alzheimer's disease (AD). To better define the relationship between IR and AD, researchers at Oxford used the SOMAscan assay to measure protein levels in plasma and cerebrospinal fluid (CSF) from cognitively healthy men with IR compared to age-matched controls. They observed differential expression of 200 proteins in CSF and 487 proteins in plasma between the IR and non-IR groups. Twenty-five proteins were associated with both IR and AD and are potential markers of shared pathology. Although promising, further investigation is needed to identify common biological pathways affected by IR and AD.

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Saleheen, D *et al.* (2017) "Human knockouts and phenotypic analysis in a cohort with a high rate of consanguinity." *Nature* **544**(7649): 235-239.
https://www.ncbi.nlm.nih.gov/pubmed/28406212

Human 'knockouts' are people who lack functional copies of a particular gene. In most populations where the parents are unrelated, natural knockouts are very rare. However, in Pakistan many people marry their first cousins, which increases the chances that children will inherit mutant copies of the same gene from both parents.

In this study, an international team led by researchers at the Broad Institute of Harvard and MIT sequenced the genes of 10,503 participants in the Pakistani Risk of Myocardial Infarction Study (PROMIS) and looked for loss of function mutations. The rate of inbreeding in PROMIS participants is 4-fold higher than in typical European or African American populations. They found 1,317 different genes that they predicted were inactivated, representing ~7% of known protein-coding genes.

To better understand the consequences of loss of function mutations in living people, the researchers measured more than 200 biochemical disease traits for 426 genes that were knocked out in two or more people. In addition, for 84 participants they analyzed blood levels of 1310 proteins using the SOMAscan assay. A detailed analysis of human knockouts of apolipoprotein C3 (apoC3) found that they had almost no circulating apoC3 protein. ApoC3 impedes fat clearance and is a drug target for heart disease. Compared to those with a functional gene, the human apoC3 knockouts had lower fasting levels of triglycerides and increased levels of high density lipoprotein (HDL) cholesterol. People lacking apoC3 also had significantly lower levels of triglycerides in their blood after eating a fatty meal. This observation demonstrates that apoC3 protein can be removed from the body without harmful effects and suggests that inhibiting apoC3 protein may be an effective therapeutic strategy cardiovascular disease.

This study serves as a proof-of-principle for future efforts to understand the biological consequences of systematically knocking out every gene in humans.

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Asai, A *et al.* (2017) "Paracrine signals regulate human liver organoid maturation from induced pluripotent stem cells." *Development* **144**(6): 1056-1064.

http://dev.biologists.org/content/144/6/1056.long

Human induced pluripotent stem cells (iPSCs) can differentiate and self-organize into a liver "organoid" in a Petri dish. Investigators at Cincinnati Children's Hospital Medical Center found that a three-dimensional architecture only forms when iPSC-derived liver cells (HE-iPSCs) are in direct contact with mesenchymal stem cells (MSCs) and human umbilical vein endothelial cells (HUVECs). However, maturation of HE-iPSCs from fetal to adult-like hepatocytes can be induced even when the cells are kept separate but allowed to exchange soluble factors. To identify these signaling molecules, the SOMAscan assay was used to analyze the supernatants of HE-iPSCs co-cultured with either MSCs, HUVECs or both. The levels of 228 proteins changed significantly (≥three-fold) when compared to HE-iPSCs cultured alone, and different proteins were secreted depending on the combination of cells that were present. These results will help further studies to dissect the mechanisms behind liver organogenesis and regeneration.

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Trausch, JJ *et al.* (2017) "Development and characterization of an HPV Type-16 specific modified DNA aptamer for the improvement of potency assays." *Anal Chem* **89**(6): 3554-3561. **(Subscription required)** https://www.ncbi.nlm.nih.gov/pubmed/28233502

Robust potency tests ensure that vaccines released to the public remain safe and effective. Most approved potency assays rely on antibody reagents, which have many drawbacks (e.g. time-consuming discovery process, limited shelf life, batch-to-batch variability, etc.). To get around these problems, researchers at Merck substituted an antibody with an aptamer in a human papilloma virus (HPV) potency assay. They worked with SomaLogic to create a custom SOMAmer reagent (named HPV-07) that binds tightly to HPV 16, a high-risk type for cervical cancer. HPV-07 was designed to bind selectively to HPV 16 in samples that contain many other HPV types. Competition experiments revealed that HPV-07 binds to the same epitope as a well-characterized HPV 16 antibody, and when used in an ELISA format, HPV-07 displayed high accuracy, precision and a wide linear range. The researchers then functionalized HPV-07 to develop a simple "mix and read" assay that was faster and cheaper to run than an ELISA. They note that the properties of SOMAmers could be exploited further to create a multiplexed assay that measures the potency of all antigens in a multivalent vaccine simultaneously.

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Wood, GC *et al.* (2017) "A multi-component classifier for nonalcoholic fatty liver disease (NAFLD) based on genomic, proteomic, and phenomic data domains." *Sci Rep* **7**: 43238. http://www.nature.com/articles/srep43238

Approximately 25% of Americans have non-alcoholic fatty liver disease (NAFLD), a disorder in which excess fat accumulates in the liver. NAFLD is often associated with obesity and can progress to more serious chronic conditions including liver inflammation, fibrosis and cirrhosis. Many people with NAFLD are asymptomatic, and commonly used tests of liver function lack the specificity and sensitivity to check for NAFLD. As obesity rates in the U.S. continue to rise, there is an urgent public health need for clinical biomarkers of NAFLD. In this study, researchers at the Geisinger Obesity Research Institute in Pennsylvania and National Jewish Health in Colorado used genomic, phenomic and proteomic data to develop an algorithm that predicts NAFLD in an extremely obese population. The data included a single nucleotide polymorphism in the PNPLA3 gene that is linked to NAFLD susceptibility, 16 clinical variables that had been shown previously to correlate with NAFLD, and 8 serum protein biomarkers of NAFLD

identified by SOMAscan assay analysis. The results represent an important step toward developing a minimally-invasive test for NAFLD diagnosis and prognosis.

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Guiraud, S *et al.* (2017) "Identification of serum protein biomarkers for utrophin based DMD therapy." *Sci Rep* **7**: 43697.
http://www.nature.com/articles/srep43697

Duchenne muscular dystrophy (DMD) is a fatal degenerative muscle disorder that is caused by mutations in the gene that encodes "dystrophin," a critical muscle structure protein. Utrophin is a protein with high similarity to dystrophin (80% homology) that can compensate for loss of dystrophin function. Overexpression of utrophin prevents disease pathogenesis in a mouse model of DMD and is of great interest as a potential therapeutic strategy in humans. Researchers at the University of Oxford performed the SOMAscan assay on blood serum samples from wild type, dystrophin-null (*mdx*) and utrophin-overexpressing *mdx* (*Fiona*) mice. They identified 83 proteins that differed significantly in concentration (>two-fold) between *mdx* and wild type mice, 34 of which were fully restored to normal levels in *Fiona* mice. These proteins represent possible biomarkers that, if validated in humans, could be used to monitor disease progression and response to therapeutics.

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Suhre, K *et al.* (2017) "Connecting genetic risk to disease end points through the human blood plasma proteome." *Nat Commun* **8**: 14357.
http://www.nature.com/articles/ncomms14357

Researchers at the Weill Cornell medical college in Qatar used the SOMAscan assay to investigate the impact of common gene variants on protein levels in human plasma. Using samples from a German cohort, they identified 539 single nucleotide polymorphism-protein associations and replicated over half of the results in an Arab and Asian cohort. The associations overlap with 57 genetic risk loci for 42 different disease endpoints. Interestingly, many of the proteins are modulated by variations that occur on different chromosomes. This study demonstrates how proteomics can help tie genomic observations to actual changes in physiology and pathology. The authors anticipate that further mining of their data will provide insights into disease-related biological pathways and therapeutic interventions.

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Escolano, JM *et al.* (2017) "Selection of aptamers to *Neisseria meningitidis* and *Streptococcus pneumoniae* surface specific proteins and affinity assay using thin film AlN resonators." *Sensors and Actuators B: Chemical* **246**: 591-596. **(Subscription required)**
http://www.sciencedirect.com/science/article/pii/S0925400517303258

Bacterial meningitis is a frightening illness—victims can die within a few hours and survivors can be left with severe afflictions such as brain damage or hearing loss. Different kinds of bacteria can cause meningitis, of which *Neisseria meningitidis* and *Streptococcus pneumoniae* are the most common. Researchers in Madrid generated polyclonal SOMAmers to two bacterial surface-expressed proteins, PavA from *S. pneumoniae* and FHbp from *N. meningitidis* and demonstrated specific binding of the SOMAmers to their target proteins. This work represents an important first step towards creating a biosensor for rapid detection of bacterial meningitis.

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van den Broek, TJ *et al.* (2017) "The impact of micronutrient status on health: correlation network analysis to understand the role of micronutrients in metabolic-inflammatory processes regulating homeostasis and phenotypic flexibility." *Genes Nutr* **12**: 5.
https://genesandnutrition.biomedcentral.com/articles/10.1186/s12263-017-0553-7

Health can be defined as the body's ability to adapt to environmental changes, such as infection, stress or exercise. Researchers in the Netherlands and Switzerland used this definition to study the roles of fat-soluble micronutrients in maintaining normal physiological processes. Plasma concentrations of vitamins A, D_3 & E and four carotenoids were measured for 36 overweight or obese males after overnight fasting and after eating a high fat shake. A proteomic analysis using the SOMAscan assay was conducted in parallel, and changes in protein levels were correlated with changes in micronutrient levels. The correlation analysis after the nutritional challenge was particularly interesting as it suggested that certain micronutrients (α-carotene, a vitamin A precursor; and γ-tocopherol, a form of vitamin E) are especially important for helping the body respond to oxidative and inflammatory stresses. This approach will be useful for quantifying the effects of diet on health.

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Di Narzo, AF *et al.* (2017) "High-throughput characterization of blood serum proteomics of IBD patients with respect to aging and genetic factors." *PLoS Genet* **13**(1): e1006565.
http://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1006565

In this article, scientists at the Icahn School of Medicine at Mt. Sinai analyzed the blood serum of patients with inflammatory bowel disease (IBD)—ulcerative colitis and Crohn's disease (CD)—as well as healthy controls. They describe using the SOMAscan assay to identify serum proteins that correlate with CD and with aging. Within a CD cohort, they found 41 proteins that associated with previously identified gene loci, including a well-known IBD susceptibility locus. This study illustrates the value of the SOMAscan assay in interpreting genome-wide association study (GWAS) results and in gaining insight into the molecular events that cause IBD.

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Sasayama, D *et al.* (2017) "Genome-wide quantitative trait loci mapping of the human cerebrospinal fluid proteome." *Hum Mol Genet* **26**(1): 44-51.
https://academic.oup.com/hmg/article-abstract/26/1/44/2595397/Genome-wide-quantitative-trait-loci-mapping-of-the?redirectedFrom=fulltext

Measuring analytes in cerebrospinal fluid (CSF) can be useful for diagnosing diseases of the central nervous system. Researchers in Japan conducted a genome-wide study of single nucleotide polymorphisms (SNPs) in the CSF of 133 physically healthy individuals and used the SOMAscan assay to look for correlated changes in protein concentrations. They identified over 400 SNP-protein pairs, of which 28 had been shown previously to associate with specific traits or diseases. Interestingly, many of the protein associations appear to be unique to CSF (i.e., they had not been previously identified from blood). This suggests that gene variants differentially control protein levels in the central vs. peripheral nervous system. These results should aid future efforts to understand brain biochemistry and to discover new biomarkers for neurological diseases.

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Jung, YJ *et al.* (2017) "Development of a protein biomarker panel to detect non-small-cell lung cancer in Korea." *Clin Lung Cancer* **18**(2): e99-e107. **(Subscription required)**
http://www.sciencedirect.com/science/article/pii/S1525730416302388

Lung cancer is the most common and most deadly cancer in the world. Early detection and treatment greatly improves chances of survival, but this can be difficult since people with early stage lung cancer are often asymptomatic. The only currently recommended screening test for lung cancer is a low-dose CT scan, which has a high false positive rate (23.3%). Investigators at the Ulsan College of Medicine in South Korea used results from the SOMAscan assay to construct a panel of seven protein biomarkers that could discriminate a Korean cohort with non-small cell lung cancer (NSLC) from negative controls. The ability of their protein panel to detect true positives was 75% overall and 61.9% for early stage (stages I & II) lung cancer. The seven-marker panel outperformed the common lung cancer marker Cyfra 21-1 in identifying NSLC at all four stages of disease, with an overall accuracy of 80.4% compared to 59.5%. The panel was also superior at distinguishing early stage NSLC from benign lung nodules. The results of this study could be useful for developing a better lung cancer diagnostic and a noninvasive test to evaluate lung nodules identified by CT screening for the Korean population.

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Qiao, Z *et al.* (2017) "Proteomic study of hepatocellular carcinoma using a novel modified aptamer-based array (SOMAscan) platform." *Biochim Biophys Acta* **1865**(4): 434-443. **(Subscription required)** http://www.sciencedirect.com/science/article/pii/S1570963916301935

Hepatocellular carcinoma (HCC) is the most common form of liver cancer and its incidence is expected to continue to grow. Accurate diagnosis and prognosis would greatly improve HCC treatments and clinical outcomes. Towards this end, researchers in Japan used the SOMAscan assay to compare global protein levels within HCC tumor and non-tumor tissue, as well as cancerous tissues with different vascular invasion status. The levels of 68 proteins were tumor-dependent, and eight proteins were associated with vascular invasion. With further validation underway, these data may help elucidate disease mechanisms and lead to improved tools for screening and evaluating HCC therapies.

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Zyba, SJ *et al.* (2017) "A moderate increase in dietary zinc reduces DNA strand breaks in leukocytes and alters plasma proteins without changing plasma zinc concentrations." *Am J Clin Nutr* **105**(2): 343-351. http://ajcn.nutrition.org/content/105/2/343.long

Researchers at the Children's Hospital Oakland Research Institute used the SOMAscan assay to analyze serum from 18 men who were fed zinc-fortified rice, a type of dietary supplement given to people in developing countries. They found that a modest increase in dietary zinc leads to an increase in the concentrations of proteins that prevent DNA damage, inflammation and oxidative stress. These results could help explain the connection between zinc deficiencies and chronic diseases such as cancer, diabetes and atherosclerosis.

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Rice, LM *et al.* (2017) "A proteome-derived longitudinal pharmacodynamic biomarker for diffuse systemic sclerosis skin." *J Invest Dermatol* **137**(1): 62-70. http://www.jidonline.org/article/S0022-202X(16)32372-7/abstract

Diffuse cutaneous systemic sclerosis (dcSSc) is an autoimmune disease that is characterized by excessive collagen deposition that causes hardening of the skin. The disease can spread to internal organs including the heart, lungs, and kidneys and cause organ failure and death. Testing for serum autoantibodies (i.e., antibodies that attack "self" tissues) can be helpful for diagnosis, but autoantibody

concentrations do not necessarily correlate with dcSSc severity, so they cannot be used to monitor disease progression or therapeutic response. The goal of this study was to use the SOMAscan assay to identify longitudinal biomarkers of dcSSc. Proteomic analysis of sera from two independent cohorts found 181 proteins with altered levels between dcSSc patients and healthy controls. Eight of the hits were subsequently validated, including three new proteins that had not been previously associated with dcSSc. A combination of two proteins (ST2 and SPON1) robustly described longitudinal changes and could prove useful for monitoring changes in dcSSc patients over time.

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De Groote, MA *et al.* (2017) "Highly multiplexed proteomic analysis of quantiferon supernatants to identify biomarkers of latent tuberculosis infection." *J Clin Microbiol* **55**(2): 391-402.
http://jcm.asm.org/content/55/2/391.long

An estimated two billion people are infected with tuberculosis (TB) worldwide, although not everyone who harbors the TB bacterium will become sick. Eliminating the disease will require better methods to identify and treat those with latent TB infection (LTBI). In this pilot study, researchers from Denver Health and SomaLogic ran the SOMAscan assay on untreated and TB antigen-stimulated plasma samples from LTBI positive and negative individuals. They identified several new proteins that distinguished those infected with TB from uninfected controls. These findings could lead to more accurate tests for diagnosing LBTI as well as the likelihood of progressing to active TB, which is a major limitation of currently available tests.

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Billing, AM *et al.* (2017) "Complementarity of SOMAscan to LC-MS/MS and RNA-seq for quantitative profiling of human embryonic and mesenchymal stem cells." *J Proteomics* **150**: 86-97.
http://www.sciencedirect.com/science/article/pii/S1874391916304006

“Dynamic range” is perhaps the single most difficult challenge in measuring the proteome in any meaningful way. In other words, proteins are present in any given biological fluid across a large range of concentrations, greater than ten logs of relative abundance. This particular challenge is the one best addressed by the SOMAscan assay, as demonstrated in this article. A research team at Weill Cornell Medical College in Dohar, Qatar (site of one of the first installations of the SOMAscan assay outside of SomaLogic), compared SOMAscan with mass spectrometry (MS) and RNA sequencing (RNA-seq) in analyzing proteins from both human embryonic and mesenchymal stem cells. In addition to validating SOMAscan results with other, more traditional approaches, their research underscores SOMAscan’s “deep reach” into the proteome to identify the “rarer” proteins that may be the most critical biomarkers for a range of diseases and conditions of interest.

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Heier, CR *et al.* (2016) "Identification of pathway-specific serum biomarkers of response to glucocorticoid and infliximab treatment in children with inflammatory bowel disease." *Clin Transl Gastroenterol* **7**(9): e192.
http://www.nature.com/ctg/journal/v7/n9/pdf/ctg201649a.pdf

Inflammatory bowel disease (IBD) is a chronic condition where the body’s immune system attacks its own digestive tract. The goal of most IBD treatments is to achieve remission, however there is increasing evidence that alleviating the symptoms does not ultimately improve outcomes. Repeated colonoscopy can be used to monitor patients’ response to IBD therapies, but the technique is costly, invasive and can

be risky, particularly for children. In order to find pharmacodynamic biomarkers of IBD, researchers at the Children's National Health Center in Washington, D.C. ran the SOMAscan assay on pediatric serum samples obtained before and after treatment with a corticosteroid (prednisone) or a biologic (infliximab) anti-inflammatory drug. They identified 18 proteins and 3 miRNAs whose levels changed in a similar manner (either increased or decreased) for both drugs. Eight of the markers that decreased are associated with inflammation, whereas many that increased are associated with resolving inflammation and tissue damage. With further validation, these protein biomarkers could be used to track treatment, optimize dosing, and accelerate new drug development for IBD patients.

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Tsim, S *et al.* (2016) "Diagnostic and Prognostic Biomarkers in the Rational Assessment of Mesothelioma (DIAPHRAGM) study: protocol of a prospective, multicentre, observational study." *BMJ Open* **6**(11): e013324.
http://bmjopen.bmj.com/content/6/11/e013324.long

This publication describes the protocol for a clinical trial to assess the performance of protein biomarkers for malignant pleural mesothelioma (MPM). MPM is a rare, aggressive, pulmonary cancer that is usually caused by asbestos exposure. Previously, scientists at SomaLogic used the SOMAscan assay to develop a panel of 13 proteins from serum that could detect MPM with 92% accuracy. The goal of this new study is to see whether the SOMAscan panel or fibulin-3 (a potential plasma biomarker of MPM) levels could provide clinically useful diagnostic and prognostic information. A non-invasive test that could distinguish MPM from confounding pleural malignancies would offer a major clinical advance over current approaches.

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Lynch, AM *et al.* (2016) "The relationship of novel plasma proteins in the early neonatal period with retinopathy of prematurity." *Invest Ophthalmol Vis Sci* **57**(11): 5076-5082.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5053115/pdf/i1552-5783-57-11-5076.pdf

Retinopathy of prematurity (ROP) is an eye disease that affects smaller premature infants and is a leading cause of childhood blindness worldwide. Not all premature babies develop ROP and not all babies affected by ROP experience impaired vision later in life. However, the risk factors for developing clinically significant (high-grade) ROP are not known. Researchers at the University of Colorado School of Medicine ran the SOMAscan assay on blood samples obtained from pre-term infants in the first week of life, and found several proteins that appear to be associated with clinically significant ROP. Although preliminary, these proteins may be diagnostic of ROP severity, as well as potential targets for future therapeutics. The authors noted that the ability to measure low abundant proteins was an important advantage of using aptamer-based technologies for this study.

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Ashley, SL *et al.* (2016) "Six-SOMAmer index relating to immune, protease and angiogenic functions predicts progression in IPF." *PLoS One* **11**(8): e0159878.
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0159878

Idiopathic pulmonary fibrosis (IPF, the thickening of lung tissue—and thus compromise of breathing leading to death—for reasons unknown) is likely several different diseases at the molecular level, requiring different therapeutic approaches. Some people with IPF manage well over time; others rapidly progress and die. Being able to tell the difference in a non-invasive manner should lead to better

treatment decisions and outcomes. A group of researchers from Medimmune and the University of Michigan applied the SOMAscan assay to blood samples from a group of IPF patients to identify potential biomarkers that distinguish long-term non-progressors from those who progressed quickly. A six-analyte index (signature) of proteins was identified, which not only suggests a better way to manage patients but also reveals some novel IPF biology to further explore.

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Welton, JL *et al.* (2016) "Proteomics analysis of vesicles isolated from plasma and urine of prostate cancer patients using a multiplex, aptamer-based protein array." *J Extracell Vesicles* **5**: 31209.
http://www.tandfonline.com/doi/full/10.3402/jev.v5.31209

Despite the high prevalence of prostate cancer, most men will die with the disease rather than of it. There is a huge unmet medical need to be able to tell the difference. The measurement of PSA (prostate-specific antigen) in the blood is a mixed success at best: Better biomarkers are needed. In this study, scientists at Cardiff University look at the protein profiles of “exosomes,” small vesicles shed by various cell types (including cancer), to determine if they can pick up prostate cancer-specific markers in the blood and urine of metastatic prostate cancer patients (and normal controls for comparison). Although a preliminary study, the researchers establish a proof of principle for this approach, and preliminary data that suggest its viability.

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Wu, D *et al.* (2016) "Incorporation of Slow Off-Rate Modified Aptamers reagents in single molecule array assays for cytokine detection with ultrahigh sensitivity." *Anal Chem* **88**(17): 8385-8389. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/27529794

Recent concerns about antibody consistency and quality in both clinical and bench research applications have many scientists looking for more reliable alternatives. In this article, researchers from Tufts University and SomaLogic demonstrate that SOMAmer reagents can be used in place of antibodies in ultrasensitive “single molecule array (Simoa) assays,” demonstrating their efficiency in measuring six different cytokine targets. The authors suggest that this combination “will greatly benefit both biomarker discovery and disease diagnostic fields.”

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Hathout, Y *et al.* (2016) "Serum pharmacodynamic biomarkers for chronic corticosteroid treatment of children." *Sci Rep* **6**: 31727.
http://www.nature.com/articles/srep31727

Corticosteroids are used effectively across a large number of diseases and conditions in which inflammation plays at least a partial role. But regular, repeated use can bring along a host of side effects, many of which can be worse than the initial disease or condition. In one particular disease, Duchenne muscular dystrophy (DMD), corticosteroids are a current standard of care, but efficacy gives way to safety issues over time, varying by patient. In this article, a multicenter group of researchers use the SOMAscan assay to identify protein biomarkers of corticosteroid efficacy and side effects, with the goal of developing a diagnostic tool to optimize the use of these powerful treatments in DMD patients—and young patients with other diseases—over time.

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Gramolini, A *et al.* (2016) "Identifying low-abundance biomarkers: Aptamer-based proteomics potentially enables more sensitive detection in cardiovascular diseases." *Circulation* **134**(4): 286-289. https://www.ncbi.nlm.nih.gov/pubmed/27444931

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Ngo, D *et al.* (2016) "Aptamer-based proteomic profiling reveals novel candidate biomarkers and pathways in cardiovascular disease." *Circulation* **134**(4): 270-285. https://www.ncbi.nlm.nih.gov/pubmed/27444932

Following closely on the publication of results from the use of SOMAscan to identify even low-concentration protein changes that foretell the personalized risk of cardiovascular events (see Ganz P *et al.* 2016, below), this set of studies by researchers at Beth Israel Deaconess Medical Center and the Broad Institute of MIT and Harvard demonstrates the power of the SOMAscan assay for finding novel biomarkers of cardiovascular disease in response to a "planned" heart attack (part of a unique treatment protocol for patients undergoing septal ablation for hypertrophic cardiomyopathy). Not only were potential low-abundance biomarkers consistently recovered from patient samples, but the proteins identified by SOMAscan were also validated by rigorous mass spectrometry analysis. The relevance of these to "unplanned" myocardial infarctions is being further investigated. As summarized in the accompanying editorial by Anthony Gramolini, Edward Lau and Peter Liu, "If these technologies continue to develop apace as expected, we can look forward to a bounty of new insights for patient care even from minute amounts of liquid biopsies."

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Sabatine, MS (2016) "Using aptamer-based technology to probe the plasma proteome for cardiovascular disease prediction." *JAMA* **315**(23): 2525-2526. **(Subscription required)** https://www.ncbi.nlm.nih.gov/pubmed/27327798

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Ganz, P *et al.* (2016) "Development and validation of a protein-based risk score for cardiovascular outcomes among patients with stable coronary heart disease." *JAMA* **315**(23): 2532-2541. https://www.ncbi.nlm.nih.gov/pubmed/27327800

Every patient diagnosed with stable coronary heart disease is currently treated aggressively in order to help prevent any future cardiovascular events. However, not every such individual is at significant risk of such events, leading to expensive overtreatment and mental anguish. In this breakthrough study, researchers from UCSF and SomaLogic used SOMAscan to discover and validate a group of nine blood proteins whose levels can reliably and accurately predict who is at high or low risk of future events. These proteins can also be used to track who is getting closer to an event, and who is benefitting from preventative interventions. The accompanying editorial by Dr. Marc Sabatine from Harvard puts these findings in the context of emerging personalized or precision medicine, as well as the possibility that several of the novel proteins uncovered could be future therapeutic targets.

Access to an electronic version of the accompanying editorial is available on request.

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Gupta, V *et al.* (2016) "An evaluation of an aptamer for use as an affinity reagent with MS: PCSK9 as an example protein." *Bioanalysis* **8**(15): 1557-1564. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/27397798

In this article, a research group at Merck Research Laboratories further demonstrates the extensive utility of individual SOMAmer reagents across multiple life science and clinical applications. They use a particular SOMAmer reagent, in this case one that binds the PCSK9 protein (a target of great interest in cardiovascular medicine), to enrich the protein from patient samples for subsequent analysis by mass spectrometry. The PCSK9 SOMAmer performed as well as—if not better than—PCSK9 antibodies, but provides significant advantages over those antibodies in terms of consistency, background, and stability.

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Lukjanenko, L *et al.* (2016) "Loss of fibronectin from the aged stem cell niche affects the regenerative capacity of skeletal muscle in mice." *Nat Med* **22**(8): 897-905.
https://www.ncbi.nlm.nih.gov/pubmed/27376579

Muscle has a remarkable ability to regenerate itself via dedicated muscle stem cells and their surrounding microenvironment of signaling and other molecules (the so-called stem cell "niche"). However, that ability decreases with age, for reasons that are still unknown. In this paper, an international research collaboration led by scientists from Nestle Institute of Health Sciences undertook a series of studies to determine the cause (and potential treatment) of aging muscle deterioration. Among those studies was a SOMAscan assay to determine what proteins might be altered in the aged muscle stem cell niche vs. younger muscle. They found that one protein in particular, fibronectin, was significantly decreased in the older muscle tissue, and addition of fibronectin could regain the regenerative capability in that muscle. They also demonstrate the structural mechanism by which fibronectin helps maintain muscle regeneration. While further studies are needed, this is an exciting insight into how to perhaps modulate one of the more devastating bodily effects of aging.

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Petek, LM *et al.* (2016) "A cross sectional study of two independent cohorts identifies serum biomarkers for facioscapulohumeral muscular dystrophy (FSHD)." *Neuromuscul Disord* **26**(7): 405-413.
http://www.nmd-journal.com/article/S0960-8966(15)30161-9/abstract

Facioscapulohumeral muscular dystrophy (FSHD), the third most common genetic disease of skeletal muscle, is usually first diagnosed, progressing towards increased disability, decreased quality of life, and death. Although there are potential treatments, the slow and often sporadic progression of FSHD makes it difficult, at best, to assess their efficacy. Thus, there is a great need for robust, reliable biomarkers. This preliminary study, using SOMAscan, identified several biomarkers that appear to correlate with clinical severity, though further studies are needed.

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Nishikawa, A *et al.* (2016) "Identification of definitive serum biomarkers associated with disease activity in primary Sjögren's syndrome." *Arthritis Res Ther* **18**(1): 106.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4868006/pdf/13075_2016_Article_1006.pdf

Sjögren's syndrome (SS), an autoimmune disease in which immune cells target the body's moisture producing cells, is the third most common rheumatic autoimmune disorder (after rheumatoid arthritis

and systemic lupus erythematosus). Despite its prevalence, SS is not well understood, and treatment interventions have had mixed success at best. In an effort to identify markers of disease and potential new drug targets, Nishikawa *et al.* used SOMAscan in samples from 88 patients with primary SS (i.e., patients without other rheumatic diseases noted). They identified 82 proteins associated with pSS, nine of which were associated with disease activity and five of these validated by traditional ELISA. Larger studies are underway to determine additional markers and to evaluate these markers as potential new therapeutic targets.

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Marion, T *et al.* (2016) "Respiratory mucosal proteome quantification in human influenza infections." *PLoS One* **11**(4): e0153674.
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0153674

Influenza virus seriously sickens three to five million people worldwide each year, causing an estimated 250,000 to 500,000 deaths annually. The degree of morbidity and mortality depends not only on the strain of virus, but also on the interaction of the virus with host factors of infected individuals. In one of the first studies of its kind, an international group of researchers used SOMAscan to understand the intricate interplay of host and virus proteins by identifying protein changes in nasal secretions during infection and disease progression. Though preliminary, this study provides a large number of new insights and potential new research directions for addressing this common but deadly virus.

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Drolet, DW *et al.* (2016) "Fit for the eye: Aptamers in ocular disorders." *Nucleic Acid Ther* **26**(3): 127-146.
http://online.liebertpub.com/doi/pdf/10.1089/nat.2015.0573

The first FDA-approved aptamer-based drug, Macugen, was developed for the treatment of the “wet form” of the eye disorder age-related macular degeneration (AMD). Two additional aptamer-based drugs for AMD are in late-stage clinical development. This review article covers not only the history of the AMD-directed aptamers, but also discusses the many other potential therapeutic opportunities for aptamers (including SOMAmer reagents) in ophthalmological indications with significant unmet medical need.

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Murota, A *et al.* (2016) "Serum proteomic analysis identifies interleukin 16 as a biomarker for clinical response during early treatment of rheumatoid arthritis." *Cytokine* **78**: 87-93. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/26700586

In this study, researchers from Keio University and Takeda Pharmaceutical Company used the SOMAscan assay to identify blood (serum)-based biomarkers of rheumatoid arthritis (RA) that could be correlated with disease progression and treatment efficacy. Comparing RA patients with non-RA volunteers, the researchers found that the serum levels of interleukin-16 (IL-16) are a better indicator than other measurement in current use, and thus IL-16 may be a more useful clinical biomarker of response to treatment. They also note that such studies have been difficult to impossible to perform prior to the availability of the “new, reliable and comprehensive” SOMAscan assay.

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Sattlecker, M *et al.* (2016) "Longitudinal protein changes in blood plasma associated with the rate of cognitive decline in Alzheimer's disease." *J Alzheimers Dis* **49**(4): 1105-1114. **(Subscription required)**

https://www.ncbi.nlm.nih.gov/pubmed/26599049

One of the more powerful uses of the SOMAscan assay is in performing "longitudinal" proteomics (i.e., tracking the changes in protein levels over time). In this study, an international group of researchers looked for changes in the blood of patients who transitioned from mild cognitive impairment (MCI) to Alzheimer's disease (AD) over the course of the year, comparing those changes to individuals with stable MCI, diagnosed AD, and controls (i.e., no MCI or AD). They found that the levels of proteins known to be involved in the complement pathway were significantly elevated in patients undergoing rapid transition from MCI to AD. These results reveal not only potential new biomarkers for testing the efficacy of investigational AD drugs, but also suggest new drug targets. Longer-term validation studies are underway.

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Hirota, M *et al.* (2016) "Chemically modified interleukin-6 aptamer inhibits development of collagen-induced arthritis in cynomolgus monkeys." *Nucleic Acid Ther* **26**(1): 10-19.
http://online.liebertpub.com/doi/pdf/10.1089/nat.2015.0567

In this manuscript, researchers from SomaLogic and Otsuka Pharmaceutical describe a series of studies that demonstrate that treatment with a novel SOMAmer reagent can significantly delay the onset and reduce the severity of rheumatoid arthritis (RA) in a cynomolgus monkey model of the disease. The SOMAmer molecule used in these studies, named SL1026, was initially selected for its ability to directly bind and block the signaling of the critical inflammatory protein interleukin-6 (IL-6), which is known to be involved in RA onset and progression. Because it is based on nucleic acids rather than amino acids, SL1026 offers certain advantages over antibody-based drugs such as tocilizumab, including the lack of an immune response to the drug itself, and a more consistent chemical rather than biological synthesis method.

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Lynch, AM *et al.* (2016) "The relationship of circulating proteins in early pregnancy with preterm birth." *Am J Obstet Gynecol* **214**(4): 517 e511-518.
https://www.ncbi.nlm.nih.gov/pubmed/26576488

Preterm birth is major global health problem, and babies born preterm (<37 weeks gestation) have an elevated risk of a spectrum of medical problems. In this paper, researchers from the University of Colorado used the SOMAscan assay to identify a signature of protein biomarkers that could foretell pre-term birth risk, with the goal of making successful early intervention possible.

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McArdle, A *et al.* (2016) "Developing clinically relevant biomarkers in inflammatory arthritis: A multiplatform approach for serum candidate protein discovery." *Proteomics Clin Appl* **10**(6): 691-698. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/26332844

Blood-based biomarkers that can distinguish between psoriatic arthritis (PsA) and rheumatoid arthritis (RA) are a significant medical need, particularly to guide treatment choice of available drugs. In this manuscript, the authors combine three proteomics approaches to identify such markers (LC-MS/MS, a Luminex immunoassay, and the SOMAscan assay), and compare the results. They found 42 (LC-MS/MS), 3 (Luminex), and 127 (SOMAscan assay) proteins respectively that distinguish between PsA and RA

patients. Besides providing the largest number of reproducible protein findings, the SOMAscan assay covered a significantly broader range of the blood proteome compared to the other two approaches.

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Olson, KA *et al.* (2015) "Association of growth differentiation factor 11/8, putative anti-ageing factor, with cardiovascular outcomes and overall mortality in humans: analysis of the Heart and Soul and HUNT3 cohorts." *Eur Heart J* **36**(48): 3426-3434.
https://www.ncbi.nlm.nih.gov/pubmed/26294790

This study is particularly notable for its demonstration that Growth Differentiation Factor-11/8 (GDF-11/8) may play a role in humans similar to that seen previously in mice (see Loffredo FS *et al.* 2013, below). The authors demonstrate that higher levels of GDF-11/8 are associated with a lower risk of cardiovascular events and death in patients with stable ischemic heart disease, suggesting that the molecular pathway represented by GDF-11/8 is a target for reducing cardiovascular risk associated with aging in humans.

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Kiddle, SJ *et al.* (2015) "Plasma protein biomarkers of Alzheimer's disease endophenotypes in asymptomatic older twins: early cognitive decline and regional brain volumes." *Transl Psychiatry* **5**: e584.
http://www.nature.com/tp/journal/v5/n6/pdf/tp201578a.pdf

Although there are no treatments known today that can delay or even prevent Alzheimer's disease (AD), having useful markers of very early onset (pre-symptomatic) is critical to testing new therapeutic interventions. Imaging approaches (e.g., MRI or PET) can detect early signs of Alzheimer's, though they are expensive and require high levels of expertise. In this study of asymptomatic older twins, the authors build on earlier work they have done by applying the SOMAscan assay to find early blood markers of AD, as well as looking at genetic contributions. They detected two proteins in particular, called "MAPKAPK5" and "MAP2K4," which are under further evaluation now as potential biomarkers for clinical trials.

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Hattori, K *et al.* (2015) "Increased cerebrospinal fluid fibrinogen in major depressive disorder." *Sci Rep* **5**: 11412.
http://www.readcube.com/articles/10.1038%2Fsrep11412

"Major depressive disorder" (MDD), like many common diseases, is a blanket term for at least several different abnormalities at the level of protein and/or genetic differences. In this manuscript, researchers describe the use of the SOMAscan assay to look for differences among patients in the levels of the protein fibrinogen in cerebrospinal fluid (CSF), one of the many biological fluids amenable to such analysis. They detected a subset of MDD patients with increased fibrinogen in CSF, which was verified using traditional protein measurement tools. They also correlated the increased level of fibrinogen in the CSF with specific damage to the brain, particularly in the white matter.

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Hathout, Y *et al.* (2015) "Large-scale serum protein biomarker discovery in Duchenne muscular dystrophy." *Proc Natl Acad Sci U S A* **112**(23): 7153-7158.
http://www.pnas.org/content/112/23/7153.full.pdf?with-ds=yes

Although we have known the genetic cause of Duchenne muscular dystrophy since 1986, our knowledge of the actual biology of the disease and its progression is still incomplete. This lack of understanding seriously compromises our efforts to find effective new treatments, as well as new diagnostic tests that can help patients and their caregivers manage disease progression. This paper, the result of a focused collaboration between industry, advocacy and Duchenne patient advocates, describes the first truly large-scale, unbiased biomarker discovery in Duchenne patients vs. controls, using the SOMAscan assay. A total of 44 proteins were identified, 24 of which are up and 20 that are down in Duchenne patients as compared to controls. Some of these were expected (and confirmatory of previous studies), but others were not, and suggest new approaches for diagnosis, prognosis and novel therapeutic discovery for this devastating disease.

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Menni, C *et al.* (2015) "Circulating proteomic signatures of chronological age." *J Gerontol A Biol Sci Med Sci* **70**(7): 809-816.
http://biomedgerontology.oxfordjournals.org/content/70/7/809.full.pdf+html

An international team of researchers used the SOMAscan assay to begin to dissect the proteomic features of aging in plasma. Initial finding from 202 subjects were subsequently replicated in 677 additional subjects. The researchers found that 11 proteins of those measured are associated with chronological age. This initial study underlines the importance of the proteome in understanding molecular mechanisms involved in human health and aging.

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Motzer, RJ *et al.* (2014) "Investigation of novel circulating proteins, germ line single-nucleotide polymorphisms, and molecular tumor markers as potential efficacy biomarkers of first-line sunitinib therapy for advanced renal cell carcinoma." *Cancer Chemother Pharmacol* **74**(4): 739-750.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4175044/pdf/280_2014_Article_2539.pdf

The drug sunitinib (SUTENT®) is approved worldwide for treatment of renal cell carcinoma. However, no good biomarkers for selecting likely responders and monitoring treatment efficacy have yet been identified. In this study, a research team lead by Pfizer scientists employed SOMAscan (and several other genomic and proteomic approaches) to discover such markers in a phase 2 clinical trial of sunitinib. Two particular protein biomarkers were identified that are now under further investigation for their predictive and prognostic value in clinical settings.

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Rohloff, JC *et al.* (2014) "Nucleic acid ligands with protein-like side chains: Modified aptamers and their use as diagnostic and therapeutic agents." *Mol Ther Nucleic Acids* **3**: e201.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4217074/pdf/mtna201449a.pdf

A comprehensive review of the development of SOMAmer reagents with an overview of the many applications for these breakthrough protein-binding molecules.

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Mehan, MR *et al.* (2014) "Validation of a blood protein signature for non-small cell lung cancer." *Clin Proteomics* **11**(1): 32.
http://www.clinicalproteomicsjournal.com/content/pdf/1559-0275-11-32.pdf

Building on previous work (see Ostroff *et al.* 2010, below), an international group of researchers led by SomaLogic scientists validated a protein signature for the detection of non-small cell lung cancer. This potential new test could be useful in particular, in follow up testing for patients diagnosed with a lung nodule using CT scanning, which has only a 4% positive rate for lung cancer detection. The work is also notable for the application of "Sample Mapping Vectors" (i.e., protein changes that are a result of blood handling rather than biological status) in validating this protein signature.

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Baumstummler, A *et al.* (2014) "Specific capture and detection of *Staphylococcus aureus* with high-affinity modified aptamers to cell surface components." *Lett Appl Microbiol* **59**(4): 422-431.
http://onlinelibrary.wiley.com/doi/10.1111/lam.12295/epdf

This study by researchers from Merck Millipore and SomaLogic demonstrates the binding ability of SOMAmer reagents created against bacterial cell surface proteins (in this case, *S. aureus*), and their applicability to the sensitive detection of the pathogen in standard biodetection, biosurveillance and food safety applications.

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Morine, MJ *et al.* (2014) "Genetic associations with micronutrient levels identified in immune and gastrointestinal networks." *Genes Nutr* **9**(4): 408.
http://link.springer.com/article/10.1007%2Fs12263-014-0408-4

This proof-of-concept study, published by researchers at Nestlé and their global collaborators, describes one of the first studies that aims to correlate metabolites, genetic variation, plasma proteomic changes, and environmental factors to begin to understand the "physiological processes for maintaining health." SOMAscan was used for longitudinal monitoring of protein changes over two years in 45 genetically unique individuals with 61 sets of metabolite, protein and diet variables.

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Monteiro, JP *et al.* (2014) "Methylation potential associated with diet, genotype, protein, and metabolite levels in the Delta Obesity Vitamin Study." *Genes Nutr* **9**(3): 403.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4026438/pdf/12263_2014_Article_403.pdf

Similar in approach to Morine *et al.* 2014 (above), this study from a global research group led by Nestlé scientists attempted to measure and correlate dietary intakes, micronutrients, and plasma proteins to identify subgroups of individuals for targeted nutritional interventions. Among other results, it is clear that measuring multiple proteins to find patterns that correlate with metabolite levels through data mining revealed the association of certain metabolic pathways (e.g., hormonal responses, neuronal responses, etc.). Protein differences in sex, age, and weight (obesity) were also seen, but further validation is required.

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Sattlecker, M *et al.* (2014) "Alzheimer's disease biomarker discovery using SOMAscan multiplexed protein technology." *Alzheimers Dement* **10**(6): 724-734.
http://www.alzheimersanddementia.com/article/S1552-5260(14)00031-4/pdf

Biomarkers that can predict the onset of Alzheimer's disease (AD) before the appearance of clinical symptoms (i.e., the "predementia phase") are critically needed for the development of early

intervention therapeutics. In this manuscript, a multinational team of researchers describes the application of SOMAscan to the unbiased discovery of potential blood-based AD biomarkers associated with various aspects of the disease. A number of protein biomarkers (including both previously described and novel biomarkers) are shown to be predictive of the various aspects of the disease, and further evaluation is underway.

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Nahid, P *et al.* (2014) "Aptamer-based proteomic signature of intensive phase treatment response in pulmonary tuberculosis." *Tuberculosis (Edinb)* **94**(3): 187-196.
https://www.ncbi.nlm.nih.gov/pubmed/24629635

The desperate need for new therapeutic agents for tuberculosis (TB) is compounded by the challenges of evaluating emerging new compounds early and effectively in clinical trials. This manuscript describes a SOMAscan-based approach to finding blood-based protein biomarkers that could speed up clinical development of new therapeutics, as well as help with monitoring patients on these new treatment regimes. The researchers identified an initial five protein-marker "signature" that differentiated between treatment responders and slow-responders, and was predictive of the current surrogate end point used in TB therapeutic trials (eight-week culture status).

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Webber, J *et al.* (2014) "Proteomics analysis of cancer exosomes using a novel modified aptamer-based array (SOMAscan™) platform." *Mol Cell Proteomics* **13**(4): 1050-1064.
http://www.mcponline.org/content/13/4/1050.full.pdf+html

Exosomes (small vesicles secreted by most, if not all, cell types into the blood) could serve as a source of biomarkers for early detection of disease. In this study, researchers from Cardiff University and SomaLogic applied SOMAscan to a prostate cancer cell line, hoping to discover better biomarkers for early detection of the disease. The unbiased protein measurement resulted in the discovery of over 300 proteins previously unassociated with prostate cancer and establishes the technology as "an effective proteomics platform for exosome-associated biomarker discovery in diverse clinical settings."

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Kiddle, SJ *et al.* (2014) "Candidate blood proteome markers of Alzheimer's disease onset and progression: a systematic review and replication study." *J Alzheimers Dis* **38**(3): 515-531.
http://content.iospress.com/download/journal-of-alzheimers-disease/jad130380?id=journal-of-alzheimers-disease%2Fjad130380

A total of 163 candidate blood-based protein biomarkers were previously described in the scientific literature for the potential diagnosis of Alzheimer's disease (AD). By applying SOMAscan (which includes SOMAmers to 94 of the 163 proteins previously described) to a large clinical sample set, researchers from King's College London and SomaLogic found that 9 of the 94 candidates are reliably associated with AD-related phenotypes, and are now being validated as a biomarker signature for the disease (as a set of protein biomarkers). Biomarkers that could predict onset and progression of AD would have great utility clinically, as well as for clinical trials and especially in the selection of subjects for preventative trials.

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Ochsner, UA *et al.* (2013) "Detection of *Clostridium difficile* toxins A, B and binary toxin with slow off-rate modified aptamers." *Diagn Microbiol Infect Dis* **76**(3): 278-285. **(Subscription required)** https://www.ncbi.nlm.nih.gov/pubmed/23680240

Clostridium difficile (*C. diff*) is a rapidly growing infectious disease health threat worldwide. A simple and highly specific diagnostic test for *C. diff* would have great utility in both the developed and developing world. This manuscript describes the generation of specific SOMAmers to several *C. diff* proteins and, equally important, the straightforward incorporation of SOMAmers into methods and platforms that are most commonly used for antibody-based tests (i.e., solution binding, pull downs with beads, dot blots, and sandwich assays).

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Xie, Y *et al.* (2013) "Interaction with both ZNRF3 and LGR4 is required for the signalling activity of R-spondin." *EMBO Rep* **14**(12): 1120-1126. http://embor.embopress.org/content/embor/14/12/1120.full.pdf

Proteins in the Wnt pathway are involved in the regulation of multiple cellular processes (proliferation, cell polarity and cell fate determination), and thus implicated in multiple cancers and other proliferative disorders. In an effort to further understand the pathway, researchers at Novartis and SomaLogic identified a SOMAmer that specifically neutralized the activity of RSPO1 (R-spondin), a critical modulator of the Wnt pathway, to determine its target and suggest new therapeutic approaches to cancer and tissue degeneration.

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Loffredo, FS *et al.* (2013) "Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy." *Cell* **153**(4): 828-839. http://ac.els-cdn.com/S009286741300456X/1-s2.0-S009286741300456X-main.pdf?_tid=f886b958-3165-11e5-909d-00000aacb35d&acdnat=1437675153_b2edc94375d8935136162c7cc44d6ecf

In this manuscript, a team of researchers led by scientists from the Harvard Stem Cell Institute, describe the discovery of a circulating protein called growth differentiation factor 11 (GDF-11), that can reverse age-related cardiac hypertrophy in mice. After failing to find the factor using lipidomic, metabolomic, and other proteomic approaches, the Harvard team turned to the SOMAscan assay, finding several proteins (including GDF-11) whose levels of expression change with age. The researchers then demonstrated that treating older mice with a recombinant version of the GDF-11 protein can rapidly reverse age-related cardiac hypertrophy. Studies aimed at extending these observations to humans are underway. It is interesting to note that, although the proteins targeted by SOMAscan are the human version, sufficient evolutionary conservation exists to make SOMAscan a useful tool for at least some non-human species applications.

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Park, NJ *et al.* (2013) "Measurement of cetuximab and panitumumab-unbound serum EGFR extracellular domain using an assay based on slow off-rate modified aptamer (SOMAmer) reagents." *PLoS One* **8**(8): e71703. http://www.plosone.org/article/fetchObject.action?uri=info%3Adoi%2F10.1371%2Fjournal.pone.0071703&representation=PDF

Epidermal growth factor receptor (EGFR) is a cell surface protein that is the target of the anticancer drugs cetuximab (Erbitux®) and panitumumab (Vectibix®). In this manuscript, scientists from Quest Diagnostics and SomaLogic describe the use of a SOMAmer that binds the extracellular domain of EGFR to determine the amount of drug-unbound EGFR in patients being treated with either drug. This assay could help determine drug efficacy and dosing for individual patients.

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De Groote, MA *et al.* (2013) "Elucidating novel serum biomarkers associated with pulmonary tuberculosis treatment." *PLoS One* **8**(4): e61002.
http://www.plosone.org/article/fetchObject.action?uri=info%3Adoi%2F10.1371%2Fjournal.pone.0061002&representation=PDF

This manuscript describes the first large-scale proteomic analysis employing SOMAscan in a study of active tuberculosis (TB). The international team of scientists identified multiple proteins that exhibit significant expression differences during the intensive phase of TB therapy, in particular discovering protein changes in conserved networks of biological processes and function (antimicrobial defense, tissue healing and remodeling, acute phase response, pattern recognition, protease/anti-proteases, complement and coagulation cascade, apoptosis, immunity and inflammation pathways). Some of these were known previously (providing validation for the work), but many novel proteins were also identified. These newly identified proteins may provide new insights for understanding TB disease, its treatment and subsequent healing processes that occur in response to effective therapy.

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Ostroff, RM *et al.* (2012) "Early detection of malignant pleural mesothelioma in asbestos-exposed individuals with a noninvasive proteomics-based surveillance tool." *PLoS One* **7**(10): e46091.
http://www.plosone.org/article/fetchObject.action?uri=info%3Adoi%2F10.1371%2Fjournal.pone.0046091&representation=PDF

This manuscript describes a set of multi-center case-control studies of serum from 117 malignant mesothelioma (MM) patients and 142 asbestos-exposed control individuals. Biomarker discovery, verification, and validation were performed using the SOMAscan assay. From 64 candidate protein biomarkers identified, the team of scientists from New York University and SomaLogic derived a 13-marker random forest classifier that demonstrated extremely high sensitivity and specificity (97%/92% in training and 90%/95% in blinded verification, and 90%/89% in a second blinded validation set). This result was far superior to that of mesothelin, the currently used biomarker for mesothelioma detection/diagnosis. The SOMAmer biomarker panel discovered and validated in these studies provides a solid foundation for surveillance and diagnosis of MM in those at highest risk for this disease.

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Baird, GS *et al.* (2012) "Age-dependent changes in the cerebrospinal fluid proteome by slow off-rate modified aptamer array." *Am J Pathol* **180**(2): 446-456.
http://ajp.amjpathol.org/article/S0002-9440(11)01014-5/abstract

This manuscript is the first published description of the use of SOMAscan to perform unbiased protein discovery in cerebrospinal fluid (CSF), a biological matrix that may provide early detection and diagnosis for several central nervous system (CNS) degenerative diseases. Scientists from the University of Washington and SomaLogic examined the CSF proteome from 90 normal adults (ages 21–85). In addition to demonstrating the applicability of SOMAscan to CSF, they discovered a set of protein

changes that correlate with increasing age, a finding that may have relevance in diagnosing age-related CNS diseases.

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Mehan, MR *et al.* (2012) "Protein signature of lung cancer tissues." *PLoS One* **7**(4): e35157. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0035157

In this first report of SOMAscan applied to tissue samples, a team of scientists from SomaLogic and the University of Washington compared the protein expression signatures of non-small cell lung cancer (NSCLC) tissues with healthy adjacent and distant tissues from surgical resections. They found that 36 proteins exhibited the largest expression differences between matched tumor and non-tumor tissues (20 proteins increased and 16 decreased in tumor tissue). Thirteen of these proteins have not been previously described in NSCLC. These tissue biomarkers also overlap with a core set of proteins identified in a large serum-based NSCLC study with SOMAscan (see Ostroff RM *et al.* 2010, below). By using the SOMAmers to the proteins identified in the study as novel histochemical probes, the scientists demonstrated that differences in protein expression are greater in tissues than in serum (as expected). The combined results of this study and the serum study present the most extensive view to date of the complex changes in NSCLC protein expression and have important implications for development of new diagnostic and therapeutic approaches.

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Gupta, S *et al.* (2011) "Rapid histochemistry using slow off-rate modified aptamers with anionic competition." *Appl Immunohistochem Mol Morphol* **19**(3): 273-278. **(Subscription required)** https://www.ncbi.nlm.nih.gov/pubmed/21217521

This manuscript is the first description of the utility of individual SOMAmers as immunohistochemical imaging reagents, both for research and potentially clinical (e.g., intraoperative) settings. The unique specificity and dissociation kinetics of the two SOMAmers used—against epidermal growth factor receptor, EGFR, and human epidermal growth factor receptor 2, HER2—allowed the two closely related protein targets to be distinguished in frozen tissue sections. Further work is underway for various imaging applications of SOMAmers.

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Ostroff, RM *et al.* (2010) "Unlocking biomarker discovery: large scale application of aptamer proteomic technology for early detection of lung cancer." *PLoS One* **5**(12): e15003. http://www.plosone.org/article/fetchObject.action?uri=info%3Adoi%2F10.1371%2Fjournal.pone.0015003&representation=PDF

This manuscript describes both the first large-scale application of SOMAscan to a specific disease and the most complete clinical serum proteome analysis of non-small cell lung cancer (NSCLC) to date. Archived serum samples from 1326 individuals (including 291 diagnosed NSCLC patients and 1,035 heavy smoker controls) from four independent studies were analyzed with SOMAscan. A 12-protein biomarker signature was found that discriminated NSCLC from controls with high specificity and sensitivity (91%/84% in training sets and 89%/83% in a separate verification set). This work, which forms the basis for a new diagnostic test in development by Quest Diagnostics, is being further extended and refined.

Katilius E *et al.* (2018) "Sperm cell purification from mock forensic swabs using SOMAmer™ affinity reagents." *Forensic Sci Int Genet* **35**: 9-13. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/29609058

Extracting sperm cells from forensic samples typically involves selectively dissolving away other contaminants, which is both time-consuming and labor-intensive. Attempts to simplify the method by using antibody-based affinity purification have not been effective because the recovery yields were too low.
In this proof-of-concept study, investigators from the Denver police department crime laboratory and SomaLogic demonstrate how SOMAmers can be used to selectively capture sperm from mock swab samples. The SOMAmer-based method was as effective as differential extractions for high sperm count samples, but less effective for low sperm count samples. This is possibly due to storage conditions or sample handling (i.e. repeated freeze/thaw, overdilution) that affect the ability of SOMAmers to bind to their native target proteins and could be resolved with further optimization.

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Duo J *et al.* (2018) "Slow Off-Rate Modified Aptamer (SOMAmer) as a Novel Reagent in Immunoassay Development for Accurate Soluble Glypican-3 Quantification in Clinical Samples." *Anal Chem* **90**(8): 5162-5170.
https://www.ncbi.nlm.nih.gov/pubmed/29605994

The protein glypican-3 (GPC3) is expressed on the surface of cells and a soluble form is elevated in the blood of hepatocellular carcinoma (HCC) patients. Characterizing the circulating forms of GPC3 (e.g. whether the soluble protein is full-length, N- or C-terminal fragment) is critical for its validation as a diagnostic biomarker for HCC. However, the only antibodies that are available bind to the C-terminal region of GPC3. This study from scientists at Bristol Myers Squibb used a SOMAmer reagent that binds to the N-terminal region of GPC3 to develop an immunoassay to determine the relevant soluble GPC3 forms in clinical samples. This work is a clear example of how SOMAmer reagents can be used for immunoassay development to address questions that cannot be answered by traditional antibody reagents alone.

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Gupta, S *et al.* (2017) "Pharmacokinetic properties of DNA aptamers with base modifications." *Nucleic Acid Ther* **27**(6): 345-353.
https://www.ncbi.nlm.nih.gov/pubmed/28961063

Rapid blood clearance currently limits the therapeutic uses of DNA aptamers. SOMAmers contain DNA bases with amino acid-like modifications that make them more resistant to breakdown by the body. The goal of this SomaLogic study was to understand how various modified groups in SOMAmers affect plasma clearance. Shorter aptamers (≤24 bases) with larger numbers of hydrophilic modifications had longer plasma residence times. These observations will help in future design of aptamers for therapeutic treatments.

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Ren, X *et al.* (2017) "Structural basis for IL-1α recognition by a modified DNA aptamer that specifically inhibits IL-1α signaling" *Nat Commun* **8**(1): 810.

https://www.nature.com/articles/s41467-017-00864-2

IL-1 alpha is an inflammatory protein involved in fever and sepsis and implicated in tumor formation and metastasis. Scientists at Yale University and SomaLogic created a SOMAmer (named SL1067) that binds tightly and specifically to IL-1 alpha, and then determined the crystal structure of the SOMAmer-protein complex. This is the first high resolution structure of IL-1 alpha and reveals the molecular details of its binding interactions with SL1067. It will be of great interest in developing new therapies that target IL-1 alpha. The researchers found that SL1067 inhibits IL-1 alpha activity by binding the same interface that IL-1 alpha uses to bind to its native receptor on cells. Thus, SL1067 represents a powerful tool for studying IL-1 alpha's role in normal inflammatory responses and those that lead to disease.

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Gawande, BN *et al.* (2017) "Selection of DNA aptamers with two modified bases." *Proc Natl Acad Sci U S A* **114**(11): 2898-2903.
http://www.pnas.org/content/114/11/2898.long

SomaLogic scientists report on the generation and characterization of SOMAmers that contain two types of modified nucleotides. The current SomaLogic technology uses bases that have been modified with amino acid-like sidechains at the 5 position of deoxyuridine (dU). Now, for the first time, researchers have created SELEX libraries that also contain 5-position modified deoxycytosine (dC). Eighteen different DNA libraries were synthesized that contained zero, one or both modified bases. SELEX was conducted against proprotein convertase subtilisin/kexin type 9 (PCSK9), a human therapeutic target protein that helps regulate cholesterol. The aptamers with the highest affinity for PCSK9 contained two modifications. Similar results were observed with another target protein, prostate-specific membrane antigen (PSMA), a predictor for progression and prognosis of prostate cancer.

The increased chemical diversity of SELEX libraries should expand the repertoire of protein targets. In addition to displaying tighter binding while maintaining high specificity, SOMAmers with two modified bases were significantly more resistant to degradation than those with a single modification. Doubly modified aptamers also showed greater epitope coverage, which should be useful for developing reagents for assays that require simultaneous binding to a given protein target.

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Cotton, RJ *et al.* (2016) "readat: An R package for reading and working with SomaLogic ADAT files." *BMC Bioinformatics* **17**(1): 201.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4857291/

The SOMAscan assay measures over 1,300 proteins in small amounts of biological samples. Experimental data from the SOMAscan assay are provided in a proprietary "ADAT" file format that is difficult to import into non-SomaLogic software packages. To overcome this limitation, two researchers at Weill Cornell Medicine in Qatar have developed "readat," a free, open source, R software package that allows users to import and analyze SomaLogic's ADAT format files.

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Gelinas, AD *et al.* (2016) "Embracing proteins: structural themes in aptamer-protein complexes." *Curr Opin Struct Biol* **36**: 122-132.
http://www.sciencedirect.com/science/article/pii/S0959440X16000129

Single stranded nucleic acids can fold into a wide variety of different shapes, many of which can recognize and bind other molecules. This review summarizes the different motifs that have been seen in structural studies of aptamer-protein complexes, including the expanded structural "vocabulary" made possible by modifying the nucleic acid bases (e.g., SOMAmer reagents).

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Gold, L (2015) "SELEX: How it happened and where it will go." *J Mol Evol* **81**(5-6): 140-143.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4661202/

In this brief mini-review, SomaLogic Founder and Chairman Larry Gold describes the origins of SELEX and aptamers, the launch of SomaLogic and SOMAmer reagents, and anticipates what is coming next.

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Carlson, M *et al.* (2015) "Improved preparation of 2 M triethylammonium bicarbonate." *Green Chem Lett Rev* **8**(3-4): 37-39.
http://www.tandfonline.com/doi/full/10.1080/17518253.2015.1091039

SomaLogic researchers describe a new method to generate a laboratory chemical used extensively in making SOMAmer reagents, resulting in a reduction of carbon dioxide waste emission by ~90% over current methods to generate the same chemical.

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Wolk, SK *et al.* (2015) "Influence of 5-N-carboxamide modifications on the thermodynamic stability of oligonucleotides." *Nucleic Acids Res* **43**(19): 9107-9122.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4627095/

The incorporation of DNA base modifications results in the high specificity for and broader range of protein types targeted by SOMAmer reagents. In this paper, the authors delve deeper into understanding the thermodynamic effects of these modifications on the stability of the SOMAmer oligonucleotides, both in their single-stranded and duplex forms. The results of these studies demonstrate that, depending on the type of modification, the addition can either destabilize or further stabilize the duplex forms, but in the single-stranded state (the usual use of SOMAmer reagents in biomarker discovery or other assays), the modifications significantly stabilized the oligonucleotide shapes as compared to unmodified single-stranded DNA.

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Jarvis, TC *et al.* (2015) "Non-helical DNA triplex forms a unique aptamer scaffold for high affinity recognition of nerve growth factor." *Structure* **23**(7): 1293-1304.
https://www.ncbi.nlm.nih.gov/pubmed/26027732

The structural explanation for the tight binding of a unique SOMAmer reagent to its target (nerve growth factor, or NGF) is described in this paper, the third in a series of manuscripts defining the precise molecular structure of specific SOMAmer:protein pairs (see Gelinas *et al.* 2014 and Davies DR *et al.* 2012, below). Like the previous two descriptions, the structure of the NGF SOMAmer is unlike any previously described traditional aptamer configuration and underlines the critical role of the DNA base modifications used in generating SOMAmer reagents.

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Rohloff, JC *et al.* (2015) "Practical synthesis of cytidine-5-carboxamide-modified nucleotide reagents." *Nucleosides Nucleotides Nucleic Acids* **34**(3): 180-198.
http://www.tandfonline.com/doi/pdf/10.1080/15257770.2014.978011

The exquisite specificity of SOMAmer reagents for their cognate proteins lies in their expanded chemical diversity over traditional aptamers via the protein-like modifications added to the chemical structure of some of the nucleotides that make up the SOMAmer sequence. This manuscript describes the further expansion of that chemical diversity through the successful efforts of SomaLogic scientists to add chemical modifications to cytidine (C). These modifications do not interfere with either solid-state synthesis or enzymatic synthesis of oligonucleotides containing such modified C bases. Modified C bases are already being incorporated into new SOMAmer discovery experiments.

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Ochsner, UA *et al.* (2014) "Systematic selection of modified aptamer pairs for diagnostic sandwich assays." *Biotechniques* **56**(3): 125-128, 130, 132-133.
http://www.biotechniques.com/multimedia/archive/00231/BTN_A_000114134_O_231855a.pdf

This manuscript is the first published description (proof-of-concept) of the use of SOMAmers in a sandwich assay. In this paper, SOMAmer pairs were generated against both *Clostridium difficile* binary toxin and for a group of seven proteins previously shown to be promising biomarkers for cardiovascular risk. The ability to use SOMAmer pairs in diagnostic applications rather than traditional antibody pairs holds promise for accelerated development of rapid tests and/or specific diagnostic panels.

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Gelinas, AD *et al.* (2014) "Crystal structure of interleukin-6 in complex with a modified nucleic acid ligand." *J Biol Chem* **289**(12): 8720-8734.
http://www.jbc.org/content/289/12/8720

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Gupta, S *et al.* (2014) "Chemically modified DNA aptamers bind interleukin-6 with high affinity and inhibit signaling by blocking its interaction with interleukin-6 receptor." *J Biol Chem* **289**(12): 8706-8719.
http://www.jbc.org/content/289/12/8706

This pair of papers, published simultaneously in the *Journal of Biological Chemistry*, describes the development of new SOMAmer reagents that can block signaling by interleukin-6 (IL-6, a critical protein involved in inflammation and cancer), as well as the structural interaction of the IL-6 SOMAmer and its target protein. This work both confirms the unique protein-binding properties of SOMAmers and underlines their potential as a new class of therapeutic reagents. The work was done in collaboration with Otsuka Pharmaceuticals and Emerald Bio.

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Brody, E *et al.* (2012) "Life's simple measures: Unlocking the proteome." *J Mol Biol* **422**(5): 595-606. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/22721953

This review article describes both the SOMAmer/SOMAscan technology and gives examples of its multiple applications in unbiased protein biomarker discovery. It also includes a description of the bioinformatics methods used to interpret the large datasets generated by SOMAscan.

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Davies, DR *et al.* (2012) "Unique motifs and hydrophobic interactions shape the binding of modified DNA ligands to protein targets." *Proc Natl Acad Sci U S A* **109**(49): 19971-19976.
http://www.pnas.org/content/109/49/19971.full.pdf+html

This manuscript is the first demonstration of the unique molecular structure of a SOMAmer reagent bound to its specific protein target. The analyses reveal the molecular basis for the vast improvement in protein binding by SOMAmers as compared to traditional aptamers, emphasizing that SOMAmers represent an entirely new class of molecular “affinity reagents” with multiple useful applications in life sciences and medicine. This work was done as a collaboration between SomaLogic and Emerald Bio.

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Gold, L *et al.* (2012) "Aptamers and the RNA world, past and present." *Cold Spring Harb Perspect Biol* **4**(3).
http://cshperspectives.cshlp.org/content/4/3/a003582.full.pdf+html

This review article clearly lays out the reasoning and the development of SOMAmers that would provide two simultaneous elements of specificity (e.g., the equivalent to a good antibody sandwich assay within a single SOMAmer reagent). Those two elements are (1) affinity for their target protein (i.e., pM or lower Kd), and (2) a kinetic component (slow off-rate, or remarkable slow dissociation rate constants). These two properties, along with the chemical basis for SOMAmers, overcome the specific technical challenges faced by other current proteomic technologies, and provide the basis for the steps comprising the SOMAscan assay.

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Kraemer, S *et al.* (2011) "From SOMAmer-based biomarker discovery to diagnostic and clinical applications: a SOMAmer-based, streamlined multiplex proteomic assay." *PLoS One* **6**(10): e26332.
https://www.ncbi.nlm.nih.gov/pubmed/22022604

This manuscript demonstrates that the SOMAscan assay provides a seamless transition from SOMAmer-based biomarker discovery to routine protein measurements for diagnostic and research purposes. Furthermore, the assay can be semi-automated (here they developed a plate-based version), and can be performed with multiple “back end” readouts (qPCR, bead-based—e.g., Luminex, etc.), underlining the compatibility of this approach with current nucleic-acid based diagnostic technologies.

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Brody, EN *et al.* (2010) "High-content affinity-based proteomics: unlocking protein biomarker discovery." *Expert Rev Mol Diagn* **10**(8): 1013-1022.
http://www.tandfonline.com/doi/full/10.1586/erm.10.89

This review article compares the SOMAscan assay directly to other current proteomic technologies (mass spectrometry and antibody-based), particularly in high-content protein biomarker discovery. It demonstrates how SOMAscan overcomes the specific technical challenges faced by these other

approaches, particularly the need for high content with high sensitivity and specificity to address the circulating proteome.

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Vaught, JD *et al.* (2010) "Expanding the chemistry of DNA for *in vitro* selection." *J Am Chem Soc* **132**(12): 4141-4151. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/20201573

This manuscript describes the fundamental biochemical steps necessary to incorporate modified nucleotides into DNA-based aptamers (and thus the first published description of "SOMAmers," though the name was subsequently coined). The manuscript also describes the identification of a modified DNA aptamer with high affinity for the tumor necrosis factor receptor superfamily member 9 (TNFRSF9), a protein that had proven refractory to aptamer selection using traditional unmodified DNA aptamers.

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Gold, L *et al.* (2010) "Aptamer-based multiplexed proteomic technology for biomarker discovery." *PLoS One* **5**(12): e15004.
https://www.ncbi.nlm.nih.gov/pubmed/21165148

This manuscript is the first published detailed description of the breakthrough SOMAmer-based SOMAscan technology and demonstrates its power through application to samples from patients with chronic kidney disease, finding not only known markers of the disease but many previously unknown protein biomarkers.

(Companion paper, Ostroff RM *et al.* 2010—above in "SOMAmer/SOMAscan Applications").

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Zichi, D *et al.* (2008) "Proteomics and diagnostics: Let's get specific, again." *Curr Opin Chem Biol* **12**(1): 78-85. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/18275862

This manuscript describes the inherent specificity limitations of antibody-based arrays for large-scale biomarker discovery, and introduces the basic idea behind the SOMAmer reagent and its two elements of specificity (i.e., high affinity and slow dissociation rates).

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Eaton, BE *et al.* (1995) "Let's get specific: The relationship between specificity and affinity." *Chem Biol* **2**(10): 633-638.
http://ac.els-cdn.com/1074552195900233/1-s2.0-1074552195900233main.pdf?_tid=2d0444ae-3169-11e5-855b00000aacb35d&acdnat=1437676530_f6803454a6da15b1b8b669dc920c5086

This review article lays out a systematic argument for selecting molecules that bind with high specificity to a particular target by screening for molecules with high affinity to that target. It applies that understanding to the selection of traditional aptamers, suggesting the critical role aptamer-based reagents can play in diagnostic and therapeutic applications.

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Jenison, RD *et al.* (1994) "High-resolution molecular discrimination by RNA." *Science* **263**(5152): 1425-1429. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/7510417

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Zimmermann, GR *et al.* (1997) "Interlocking structural motifs mediate molecular discrimination by a theophylline-binding RNA." *Nat Struct Biol* **4**(8): 644-649. **(Subscription required)**
https://www.ncbi.nlm.nih.gov/pubmed/9253414

These two manuscripts together describe (1) the isolation of an RNA-based aptamer that can bind theophylline with a 10,000-fold better affinity than it binds the closely related caffeine molecule (which differs from theophylline by only an extra methyl group) and (2) the structural basis of that affinity. These early studies of the incredible specificity that can be achieved with traditional aptamers are being even more fully realized with the work being done with SOMAmer reagents today.