Discovery of neurological biomarker signatures using SOMA
c™ Multiplex Proteomic Technology

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Abstract

The potential of blood-based protein biomarkers to diagnose disease early and accurately could greatly enhance medical care and offer new avenues for therapeutic discovery and development. However, the discovery of such biomarkers is complicated by the technological challenges of profiling the circulating proteome: a complex mixture of thousands of proteins that range from high (µM) to exceedingly low (fM) concentrations. Using a new high-content multiplex proteomics assay (SOMAscan), we assayed thousands of blood samples for biomarker discovery and algorithm training. The archived samples spanned 30 sites in the US and Europe, with blood and CSF samples from a wide range of central nervous system diseases that include Amyotrophic Lateral Sclerosis (ALS), Multiple Sclerosis (MS), Parkinson’s Disease (PD) and Alzheimer’s Disease (AD). Robust biomarkers were identified in ALS to develop preliminary classifiers. Each performed well on our test sets with differential expression seen between samples from disease patients and healthy controls with an estimated AUC = 0.93 (0.83, 0.97) and 10-fold cross-validation, giving a mean AUC < 0.89 ± 0.02, and an associated sensitivity of 0.89 ± 0.02 and specificity of 0.8 ± 0.03. These biomarkers were specific to ALS as they were not affected by neurodegeneration-based symptom mimics such as MS or AD.

A Rule-Out test for Amyotrophic Lateral Sclerosis (ALS) in Subjects Presenting With Neurological Symptoms

Establishing the diagnosis in people who have the disease is a slow, invasive and costly process, and establishing that an individual with neurological symptoms does not have ALS is equally slow, invasive and costly. The most differential expression was seen between samples from ALS patients and healthy controls with an estimated AUC = 0.93 (0.83, 0.97) and 10-fold cross-validation, giving a mean AUC < 0.89 ± 0.02, and an associated sensitivity of 0.89 ± 0.02 and specificity of 0.8 ± 0.03. These biomarkers were specific to ALS as they were not affected by neurodegeneration-based symptom mimics such as MS or AD.

Study design:

Initial discovery of ALS biomarkers (Figs 2A-D): 223 patients with early ALS; 100 healthy controls without ALS; 50 neurological disease mimics

Confirmation of disease specificity of ALS biomarkers (Fig 2E): 50 subjects with early MS; 50 subjects with neurological symptoms but not MS or ALS; 182 subjects with Alzheimer’s disease

Fig. 2A. ALS vs. Healthy Controls

2C. ROC curve (left) and log odds plot (right) generated by four protein logistic regression model applied to the test set

2E. ALS classifier correctly identifies other neurological diseases and Healthy Controls as not ALS

Cerebrospinal Fluid Analysis using SOMA
c™

100% of Biological Samples

1129 SOMAmer Reagents

Table 1. SOMA
c™ Assay Overall Performance

| Metric               | Conditions          | Current SOMA
c™ v3.0 | Sensitivity | Specificity |
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<tbody>
<tr>
<td></td>
<td>Median LOD (buffer)</td>
<td>Median LOD (plasma)</td>
<td>10 M</td>
<td>1.6 pg/ml</td>
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<tr>
<td></td>
<td>Precision</td>
<td>Median Total %CV</td>
<td>5%</td>
<td>10%</td>
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<td>Dynamic Range</td>
<td>Over all proteins in serum or plasma</td>
<td>Median individual SOMAmer</td>
<td>10%</td>
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<tr>
<td>Sample Volume</td>
<td>Per sample</td>
<td>50 µl</td>
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<tr>
<td>Multiplex Size</td>
<td>Current # targets per sample</td>
<td>1129</td>
<td></td>
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<tr>
<td>Time to result</td>
<td>Automated assay &amp; hybridization detection</td>
<td>~30 hours</td>
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Fig. 1. SOMA
c: Empirical Biomarker Discovery In 5 weeks

B. SOMA
cAssay Overview

The SOMA
cassay is a powerful tool, allowing the use of conventional, fully validated DNA detection methods for detection (Fig. 1). The most recent version of SOMA
cassay, v3.0, is multiplexed to 1129 analytes per sample, automated using a Biomek FXP (2), and detected using custom Agilent 15,000 spot microarrays. Once developed, a panel of biomarkers can be replicated to a streamlined assay in just a few weeks with a standardized protocol. The overall assay performance and throughput are detailed in Table 1.

References