Development of multiplexed MSIA (Mass Spectrometric Immunoassay)-SRM assays for proteins associated with Alzheimer’s Disease and application to clinical samples.

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Overview


Methods: Mass Spectrometric Immunoassay coupled to selected reaction monitoring (MSIA-SRM) was applied to rapid and quantitative enrichment of multiple protein and peptide fragments and isoforms.

Results: The results demonstrated assay linearity, sensitivity and differential protein abundance in a small clinical cohort of AD and control plasma samples.

Introduction

Several protein biomarkers are known to be associated with Alzheimer’s Disease (AD). The leading theory of AD pathophysiology is the Amyloid Cascade Hypothesis, which was originally focused on the extracellular deposition of beta amyloid peptides (Aβ) in large fibrillar aggregates [1]. To date, several variants of Aβ have been found in brain and cerebrospinal fluid (CSF) while data from blood and plasma are more unambiguous [2]. Another possible biomarker of AD, apolipoprotein E (Apo E) may also play a central role in the AD amyloid cascade leading to cognitive decline [3]. APO-E binds to Aβ and promotes both fibril formation in the brain and Aβ clearance from the blood [3-4]. The apparently contradictory fibril promotion and clearance roles of Apo E might both promote Aβ fibril formation in the brain and Aβ clearance from the blood [3,4]. The apparent mechanisms include fibril promotion and clearance roles of Apo E might be due to the different binding capacities of Apo E isoforms (Apo E2,3,4) for Aβ [4]. Distances of copper homeostasis may also contribute to the neurodegeneration associated with AD and the level of the copper enzyme ceruloplasmin (CP) is increased in the CSF of AD affected individuals [5]. In order to further investigate the relationship of these markers to AD, we developed multiplexed, MSIA-SRM assays that allow quantification of CP, APOE monitoring and isoform- specific peptides and several Aβ isoforms. The MSIA technology provides rapid enrichment for low abundance analytes from fluids such as plasma, serum and cerebrospinal fluid (CSF). We used these assays to interrogate a small cohort of clinical plasma samples from patients with AD and matched controls. The results demonstrated (for the first time) quantitative MS detection of Aβ and other peptides in human plasma.

Methods

Clinical Samples

Alzheimer’s and Controls patients were recruited following IRB-approved protocol (Table 1). MSIA enrichment and SRM assay development

Serum samples were thawed on ice and processed on MSIA tips using commercially available antibodies (Aβ, Covance; Apo E, Academy Biochemical, CP, DAKO), recombinant proteins (ApoE, ABCAM, CP, MP Biomedicals) and isoelectrically labeled heavy peptide standards (Thermo Fisher Scientific) as previously described [6]. One hundred nanoliters of plasma from each sample was used for the CP and one microliter plasma for the APOE multiplexed assays. Due to low available sample volume, four 500ul plasma pools of randomly selected AD and control samples each were created for MSIA extraction in the Aβ assays. When Aβ was assayed in CSF, 200ul was extracted with each MSIA tip. SRM assays (individually for AD, CP and Apo E) were developed for targeted peptides (Table 2) as previously described [7,8], and run on a TSQ Vantage triple quadrupole mass spectrometer using high flow LC. Peptides were chosen based upon 4 criteria: literature references, previously obtained LC-MS/MS discovery data, positive control results, and algorithmic prediction. Total MS assay time for Aβ, APOE and CP was 20 min per sample.

Data Analysis

Data were analyzed with Pinpoint as previously described [7].

RESULTS

Peptide Selection

Table 2 shows the list of surrogate peptides chosen for the three multiplexed SRM assays. Peptide selection was based on literature references, previously obtained LC-MS/MS discovery data (not shown), optimization using recombinant proteins or algorithmic prediction in Pinpoint. We selected three Aβ peptides for quantification, 1-38, 1-40 and 1-42 (cited as correlated with the pathology of AD and deposition of plaques). Five tryptic peptides providing the best signal-to-noise were chosen for CP based on iterative optimization (in Pinpoint) using a recombinant CP digest.

The APO-E surrogate peptides fell into 2 groups: Monitoring and isoform-specific. The monitoring peptides are metabolite-like or common peptides associated with AD, and diversity is provided by the isoform-specific peptides (Table 3). Some of the isoforms, specifically Apo E4, 5 are specifically associated with AD [3], however, the presence of the Apo E4 allele does not always predict AD pathology.

Sensitivity and Precision of the MSIA-SRM assays

The assays demonstrated excellent linearity and precision. Figure 2A shows a calibration curve for one of the Aβ monitoring target peptides. In general, the CV’s of full technical replicates (complete MSIA-SRM process) were <20% at or above the LOQ. Figure 2B shows the sensitivity of the three protein/peptide assays (Table 3). Some of the isoforms, specifically Apo E4, 5 are specifically associated with AD [3], however, the presence of the Apo E4 allele does not always predict AD pathology.

FIGURE 3. MSIA-SRM detection and quantification of Aβ peptides in human CSF. All three fragments 1-38,1-40 and 1-42 were easily detected in CSF samples using the described assay.

TABLE 1. Clinical samples.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Number</th>
<th>Gender</th>
<th>Age</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>30</td>
<td>Male</td>
<td>70</td>
<td>Alzheimer's disease</td>
</tr>
<tr>
<td>Normal</td>
<td>30</td>
<td>Female</td>
<td>70</td>
<td>Normal</td>
</tr>
</tbody>
</table>

*Average of all monitoring peptides

*Average of 5 monitoring peptides

Detection of isoform-specific APO E peptides in clinical samples.

Three isoform-specific APO E peptides were detected in the clinical sample cohort (Table 3), although they were at a very low frequency (5-15% in controls only) in our sample set. However, peptide LSG4MEDVR, present in APO E3, was found at approximately 1.4 X higher frequency in the AD samples.

TABLE 3. MSIA-SRM detection of isoform-specific APO E peptides in AD and control clinical samples.

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Metabolite</th>
<th>Peptide sequence</th>
<th>Frequency of detection (A4) in normal samples</th>
<th>Frequency of detection (A4) in AD samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>APO E3</td>
<td>LSG4MEDVR</td>
<td></td>
<td>0.1ug/mL</td>
<td>0.3ug/mL</td>
</tr>
<tr>
<td>APO E4</td>
<td>PPO4MEDVR</td>
<td></td>
<td>0.01ug/mL</td>
<td>0.05ug/mL</td>
</tr>
<tr>
<td>APO E5</td>
<td>APO E5</td>
<td></td>
<td>0.001ug/mL</td>
<td>0.005ug/mL</td>
</tr>
</tbody>
</table>

Conclusions

- We have developed novel, multiplexed, high-throughput MSIA-SRM assays for 3 AD associated proteins.
- The assays demonstrate excellent linearity, sensitivity and precision for Aβ1-38, 1-40 and 1-42, and CP and APO E in plasma.
- We tested the assays with a small clinical cohort and demonstrated differential abundance of Aβ1-42 and APO E in AD vs control, consistent with previously published data and the Amyloid Cascade Hypothesis [1].
- We demonstrated detection of APO E specific isoforms in blood plasma using MS.
- One of the APO E isoforms, specific for E3,4 but not E2 displayed higher frequency in AD vs control samples. This observation is consistent with previously published findings [3].
- Despite previously published results [5], we did not observe differential expression/ abundance of ceruloplasmin in our cohort.

REFERENCES