A BLOOD-BASED GENE EXPRESSION TEST FOR ALZHEIMER’S DISEASE IDENTIFIES LIKELIHOOD OF PROGRESSION IN MCI PATIENTS

Anders Lönnemann1, Peter Wetterberg2, Solve Sæbø1, Torbjörn Lindahl3, Magdalena Kauczynska1, Phil D. Rey4, Marianne Jensen1, Ken Bärdén1, Lena Kristensen1, Birgitte Boije5, Praveen Sharma1

1DiAGenic ASA, Grenevæk 92, NO-6663 Oslo, Norway; 2Department of Geriatrics, University Hospital, Oslo, Norway; 3Dept. of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 3054, NO-1432 Ås, Norway

Introduction

Although there is currently a lack of treatment options to arrest the most devastating symptoms of Alzheimer’s disease (AD), early diagnosis and active management strategies can potentially delay or slow their development. The Mini-Mental State Examination (MSE) is characterized by minor but worsening memory changes. A significant percentage of MCI subjects (8-15% per year) progress to dementia, but there is no reliable test to indicate which of these patients are most susceptible. The DiAGenic blood-based gene expression assay has previously demonstrated its value in identifying AD from non-AD patients (see adjoining poster for supplemental information) and distinguishing AD from other neurological disorders.

Several independent studies have recently indicated that a peripheral blood-based test could be used to detect neurodegenerative diseases [1-6]. Indeed, we have more recently shown that a blood-based gene signature can accurately identify AD patients (see Figure 3) using the in house blood assay UV-7000IQ (platform using a 96-assay format on TriageTrack Dementia Array (OTA) cards [4-5]). Moreover, AD patients could be distinguished from MCI and controls using other peripheral blood-based assays such as Pathology Detection. Prediction accuracies using independent patient cohorts on different technology platforms ranged between 81-87% and was within the acceptable limits recommended for clinical diagnostic tests [6-12] (but, however, it was not clear if this same signature could be used to identify differences in clinically graded AD samples. Therefore, the aim of this study was to determine if any of the gene signatures used in the development of the prototype could correlate with AD severity and whether these MCI patients with an elevated risk of converting to AD could be predicted.

Results and Discussion

The data presented show the lack of a definite trend of AD classification with AD grade from 1-4 indicating the complexity in predicting AD-grade solely from the currently available gene expression data (Figure 2). The differences between grade 1, 2 and 3 are subtle, with trained predictions tending to classify samples as grade 2 which is the dominating class in the data set.

Materials and Methods

Patient samples

Whole blood was collected prior to diagnosis from 251 individuals in PAXGene™ blood RNA tubes from memory clinic in Norway. These included 125 patients subsequently diagnosed AD (based on the ICD-10 criteria for dementia syndrome), 88 age-matched healthy controls and 28 young healthy controls (see Table 1). In addition 10 MCI patients were included in the study.

Sample preparation

An overview of the sample preparation and processing is shown in Figure 1. Total RNA was extracted from blood samples using PAXGene™ Blood RNA kit according to manufacturers instructions and quality assessed by Nanodrop spectrophotometer and Agilent 2100 Bioanalyzer. cDNA was prepared using the high-capacity cDNA archive kit from Applied Biosystems.

Sample Collection

Total RNA Extraction

RNA Quality Check

Label synthesis

Hybridization

MG-Array

Model Building

Screening of Biomarkers 1229 informative probes

Raw data

Quality Filtered

Informative probes

Cross validation set

Validating set

AB 1700 Overexpression analysis

Prediction results from the AB1700 Human whole genome array [7]. (95% Confidence interval).

Figure 1. Overview of ABI 1700 whole genome survey microarray

Table 1. Demographic information of patient and control samples [7].

<table>
<thead>
<tr>
<th>Samples</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s disease (N=125)</td>
<td>77.3</td>
<td>7.9</td>
<td>21.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Age (≥75 years)</td>
<td>7.6</td>
<td>7.8</td>
<td>23.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Normal controls (N=20)</td>
<td>23.3</td>
<td>2.7</td>
<td>67%</td>
<td>33%</td>
</tr>
<tr>
<td>Mild-Cognitive Impairment (N=10)</td>
<td>71.1</td>
<td>8.2</td>
<td>22</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Table 2. 4D grading structure.

<table>
<thead>
<tr>
<th>4D grade</th>
<th>Healthy controls</th>
<th>Very mild AD</th>
<th>Moderate AD</th>
<th>Severe AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>N=100</td>
<td>N=30</td>
<td>N=15</td>
<td>N=10</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mild AD</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Moderate AD</td>
<td>15</td>
<td>10</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Severe AD</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

In retrospective grading the patients, the following factors were considered: MMSE score, clock drawing test score and frontal object manipulation test score. For each patient the AD grade was assigned according to the Clinical Dementia Rating Scale criteria [10]. Mild AD was primarily used to determine the most appropriate AD grade (Table 2) for MCI classification was based on the same range of tests, as in non-demented individuals that were tested with mild memory complaints but without the loss of functions characterized as associated with clinical AD.

Gene selection and expression analysis

The expression analysis was done on the AB 1700 System, which contained an AD-specific gene signature in a custom format. The genes were selected based on the performance characteristics from previous studies using an oligo-array platform [11]. A total of 936 genes probes remained after data normalization. Some subsets of variables were selected previously for prototype development. Although 4P-genotyping was not used in the selection process (only AD/Healthy status). Subsequent testing of the following variables (probe-set) was done with respect to the assigned AD-grading. Adjacent samples (AD: 404, non-AD: 384 etc. and 82-set).

AD-grading was used in multiple model building. Cross-validation using Soft Threshold-PLS (12) regression on AD grades was conducted to see whether these grades (as continuous measures) were predictable from gene expression data.

Table 4. MCI prediction data.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Prediction Value</th>
<th>Prediction result</th>
<th>ACU (95% CI)</th>
<th>FS (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.73</td>
<td>Vascular dementia</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>MCI</td>
<td>0.14</td>
<td>Severe Dementia</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>AD</td>
<td>0.03</td>
<td>AD</td>
<td>0.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

In Figure 3 and Table 4 the prediction of individuals in the MCI group appear to support the possibility that we can identify a subset with elevated risk of progression to AD. After an average follow-up time of 21 months, 5/10 of these subjects have been correctly predicted (highlighted in green). Subject 242 was technically defined as mild AD at follow-up but still had a substantially lower MMSE score and showed a slower progression from the original diagnosis. None of the additional clinical data available in the sample size was applicable. This subject was probably misdiagnosed as MCI at the time of inclusion in this study.

The at the time of original diagnosis, two of these subjects (141, 242) were correctly predicted as non-AD, using the gene expression assay and these later progressed to non-AD dementia (vascular and senile).

A further two subjects were still identified as MCI after 12 months, but we are currently awaiting more recent follow-up data. From the MCI group only 2/10 subjects were incorrectly predicted (highlighted in red) and was within the acceptable limits recommended for molecular biomarkers in AD [11]. However, it was noted that cognitive status was a complicating factor. Nevertheless, the prediction summary has been defined as uncertain.

Conclusions

The gene expression signature appears to detect AD grades 1-4 with a similar level of accuracy independent of disease severity.

A linear increasing trend from the healthy controls to the MCI group to AD grade 1 suggests some predictive value.

Absence of a clear trend with increasing AD grade 1-4 may reflect the biological nature of the disease progression.

Individuals within the MCI group may be associated with a tendency for conversion to AD grade 1.

References


