DEVELOPMENT OF A BLOOD BASED GENE EXPRESSION TEST FOR THE EARLY DETECTION OF ALZHEIMER’S DISEASE

Anders Lönneborg1, Birgitte Booj1, Marianne Jensen1, Ken Bårdsen1, Lena Kristiansen1, Torbjørn Lindahl1, Magdalena Kauczynska1, Phil D. Rye1, Guri Feten1, Praveen Sharma1

1DiaGenic ASA, Grenseveien 92, NO-0663 Oslo, Norway; 2Dept. of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O.Box 5003, NO-1432 Ås, Norway

Introduction

The Disease

It has been reported that there are currently 5.1 million Americans diagnosed with Alzheimer’s Disease (AD). It is the most common form (20-60%) of all dementia types and is the seventh leading cause of death in all ages in the USA. It is estimated that there are currently 24.3 million people worldwide with dementia today [1], but this prediction is projected to double by 2025 because of population demographics.

- Every 72 seconds someone in America develops Alzheimer’s
- Over 24 million people worldwide with dementia today
- Worldwide estimates of one new case of dementia every 7 seconds

The socio-economic costs are huge with the average lifetime cost of care for an individual with Alzheimer’s estimated to be $174,000. Moreover, this does not include the additional costs to business for employees who are caregivers.

The Technology

Several independent studies have recently indicated that a peripheral blood based test could be used for diagnostic profiling in neurological diseases [2-6]. Indeed, our own previous studies based on a 1536 gene macroarray derived from normal mononuclear cells in peripheral blood have shown that AD patients could be identified with a blood-based gene expression test [7].

This approach has recently been developed further using a whole genome array in a larger cohort of 330 individual samples and showed that a test set validated signature gave a high degree of specificity (91%), sensitivity (84%) and accuracy (87%). Moreover, AD patients could be accurately distinguished from other neurodegenerative diseases such as Parkinson’s Disease [8].

Materials and Methods

Patient samples

Whole blood was collected from 91 individuals in PAXgene® Blood RNA tubes at 4 different hospitals and institutions in Norway. These included 46 patients diagnosed with AD (based on the ICD-10 criteria for dementia syndrome) and 45 age-matched controls, see Table 1.

Table 1. Demographic information of patient and control samples.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Age (years)</th>
<th>MMSE score</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s disease</td>
<td>Mean</td>
<td>SD</td>
<td>Min</td>
</tr>
<tr>
<td>N=46</td>
<td>73.0</td>
<td>5.4</td>
<td>59</td>
</tr>
<tr>
<td>Age matched controls</td>
<td>69.3</td>
<td>6.2</td>
<td>52</td>
</tr>
</tbody>
</table>

Sample preparation

Total RNA was extracted from blood samples using PAXgene® Blood RNA kit and quality assessed by NanoDrop spectrophotometer and Agilent 2100 Bioanalyzer. cDNA was prepared using the high-capacity cDNA archive kit from Applied Biosystems.

Gene sets and expression analysis

The gene expression analysis was done on the ABI Prism 7900HT Fast System using TaqMan® LDA with both 96 gene assay format, which contained an AD-specific gene signature in a custom format, such that 4 individual samples could be run in parallel on each 384 LDA card (Figure 1). The genes were selected based on the performance characteristics from previous studies using an Applied Biosystems Whole Genome Array [8] and TaqMan® LDA [9]. In addition to the pre-selected AD-specific gene signature a range of other genes were included as technical controls.

Data processing

Data processing was performed as outlined below in Figure 3.

Results and Discussion

The prediction accuracy of the developed signature is shown in Table 2. The gene expression signature in the 48-assay format correctly predicted the class of 68/91 samples (accuracy 75%), including 35/46 AD samples (sensitivity 76%) and 33/45 Non-Alzheimer controls (specificity 73%). This is comparable to the results previously achieved using the same gene assays (Table 2) suggesting that the expression of the selected genes are highly informative for the early classification of AD.

Table 2. Prediction results from the ABI7000 Human whole genome array and the current TaqMan® LDA. LR+: positive likelihood ratio, (95%) 95% confidence interval. The 96 and 48 assay formats include both the gene signature and reference control genes.

<table>
<thead>
<tr>
<th>Assay/Array Format</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>LR+</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABI7000 Whole Genome Array [8]</td>
<td>85%</td>
<td>88%</td>
<td>87% (75%)</td>
<td>7.3</td>
</tr>
<tr>
<td>TaqMan® LDA 96-assy [7]</td>
<td>74%</td>
<td>86%</td>
<td>81% (71%)</td>
<td>5.3</td>
</tr>
<tr>
<td>TaqMan® LDA 48-assy</td>
<td>76%</td>
<td>73%</td>
<td>75% (9%)</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Conclusions

- An Alzheimer’s specific gene expression signature has been developed that has diagnostic value in a clinical setting.
- The diagnostic gene signature can be reduced to fit a 48-assay format without loss in sensitivity and only a slight reduction in overall accuracy.
- Using a 48-assay format size means that 8 samples can be run on each LDA. This in turn will significantly reduce the overall operating costs of the AD assay.
- Our peripheral blood test represents a robust method for accurate detection of AD.
- Studies are currently ongoing with patients exhibiting mild cognitive impairment (MCI) to determine if the same signature can be applied to pre-symptomatic AD

References

7. More debilitating symptoms. Early and accurate detection of AD is therefore critical to improving the quality of life of the patient and caregivers.
8. The prediction results reported here are also comparable to those previously presented using ABI7000 Human Whole Genome Microarrays. The comparative ROC and AUC obtained in these studies is also presented in Figure 4. The AUC in these studies ranged between 0.82 to 0.91 thus demonstrating improved diagnostic value compared to existing clinical diagnostic practice.

Figure 1. Projected number of people with dementia in developing and developed countries

Figure 2. Outline of the prototype test

Figure 3 Schematic representation of data processing.

Figure 4 ROC curves for AB1700 WGA and TaqMan® LDA with both 96- and 48-assay formats.

A more extensive validation of this peripheral blood test, using a smaller number of genes, has now been performed on the same technical platform. This new data, from an independent cohort of 91 patients, confirms our previous studies indicating that our unique gene expression signature on an LDA platform represents a novel and promising diagnostic approach for the early detection of AD.