Introduction

Alzheimer’s Disease (AD), the most common form of dementia, is the seventh leading cause of death in all ages in the USA. A recent report by the ADI [1] highlighted a disturbing trend for dementia in the Asia Pacific Region:

- The number of new cases of dementia is projected to increase from 4.3 million new cases per year in 2015 to 9.7 million new cases by 2050.
- The number of people with dementia in the Asia Pacific Region will rise from about 14 million today to 65 million by 2050.
- Those aged over 60 in the Asia Pacific Region will increase from 10% today to 25% of the total population by 2050.
- In Japan alone the incidence is expected to double from 570,200 in 2005 to 1,417,700 by 2050.

Although there is currently a lack of treatment options to arrest the disease, early diagnosis and active management strategies can temporarily delay the onset of the debilitating symptoms. Early and accurate detection of AD is therefore critical to improving the quality of life of the patient and caregivers.

Current diagnosis of AD involves detailed clinical interviews, cognitive tests and imaging techniques (e.g., MRI, CT, PET, SPECT). However, despite this variety of testing approaches it is still difficult to make an accurate diagnosis at an early stage of the disease.

Several independent studies have recently indicated that a peripheral blood based test could be used for diagnostic profiling in neurological diseases [2-4]. 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 [5].

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 sensitivity (83%), specificity (97%) and accuracy (92%). Moreover, AD patients could be accurately distinguished from other neurodegenerative diseases such as Parkinson’s Disease [8].

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 330 individual samples and showed that a test set validated signature gave a high sensitivity (76%), specificity (73%) and accuracy (75%).

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%).

The prediction results presented here are also comparable to those previously presented using AB1700 Human Whole Genome Microarrays. The comparative ROC and AUC obtained in these studies is presented in Figure 2. The AUC in these studies ranged between 0.82 to 0.91 suggesting that the expression of the selected genes are highly informative for the early classification of AD.

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.

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), 45 age-matched controls, see Table 1.

Sample preparation

Total RNA was extracted from blood samples using PAXgene™ Blood RNA kit and quality assessed by NanoDrop spectrophotometer and Agilent 2100 Bioanalyzer. One sample did not pass the quality criteria. cDNA was prepared using the high-capacity cDNA archive kit from Applied Biosystems. The RNA extraction was slightly modified compared to our previous studies with the extraction done from individual PAXGene™ tubes without pooling.

Gene sets and expression analysis

The gene expression analysis was done on the ABI Prism 7900HT Fast System using TaqMan® LDA with a 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.

Figure 1. TaqMan® LDA customized arrays

<table>
<thead>
<tr>
<th>Study / Assay Format</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>LR+</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB1700 Whole Genome Array [8]</td>
<td>85%</td>
<td>88%</td>
<td>87% (5%)</td>
<td>7.3</td>
</tr>
<tr>
<td>TaqMan® LDA 96-assay [7]</td>
<td>74%</td>
<td>86%</td>
<td>81% (5%)</td>
<td>5.3</td>
</tr>
<tr>
<td>TaqMan® LDA 48-assay</td>
<td>66%</td>
<td>73%</td>
<td>75% (8%)</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Table 1. Demographic information of patient and control samples.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>M/F</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>50%</td>
<td>50%</td>
<td></td>
<td>90%</td>
</tr>
<tr>
<td>Controls</td>
<td>50%</td>
<td>50%</td>
<td></td>
<td>90%</td>
</tr>
</tbody>
</table>

References


All authors from Diagenic have competing interests. Authors from other institution have no competing interest.