Detection of Alzheimer’s disease based on gene expression patterns in peripheral blood cells.

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Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder affecting about 7% of the population over 67 years. A recent study estimated the direct-care cost for the 28 million sufferers from Alzheimer’s related dementia worldwide, to be staggering 156 billion $ annually.

AD is characterized by a progressive loss of intellectual function and accumulation of neuritic plaques, neurofibrillary tangles and neurochemical alterations. Before a diagnosis for AD is accepted, the clinicians have to rule out the presence of other forms of dementia. An accurate, convenient and objective method to detect AD is thus highly desired.

A vast amount of literature is already available describing the potential use of large-scale gene expression analysis in disease diagnosis. Also, the potential use of blood-based gene expression profiling for diagnosis of brain disorders has been contemplated and described [1-3, 6]. The rationale for using blood cells as monitors for brain disorders is based on the hypothesis that there is a bidirectional communication between the immune and nervous system. Characteristic changes in nervous system will cause characteristic changes in the biochemical environment of blood affecting also the expression pattern of certain genes in blood cells.

In this pilot study, we have analyzed gene expression patterns in peripheral blood cells of patients diagnosed with AD and age-matched controls. We have identified a panel of genes that correctly predicted the diagnostic class in 34/37 samples. Our findings show that a blood-based gene expression test can potentially be developed to detect AD. Additional studies with a large sample size are warranted to confirm this finding.

Material and Methods

Clinical samples and subjects

10 ml blood was obtained from 19 patients diagnosed with AD and 24 age-matched controls with their informed consent under an approval from the Regional Ethical Committee of Norway (573-04/074). The blood was drawn by skilled personnel in vacuum tubes containing EDTA (Becton Dickinson, Baltimore, USA) and immediately stored at - 80°C.

The mean age of patients with AD was 72.3 (age range, 69-76) and the mean MMSE score was 22.0 (the maximum score attainable being 30). All individuals belonging to the control group were tested with MMSE and had a minimum score of 28 (mean 28.4). The mean age of control group was 73.0 (age range, 66-81).

AD was diagnosed according to NINCDS-ADRDA criteria based on the following assessment: standardized interview with a care-giver using ICQODI, an ADL scale and a scale measuring behavior of the patient (Green scale); neuropsychological evaluation using MMSE, Clock drawing test, Trailmaking test A and B (TMT A and B), Kendrick object learning test (visual), TMT A and B, Kendrick object learning test (visual), a psychiatric evaluation using scales for detection of dementia (MMSE), part of the Wechsler battery and Benton test; A psychiatric evaluation using scales for detection of dementia (MMSE), part of the Wechsler battery and Benton (TMT A and B), Kendrick object learning test (visual).

Expression profiling using macroarrays and statistical analysis

The experimental methodology employed has recently been described in detail [4]. However, in the present work, only 663 DNA clones were randomly picked, amplified and spotted in duplicates onto Hybond-N+ membranes. mRNA isolation, probe synthesis, hybridization and pre-processing of the expression data were performed as described. The data were analyzed using the nearest shrunken centroid method [5] and results validated using a leave-one-out cross-validation (LOOCV) statistical method.

To determine the optimal shrinkage threshold a standard leave-one-out cross-validation approach was used. Since, we had 43 unique blood samples, expression data of 94 experimental samples were divided into 43 non-overlapping subsets, where each subset represented one unique blood-sample and included all the corresponding technical replicates. The model was trained 42 times on one of the subsets (unique blood-sample) from the training data, but using only the omitted subset to compute the prediction error. The error obtained on all parts were added together and used to compute the overall misclassification error. As shown in figure 1, the minimum overall misclassification error was observed at a threshold value of 2.46, yielding a subset of 33 genes.

Table 1. Experimental detail

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number</th>
<th>Non Decision</th>
<th>Correctly predicted</th>
<th>Incorrectly predicted</th>
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<tbody>
<tr>
<td>Alzheimer</td>
<td>19</td>
<td>3</td>
<td>15</td>
<td>2</td>
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<td>Non-Alzheimer</td>
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Table 2. Prediction results. When there was no majority for either the Alzheimer or non-Alzheimer class the prediction was regarded as non-decisions.

The relative expression of 12/33 genes identified as good predictors is shown in (2). The identity of these genes is being determined.

Results and Discussion

We analyzed gene expression patterns in the peripheral blood cells (PBC) of 19 patients diagnosed with Alzheimer and 24 age-matched controls. Expression data was generated from a total of 94 experimental samples in five different experimental batches. To investigate the reproducibility of results, 18 samples from Alzheimer’s patient and 24 control were analyzed at least twice in different batches using aliquots from the same mRNA pool (Table 1).

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Figure 2. Relative expression of 12/33 genes with highest shrunken T statistic scores. Red circles represent samples from controls and green circles represent samples from Alzheimer patients. The number in the upper axis represents the position ID of predictive genes in the array.

Conclusion

- AD affects gene expression patterns in PBC.
- A blood-based gene expression test can potentially be developed for AD.
- Study in progress: We are now conducting a large scale study where gene expression patterns in PBC of 400 samples will be analysed using ABI microarray platform.

References


Competing interests

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