A Blood Gene Expression Marker of Early Alzheimer’s Disease


1 King’s College London, Institute of Psychiatry, King’s Health Partners Centre for Neurodegeneration, Research, London, UK
2 NIHR Biomedical Research Centre for Mental Health at South London and Maudsley NHS Foundation Trust and Institute of Psychiatry, King’s College London, London, UK
3 Program in Neurogenetics, Department of Neurology, David Geffen School of Medicine, University of California at Los Angeles, CA, USA
4 Department of Neurology, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland
5 Medical University of Lodz, Lodz, Poland
6 Institute of Gerontology and Geriatrics, University of Perugia, Perugia, Italy
7 3rd Department of Neurology, “G. Papanicolaou” Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece
8 INSERM U 558, University of Toulouse, Toulouse, France

Accepted 21 August 2012

Abstract. A marker of Alzheimer’s disease (AD) that can accurately diagnose disease at the earliest stage would significantly support efforts to develop treatments for early intervention. We have sought to determine the sensitivity and specificity of peripheral blood gene expression as a diagnostic marker of AD using data generated on HT-12v3 BeadChips. We first developed an AD diagnostic classifier in a training cohort of 78 AD and 78 control blood samples and then tested its performance in a validation group of 26 AD and 26 control and 118 mild cognitive impairment (MCI) subjects who were likely to have an AD-endpoint. A 48 gene classifier achieved an accuracy of 75% in the AD and control validation group. Comparisons were made with a classifier developed using structural MRI measures, where both measures were available in the same individuals. In AD and control subjects, the gene expression classifier achieved an accuracy of 70% compared to 85% using MRI. Bootstrapping validation produced expression and MRI classifiers with mean accuracies of 76 and 82%, respectively, demonstrating better concordance between these two classifiers than achieved in a single validation population. We conclude there is potential for blood expression to be a marker for AD. The classifier also predicts a large number of people with MCI, who are likely to develop AD, are more AD-like than normal with 76% of subjects classified as AD rather than control. Many of these people do not have

1 These authors contributed equally.
2 Present address: The Institute of Cancer Research, London, UK.
3 Correspondence to: Angela Hodges, King’s College London, Institute of Psychiatry, De Crespigny Park, London, UK. Tel.: +44 207 848 0772; Fax: +44 207 848 0632; E-mail: angela.k.hodges@kcl.ac.uk.
INTRODUCTION

Alzheimer’s disease (AD) is a common chronic neurodegenerative disorder, accounting for ~60% of dementia cases. Dementia affects 63 million patients worldwide with numbers set to rise to 114 million by 2050 resulting in dramatic social and economic consequences as our care systems struggle to cope [1]. Although much progress has been made in understanding the molecular pathology of AD, the treatments currently available only temporarily alleviate some symptoms and do not modify pathology. The use of biomarkers for identification of individuals with AD prior to the appearance of clinical symptoms, the so-called pre-dementia phase of the disease [2], will be essential to the development of drugs for early intervention [3–5]. Furthermore, if sufficiently powered, some biomarkers could be used as part of a screening program for at-risk elderly people [6].

By the time an individual is diagnosed with AD, there is already considerable neuronal cell loss, plaque deposition, and neurofibrillary tangles within the brain [7, 8], which may have emerged up to 10 years or more before clinical diagnosis [9–41]. Biomarkers linked to the pathophysiological process in AD can greatly increase the confidence of concluding a person will have an AD-endpoint even in the pre-dementia phase [12]. Cerebrospinal fluid (CSF) biomarkers such as increased tau and decreased levels of amyloid-β (Aβ)₄₂ in the CSF correlate with postmortem AD pathology [13, 14]. These measurements together not only differentiate AD from normal elderly controls with high accuracy, but also predict which subjects with mild cognitive impairment (MCI) are likely to progress to AD within 5 years [15]. However, a lumbar puncture to collect CSF is an invasive procedure, which may not be suitable for use in large-scale trials or for screening populations. Similarly, positron emission tomography (PET) imaging of amyloid burden in the brain correlates with clinical diagnosis of AD, Aβ neuropathology at autopsy [13, 14, 16], and CSF Aβ₁₋₄₂ levels [17–20]. PET imaging is expensive and impractical to be used in large groups of frail elderly patients and may be restricted to specialist centers.

Although AD is a disease of the brain, it is increasingly accepted that there is communication between the brain and the periphery, and we therefore hypothesize that there will be blood-associated changes detectable in disease which could be used to develop a diagnostic marker. Blood is easily obtainable in frail elderly people and relatively inexpensive to analyze, making it an attractive source for developing a biomarker [21]. Many studies, including those from our own group, have identified AD- and/or MCI-related protein changes in blood plasma using 2DE/Mass Spectrometry and luminex technology [22–30], and are undergoing further rounds of testing to establish their validity as the basis for an accurate marker. Furthermore, companies specializing in AD diagnostics have recently published reports of gene expression changes in blood that are able to distinguish AD subjects from cognitively normal people. Diagenic ASA published a discovery and validation study consisting of a 96 gene classifier with 72% accuracy for AD diagnosis. The marker could also correctly predict the outcome of 7 out of 10 MCI subjects after a 2 year follow-up. Although these numbers are too small to draw firm conclusions about its performance in identifying pre-dementia in MCI subjects, these results are encouraging [31, 32]. ExmonHi Therapeutics used the additional splice variant discriminatory power of Genome-Wide Splice Arrays to identify a blood expression classifier consisting of 133 genes able to distinguish AD from normal elderly control subjects with 98% accuracy [33]. Combining blood measures across different modalities such as proteins, metabolites, and gene expression may further improve biomarker accuracy, although the outcomes of these studies have yet to be reported.

Our aim was to first identify and validate an AD diagnostic gene expression marker in blood able to distinguish people clinically diagnosed with AD from normal elderly controls with high sensitivity and specificity. We then sought to evaluate its specificity...
and sensitivity compared to another marker which incorporates structural magnetic resonance imaging (MRI) measures of regional brain atrophy and cortical thickness assessed in the same individuals, using a hypothesis-free approach. To date, the outcome of comparing a gene expression marker of AD alongside a classifier using measures of brain atrophy has not yet been reported. Classifiers combining measures of cortical thickness and/or regional brain atrophy changes in AD have previously been shown to very accurately distinguish AD from normal elderly controls [34]. Structural brain changes can also be used to identify people with MCI who will subsequently receive a diagnosis of AD or experience a more rapid drop in Mini-Mental Status Examination (MMSE) score over time [35, 36]. Structural brain changes generally appear after changes in other modalities such as CSF Aβ and tau and coincide with neuronal cell loss and the onset of clinical symptoms [11, 37]. Individuals with MCI are likely to have an AD-endpoint and therefore are more likely to be classified as AD-like rather than normal using an appropriate biomarker. The proportion of people with MCI having prodromal AD varies between studies as it depends on the method of ascertainment, the criteria for identifying people with MCI, the period of follow-up, and the confirmatory methods for subsequent AD diagnosis. Typical estimates suggest about 40–75% of people who have cognitive symptoms consistent with MCI will eventually progress to pathologically confirmed AD [38–40], with only ∼5% “reverting” to normal, [41, 42] and ∼30–55% developing other dementias [43–45]. We were therefore interested to investigate whether a set of blood gene expression changes found in AD patients also exist in a MCI population and whether or not there was any distinction between MCI subjects close to receiving a clinical diagnosis of AD and those who remained MCI within the subsequent two year follow-up period. While the AD diagnostic classifier was adequately powered, we did not have sufficient power to build a separate predictive classifier within our MCI population to predict which subjects would develop AD within a two year period.

METHODS AND MATERIALS

Clinical subjects and samples

Subjects used were from AddNeuroMed, a large cross-European prospective biomarker study (2005–2007) [46, 47]. Informed consent was obtained for all subjects according to the Declaration of Helsinki (1991) and protocols and procedures were approved by the relevant local ethical committees at each site. Subjects were excluded from the study if they were younger than 65 years, had significant neurological or psychiatric illness other than AD, significant unstable systemic illness or organ failure, or a geriatric depression rating scale score ≥45 [48].

Normal elderly control subjects were recruited from non-related family members of AD patients, caregivers’ relatives, social centers for the elderly, or GP surgeries and had no evidence of cognitive impairment. A small number of individuals recruited in this way met the MCI criteria detailed below and were therefore included in the MCI cohort for analyses. AD and MCI subjects were recruited primarily from local memory clinics, and as such the MCI cohort was expected to be composed largely of subjects with a likely AD-endpoint. AD subjects were diagnosed using the National Institute of Neurological and Communicative Disease and Stroke and Alzheimer’s disease (NINCDS-ADRDA) [49] and Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [50] criteria for possible or probable AD. All MCI subjects reported problems with memory, corroborated by an informant, but had normal activities of daily living as specified in the Petersen’s criteria for amnestic MCI [51, 52]. MCI subjects scored 0.5 on the total Clinical Dementia Rating Scale or had a memory score of 0.5 or 1 [53].

Within 2 years of the baseline visit, some MCI subjects progressed to a clinical diagnosis of AD (MCIc), while others remained MCI (MCInc). Diagnoses were made by trained researchers following a previously validated protocol [54]. All subjects underwent a structured interview and a battery of neuropsychological assessments including the Mini Mental State Examination (MMSE) [55], Global Deterioration Scale (GDS) [56], and Clinical Dementia Rating Scale (CDR) [53] by trained researchers. Control and MCI subjects were further assessed using the CERAD battery [57]. Detailed information on subject recruitment and assessments can be found in other published studies [46, 47]. Where possible, whole blood samples were collected from each subject for DNA (APOE genotyping) and RNA analyses (gene expression). Consecutive subjects who met MRI inclusion criteria (no claustrophobia, no psychiatric illness other than AD, no prior or pending neurological or psychiatric illness other than AD, no significant organ failure, or geriatric depression rating scale score ≥45) [48].

All subjects underwent a structured interview and a battery of neuropsychological assessments including the Mini Mental State Examination (MMSE) [55], Global Deterioration Scale (GDS) [56], and Clinical Dementia Rating Scale (CDR) [53] by trained researchers. Control and MCI subjects were further assessed using the CERAD battery [57]. Detailed information on subject recruitment and assessments can be found in other published studies [46, 47]. Where possible, whole blood samples were collected from each subject for DNA (APOE genotyping) and RNA analyses (gene expression). Consecutive subjects who met MRI inclusion criteria (no claustrophobia, no psychiatric illness other than AD, no prior or pending neurological or psychiatric illness other than AD, no significant organ failure, or geriatric depression rating scale score ≥45) [48].

All subjects underwent a structured interview and a battery of neuropsychological assessments including the Mini Mental State Examination (MMSE) [55], Global Deterioration Scale (GDS) [56], and Clinical Dementia Rating Scale (CDR) [53] by trained researchers. Control and MCI subjects were further assessed using the CERAD battery [57]. Detailed information on subject recruitment and assessments can be found in other published studies [46, 47]. Where possible, whole blood samples were collected from each subject for DNA (APOE genotyping) and RNA analyses (gene expression). Consecutive subjects who met MRI inclusion criteria (no claustrophobia, no psychiatric illness other than AD, no prior or pending neurological or psychiatric illness other than AD, no significant organ failure, or geriatric depression rating scale score ≥45) [48].

All subjects underwent a structured interview and a battery of neuropsychological assessments including the Mini Mental State Examination (MMSE) [55], Global Deterioration Scale (GDS) [56], and Clinical Dementia Rating Scale (CDR) [53] by trained researchers. Control and MCI subjects were further assessed using the CERAD battery [57]. Detailed information on subject recruitment and assessments can be found in other published studies [46, 47]. Where possible, whole blood samples were collected from each subject for DNA (APOE genotyping) and RNA analyses (gene expression). Consecutive subjects who met MRI inclusion criteria (no claustrophobia, no psychiatric illness other than AD, no prior or pending neurological or psychiatric illness other than AD, no significant organ failure, or geriatric depression rating scale score ≥45) [48].
Whole blood RNA extraction

Venous blood (2.5 ml) was collected into a PAXgene™ Blood RNA Tube (BD) for RNA analysis from subjects who had fasted 2 hours prior to collection. The sample was frozen at −20°C overnight and then transferred to −80°C for long-term storage. Prior to RNA extraction, samples were thawed at room temperature overnight. RNA was extracted using the PAXgene™ Blood RNA Kit (Qiagen), according to the manufacturer’s protocol. The yield and quality of extracted RNA were assessed using the NanoDrop™ 1000 spectrophotometer (NanoDrop Technologies) and the Agilent 2100 Bioanalyzer (Agilent Technologies) respectively. Only samples with an RNA Integrity Number ≥ 7.0 were analyzed in this study.

Microarray analysis

Illumina Human HT-12 v3 Expression BeadChips (Illumina) were used to analyze whole genome transcript expression according to the protocol supplied by the manufacturer for 356 subjects (116 control, 127 MCI, and 113 AD). Samples from subjects who had also undergone an MRI scan or who had subsequently changed their diagnosis from MCI to AD within 2 years of the sample being analyzed were prioritized for inclusion. The remaining control, MCI, and AD subjects were randomly chosen from the cohort available at the time of analysis to provide roughly equal numbers across the three groups and to allow 356 samples to be analyzed in total, a number chosen for practical reasons. The beadchips contained 48,803 probes designed using data from RefSeq (Build 36.2, Rel 22) and the UniGene (Build 199) databases. Briefly, the TotalPrep RNA Amplification Kit (Ambion) was used to synthesize cDNA from 200 pg total RNA followed by amplification and biotinylation of cRNA and hybridization. Following hybridization, gene expression values were variance-stabilization transformed and quantile normalized using the R Bioconductor package limma [58]. A total of 30 chips were excluded from further analysis for a number of reasons including very low beadChip detection rate, disparity in XIST gene expression gender calling (Illumina probe ID: ILMN_1764573) with recorded gender and/or gender markers derived from SNP chip data from the same individuals or diagnostic re-classification at subsequent visits prior to final data analysis (other than MCI-AD). Thus a total of 326 subjects (104 AD, 118 MCI [77 MCInc, 41 MCIc], and 104 normal elderly control subjects) which were used for classifier development and validation. Probes that were not expressed in any samples were removed from further analysis, leaving 38,314 probes for classification purposes. Data were adjusted for the independent variables of age, gender, collection site, and RNA quality (RIN).

Neuroimaging

Highly automated bilateral regional cortical thickness measures from 34 areas and regional brain volume measures from 41 areas totaling 109 measures were obtained from subjects who underwent MRI. Detailed information about data acquisition, pre-processing, and quality control assessment have been described for this cohort in detail elsewhere [34, 36, 47, 59, 60]. Data from 91 AD, 92 MCI, and 90 control subjects were included in the analyses.

Diagnostic classifier development

Using the clinical diagnosis of AD as the diagnostic standard, we developed a classifier containing those measures of blood gene expression able to achieve the greatest sensitivity and specificity in a training cohort. We based our methods on those recommended by the MicroArray Quality Control II (MAQC-II) study [61]. Samples from AD and normal elderly control subjects were randomly divided into a 75% training cohort (78 AD, 78 Control; Table 1) and a 25% independent validation cohort (26 AD, 26 Control). Initially a t-test was performed in the training cohort to reduce the number of gene expression features, leaving a total of 203 probes with p-value < 0.01 (False Discovery rate (FDR)-corrected). These 203 probes were then used to optimize the parameters of a random forest model as the Random Jungle [62] implementation. These 203 probes were included in the analyses.

Diagnostic classifier development

Using the clinical diagnosis of AD as the diagnostic standard, we developed a classifier containing those measures of blood gene expression able to achieve the greatest sensitivity and specificity in a training cohort. We based our methods on those recommended by the MicroArray Quality Control II (MAQC-II) study [61]. Samples from AD and normal elderly control subjects were randomly divided into a 75% training cohort (78 AD, 78 Control; Table 1) and a 25% independent validation cohort (26 AD, 26 Control; Table 1). Initially a t-test was performed in the training cohort to reduce the number of gene expression features, leaving a total of 203 probes with p-value < 0.01 (False Discovery rate (FDR)-corrected). These 203 probes were then used to optimize the parameters of a random forest model as the Random Jungle [62] implementation, requiring in the optimization procedure, various combinations of the random forest parameters including mTry (number of randomly selected variables) and nTrees (number of trees) were used. In addition, we applied the Meng score as a measure of variable importance [63], backward elimination, and a conditional importance setting of 0.8, which was kept constant throughout the optimization procedure. The best training result was achieved by setting of nTrees = 750 and mTry = 15, with 50 probes included in the model. These settings and the 50 highest ranked probes were then further used into the final model. The final optimized random forest parameters, 50 highest ranked probes, and all samples in the training cohort were then used to build a final random forest model.
which was then tested with the independent validation cohort.

In order to allow us to directly compare the predictive power of gene expression with sMRI, we re-developed the expression classifier to include only those samples for which both imaging and gene expression data were available. AD and normal elderly control subjects were divided into a 75% training cohort (68 AD, 67 Control; Table 1) and a 25% independent validation group of 23 AD, 26 Control (Table 1), maintaining sample groups as before and removing those where only expression data were available in order to achieve a similar sample distribution across training and test cohorts. The reduced training cohort was then used to develop three additional diagnostic models; the first included only sMRI imaging measures, the second included sMRI and gene expression, and the third only gene expression in these samples. We found 48 genes, represented by 50 Illumina probes selected through backward elimination of 203 probes identified by t-tests (Supplementary Table 2; available online: http://www.j-alz.com/issues/33vol33-3.html#supplementarydata03), and achieved the highest “out of bag” accuracy in our training cohort. The 50 probes along with their Meng importance scores/33/vol33-3.html#supplementarydata03), and achieved the highest “out of bag” accuracy in our training cohort. The 50 probes along with their Meng importance scores were listed in Table 2. When this AD-Control classifier was applied to the independent validation group of samples, it was able to correctly classify 39/52 subjects (75.0% accuracy), of which 21/26 AD subjects were correctly classified (80.8% sensitivity) and 18/26 normal elderly control subjects were correctly excluded (69.2% specificity) (Table 3A), achieving a positive predictive value (PPV) of 72.4% and negative predictive value (NPV) of 75.0%.

### RESULTS

#### Performance of diagnostic classifier in distinguishing AD from normal elderly control subjects

We sought to identify and evaluate a blood gene expression diagnostic classifier of AD that could distinguish people with AD from normal elderly control subjects. We found 48 genes, represented by 50 Illumina probes selected through backward elimination of 203 probes identified by t-tests (Supplementary Table 2; available online: http://www.j-alz.com/issues/33vol33-3.html#supplementarydata03), and achieved the highest “out of bag” accuracy in our training cohort. The 50 probes along with their Meng score of relative importance within the classifier are listed in Table 2. When this AD-Control classifier was applied to the independent validation group of 23 AD, 26 Control subjects, it was able to correctly classify 21/26 AD subjects (80.8% sensitivity) and 18/26 normal elderly control subjects were correctly excluded (69.2% specificity) (Table 3A), achieving a positive predictive value (PPV) of 72.4% and negative predictive value (NPV) of 75.0%.

#### Table 1

Subject characteristics of the out of bag (training) and validation (test) cohorts used in the development of an AD-Control classifier.

<table>
<thead>
<tr>
<th>AD-Control classifier subject characteristics</th>
<th>Out of bag cohort (Training)</th>
<th>Validation cohort (Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>AD</td>
<td>Control</td>
</tr>
<tr>
<td>Samples used in analysis</td>
<td>76</td>
<td>76</td>
</tr>
<tr>
<td>No. with MRI measures</td>
<td>45/33</td>
<td>52/68</td>
</tr>
<tr>
<td>Age in years (±SD)</td>
<td>72.1 (6.2)</td>
<td>75.5 (6.9)</td>
</tr>
<tr>
<td>Duration in years (±SD)</td>
<td>N/A</td>
<td>3.7 (2.5)</td>
</tr>
<tr>
<td>MMSE (±SD)</td>
<td>29.0 (1.2)</td>
<td>21.1 (4.4)</td>
</tr>
<tr>
<td>ADAS-Cog (±SD)</td>
<td>N/A</td>
<td>23.6 (9.4)</td>
</tr>
<tr>
<td>CDR sum of boxes (±SD)</td>
<td>0.1 (0.2)</td>
<td>6.4 (3.0)</td>
</tr>
</tbody>
</table>

Summary of sample characteristics of the development and validation cohorts. The reduced training cohort was then used to develop three additional diagnostic models; the first included only sMRI imaging measures, the second included sMRI and gene expression, and the third only gene expression in these samples. We found 48 genes, represented by 50 Illumina probes selected through backward elimination of 203 probes identified by t-tests (Supplementary Table 2; available online: http://www.j-alz.com/issues/33vol33-3.html#supplementarydata03), and achieved the highest “out of bag” accuracy in our training cohort. The 50 probes along with their Meng score of relative importance within the classifier are listed in Table 2. When this AD-Control classifier was applied to the independent validation group of 23 AD, 26 Control subjects, it was able to correctly classify 21/26 AD subjects (80.8% sensitivity) and 18/26 normal elderly control subjects were correctly excluded (69.2% specificity) (Table 3A), achieving a positive predictive value (PPV) of 72.4% and negative predictive value (NPV) of 75.0%.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMSE</td>
<td>29.0 ± 1.2</td>
<td>21.1 ± 4.4</td>
</tr>
<tr>
<td>ADAS-Cog</td>
<td>N/A</td>
<td>23.6 ± 9.4</td>
</tr>
<tr>
<td>CDR Sum of Boxes</td>
<td>0.1 ± 0.2</td>
<td>6.4 ± 3.0</td>
</tr>
</tbody>
</table>

### Table 3A

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAS-Cog</td>
<td>N/A</td>
<td>3.7 ± 2.5</td>
</tr>
<tr>
<td>CDR Sum of Boxes</td>
<td>0.1 ± 0.2</td>
<td>6.4 ± 3.0</td>
</tr>
</tbody>
</table>

### Table 3B

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAS-Cog</td>
<td>N/A</td>
<td>3.7 ± 2.5</td>
</tr>
<tr>
<td>CDR Sum of Boxes</td>
<td>0.1 ± 0.2</td>
<td>6.4 ± 3.0</td>
</tr>
</tbody>
</table>

### Table 3C

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAS-Cog</td>
<td>N/A</td>
<td>3.7 ± 2.5</td>
</tr>
<tr>
<td>CDR Sum of Boxes</td>
<td>0.1 ± 0.2</td>
<td>6.4 ± 3.0</td>
</tr>
</tbody>
</table>

### Table 3D

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAS-Cog</td>
<td>N/A</td>
<td>3.7 ± 2.5</td>
</tr>
<tr>
<td>CDR Sum of Boxes</td>
<td>0.1 ± 0.2</td>
<td>6.4 ± 3.0</td>
</tr>
</tbody>
</table>

### Table 3E

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAS-Cog</td>
<td>N/A</td>
<td>3.7 ± 2.5</td>
</tr>
<tr>
<td>CDR Sum of Boxes</td>
<td>0.1 ± 0.2</td>
<td>6.4 ± 3.0</td>
</tr>
</tbody>
</table>

### Table 3F

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAS-Cog</td>
<td>N/A</td>
<td>3.7 ± 2.5</td>
</tr>
<tr>
<td>CDR Sum of Boxes</td>
<td>0.1 ± 0.2</td>
<td>6.4 ± 3.0</td>
</tr>
</tbody>
</table>
Fig. 1. Sample flow, model development, and model validation scheme for classification. Samples with a minimum RNA integrity number of 7 were selected from AddNeuroMed participants, with priority given to those who had undergone MRI scans and MCI subjects known to have converted from MCI to AD. The remaining control, MCI, and AD subjects were randomly chosen from available samples to provide approximately equal numbers across the three groups. When generating the AD-Control classifier, AD and Control samples were randomly categorized as training (out of bag) (75%) or validation (25%) from samples passing quality control. Samples from all MCI subjects were examined as part of the validation cohort to assess how they were categorized relative to AD and normal elderly control subjects. Each classifier was developed in the training cohort and then tested in the validation cohorts to generate a final probe (gene) or imaging measure list along with a measure of accuracy, sensitivity, and specificity. Finally, data underwent bootstrapping, whereby in each bootstrap iteration (total of 200) a new development and validation set was drawn from the complete data set used for each model. In each round of bootstrapping, the training set was used to build a classification model, which was then assessed with the validation set. This method gives a range of classifier accuracies, reflecting variation in the population, and allowing an additional assessment to the traditional independent test set approach.
Table 2
The 50 Illumina probes (48 genes) that best classify AD from control samples in the training cohort

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Illumina probe</th>
<th>Meng score*</th>
<th>q-valueb</th>
<th>Bootstrap countc</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPS27A</td>
<td>ILMN:2048326</td>
<td>1.67E-02</td>
<td>2.11E-05</td>
<td>200</td>
</tr>
<tr>
<td>CHMP4A</td>
<td>ILMN:015607</td>
<td>1.71E-02</td>
<td>4.01E-03</td>
<td>200</td>
</tr>
<tr>
<td>SFRS17A</td>
<td>ILMN:067737</td>
<td>1.66E-02</td>
<td>9.79E-03</td>
<td>198</td>
</tr>
<tr>
<td>POMP</td>
<td>ILMN:0693287</td>
<td>1.67E-02</td>
<td>3.62E-05</td>
<td>147</td>
</tr>
<tr>
<td>CSF4B1</td>
<td>ILMN:2345821</td>
<td>1.69E-02</td>
<td>9.49E-05</td>
<td>142</td>
</tr>
<tr>
<td>FTH1</td>
<td>ILMN:234016</td>
<td>1.65E-02</td>
<td>2.26E-05</td>
<td>147</td>
</tr>
<tr>
<td>LOC401206</td>
<td>ILMN:2752528</td>
<td>1.59E-02</td>
<td>3.62E-05</td>
<td>115</td>
</tr>
<tr>
<td>AK</td>
<td>ILMN:2716052</td>
<td>1.58E-02</td>
<td>2.34E-05</td>
<td>118</td>
</tr>
<tr>
<td>UQCRB</td>
<td>ILMN:759453</td>
<td>1.55E-02</td>
<td>3.64E-03</td>
<td>200</td>
</tr>
<tr>
<td>LOC635505</td>
<td>ILMN:776360</td>
<td>1.55E-02</td>
<td>4.93E-05</td>
<td>134</td>
</tr>
<tr>
<td>PSD1</td>
<td>ILMN:2075051</td>
<td>1.51E-02</td>
<td>2.34E-03</td>
<td>58</td>
</tr>
<tr>
<td>NRBP2</td>
<td>ILMN:233248</td>
<td>1.50E-02</td>
<td>1.85E-03</td>
<td>197</td>
</tr>
<tr>
<td>UTF14A</td>
<td>ILMN:20695820</td>
<td>1.47E-02</td>
<td>2.34E-05</td>
<td>157</td>
</tr>
<tr>
<td>Dicer1</td>
<td>ILMN:772629</td>
<td>1.46E-02</td>
<td>4.32E-05</td>
<td>180</td>
</tr>
<tr>
<td>RPL5 AL</td>
<td>ILMN:2189936</td>
<td>1.45E-02</td>
<td>3.62E-05</td>
<td>200</td>
</tr>
<tr>
<td>ATP5PR2</td>
<td>ILMN:2355892</td>
<td>1.42E-02</td>
<td>5.70E-03</td>
<td>145</td>
</tr>
<tr>
<td>SIEP1</td>
<td>ILMN:771801</td>
<td>1.39E-02</td>
<td>2.34E-03</td>
<td>200</td>
</tr>
<tr>
<td>LSM3</td>
<td>ILMN:2232942</td>
<td>1.36E-02</td>
<td>8.79E-03</td>
<td>200</td>
</tr>
<tr>
<td>APBD3</td>
<td>ILMN:2302153</td>
<td>1.33E-02</td>
<td>7.40E-04</td>
<td>177</td>
</tr>
<tr>
<td>MRPL54</td>
<td>ILMN:2074721</td>
<td>1.32E-02</td>
<td>2.34E-05</td>
<td>100</td>
</tr>
<tr>
<td>REL12</td>
<td>ILMN:663240</td>
<td>1.30E-02</td>
<td>5.38E-03</td>
<td>187</td>
</tr>
<tr>
<td>CETN2</td>
<td>ILMN:2355892</td>
<td>1.29E-02</td>
<td>2.34E-05</td>
<td>195</td>
</tr>
<tr>
<td>PRP1</td>
<td>ILMN:234099</td>
<td>1.18E-02</td>
<td>5.07E-03</td>
<td>81</td>
</tr>
<tr>
<td>KIAA0146</td>
<td>ILMN:2048326</td>
<td>1.15E-02</td>
<td>7.55E-04</td>
<td>100</td>
</tr>
<tr>
<td>LOC788720</td>
<td>ILMN:2074721</td>
<td>1.14E-02</td>
<td>2.12E-04</td>
<td>101</td>
</tr>
<tr>
<td>CEP9</td>
<td>ILMN:468096</td>
<td>1.11E-02</td>
<td>5.40E-03</td>
<td>191</td>
</tr>
<tr>
<td>LOC636261</td>
<td>ILMN:677262</td>
<td>1.11E-02</td>
<td>4.75E-03</td>
<td>139</td>
</tr>
<tr>
<td>SFRS17A</td>
<td>ILMN:2117136</td>
<td>1.10E-02</td>
<td>4.87E-04</td>
<td>182</td>
</tr>
<tr>
<td>PCBP1</td>
<td>ILMN:1673215</td>
<td>1.04E-02</td>
<td>4.32E-04</td>
<td>100</td>
</tr>
<tr>
<td>ATP5P1</td>
<td>ILMN:233062</td>
<td>1.03E-02</td>
<td>3.09E-05</td>
<td>65</td>
</tr>
<tr>
<td>HZAFY</td>
<td>ILMN:1673215</td>
<td>1.01E-02</td>
<td>6.08E-03</td>
<td>163</td>
</tr>
<tr>
<td>COX17</td>
<td>ILMN:218771</td>
<td>1.01E-02</td>
<td>2.15E-05</td>
<td>5</td>
</tr>
<tr>
<td>IAS</td>
<td>ILMN:273662</td>
<td>1.01E-02</td>
<td>2.34E-05</td>
<td>162</td>
</tr>
<tr>
<td>SHFM1</td>
<td>ILMN:232128</td>
<td>1.07E-02</td>
<td>2.80E-04</td>
<td>173</td>
</tr>
<tr>
<td>LOC755164</td>
<td>ILMN:2741217</td>
<td>1.01E-02</td>
<td>6.09E-03</td>
<td>53</td>
</tr>
<tr>
<td>STX16</td>
<td>ILMN:2741942</td>
<td>9.95E-03</td>
<td>1.91E-03</td>
<td>160</td>
</tr>
<tr>
<td>GDPD1</td>
<td>ILMN:2106265</td>
<td>9.93E-03</td>
<td>4.80E-03</td>
<td>179</td>
</tr>
<tr>
<td>CSFR1F66</td>
<td>ILMN:2384847</td>
<td>9.85E-03</td>
<td>5.31E-03</td>
<td>27</td>
</tr>
<tr>
<td>NG3</td>
<td>ILMN:235744</td>
<td>9.83E-03</td>
<td>5.42E-03</td>
<td>158</td>
</tr>
<tr>
<td>CACNA2D4</td>
<td>ILMN:2096342</td>
<td>9.61E-03</td>
<td>7.73E-03</td>
<td>47</td>
</tr>
<tr>
<td>PLEK</td>
<td>ILMN:279576</td>
<td>9.22E-03</td>
<td>2.84E-03</td>
<td>3</td>
</tr>
<tr>
<td>NOV1A1</td>
<td>ILMN:216198</td>
<td>9.04E-03</td>
<td>2.45E-06</td>
<td>198</td>
</tr>
<tr>
<td>CDKN1B</td>
<td>ILMN:272841</td>
<td>7.90E-03</td>
<td>9.35E-03</td>
<td>199</td>
</tr>
<tr>
<td>RGS19</td>
<td>ILMN:27753</td>
<td>7.62E-03</td>
<td>5.40E-03</td>
<td>165</td>
</tr>
<tr>
<td>RPS27A</td>
<td>ILMN:25554</td>
<td>7.61E-03</td>
<td>2.77E-04</td>
<td>180</td>
</tr>
<tr>
<td>LOC731604</td>
<td>ILMN:663174</td>
<td>7.41E-03</td>
<td>8.33E-03</td>
<td>66</td>
</tr>
<tr>
<td>AHS1A</td>
<td>ILMN:205363</td>
<td>6.98E-03</td>
<td>1.74E-03</td>
<td>58</td>
</tr>
<tr>
<td>BXDC1</td>
<td>ILMN:664187</td>
<td>6.43E-03</td>
<td>4.29E-04</td>
<td>200</td>
</tr>
<tr>
<td>KARS</td>
<td>ILMN:277364</td>
<td>5.54E-03</td>
<td>2.56E-03</td>
<td>5</td>
</tr>
<tr>
<td>UBE2D1</td>
<td>ILMN:214485</td>
<td>4.62E-04</td>
<td>1.74E-03</td>
<td>105</td>
</tr>
</tbody>
</table>

*aAn estimate of variable (probe) importance in the AD versus Control classifier Random Forest model. bq-value from a t-test comparing AD and control samples. cThe number of times each probe appeared in the 200 bootstrap iterations.

predictive value (NPV) of 78.3%. As APOEε4 dosage is a known risk factor for developing AD, we repeated the model creation step including the APOEε4 genotype in the list of attributes. During backward elimination, we found this attribute was removed in the early rounds of the iterative process and therefore excluded it from further analysis. As the accuracy of a classifier varies depending on the population sampled, we bootstrapped the sampled population 200 times to assess the accuracy of the 203 probes identified.
Table 3: Classifiers' performance in out of bag and validation data sets following Random Forests modeling, implemented in Random Jungle

(A) Performance of AD-Control classifier in AD and Control samples

<table>
<thead>
<tr>
<th>Classifier</th>
<th>Accuracy</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total correctly classified %</td>
<td>AD correctly classified %</td>
<td>Control correctly classified %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD-Control training cohort (expression)</td>
<td>128/156 (82.1)</td>
<td>86/78 (68.7)</td>
<td>60/78 (76.9)</td>
<td>79.7</td>
<td>85.5</td>
</tr>
<tr>
<td>AD-Control validation cohort (expression)</td>
<td>36/52 (75.0)</td>
<td>21/26 (80.8)</td>
<td>15/26 (73.1)</td>
<td>72.4</td>
<td>79.3</td>
</tr>
<tr>
<td>AD-Control validation cohort (expression + imaging)</td>
<td>36/52 (78.3)</td>
<td>21/26 (80.8)</td>
<td>15/26 (73.9)</td>
<td>76.0</td>
<td>81.0</td>
</tr>
<tr>
<td>AD-Control validation cohort (expression in imaging subset)</td>
<td>12/16 (75.0)</td>
<td>6/8 (75.0)</td>
<td>6/8 (75.0)</td>
<td>75.0</td>
<td>75.0</td>
</tr>
</tbody>
</table>

(B) Performance of AD-Control classifier in MCI samples

<table>
<thead>
<tr>
<th>Classifier</th>
<th>Accuracy</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total correctly classified %</td>
<td>AD correctly classified %</td>
<td>Control correctly classified %</td>
</tr>
<tr>
<td>MCI cohort (expression)</td>
<td>90/121 (74.6)</td>
<td>32/41 (78.0)</td>
<td>58/73 (79.5)</td>
</tr>
<tr>
<td>MCI cohort (expression + imaging)</td>
<td>59/91 (64.9)</td>
<td>15/26 (80.8)</td>
<td>44/73 (52.1)</td>
</tr>
<tr>
<td>MCI cohort (expression in imaging subset)</td>
<td>6/8 (75.0)</td>
<td>6/8 (75.0)</td>
<td>6/8 (75.0)</td>
</tr>
</tbody>
</table>

Mean 95% CI: Mean 95% CI: Mean 95% CI

For bootstrapped data, the mean value and 95% confidence interval (95% CI), measured over 200 models is shown.
Fig. 2. Bootstrap results for AD-Control classifier in classifying AD and Control samples. The graphs demonstrate the mean accuracy of the classifier measured from bootstrapping (black dotted line) and the accuracy measured using the "out of bag" classifier in the validation cohort (red hashed line). Results are shown for (A) expression classifier alone; (B) expression and imaging classifiers combined; (C) imaging classifier alone, and (D) expression classifier alone in the subset of individuals with sMRI measures.

Structural MRI is able to accurately detect subtle changes in the thickness or volume of particular brain areas that occur during AD pathogenesis and is believed to be associated with the onset of neuronal dysfunction and cell loss. We first investigated the power of sMRI to classify the AD and control subjects using 68 local cortical thickness and 41 brain volume MRI measures collected in a subset of our cohort around the time of blood sampling [34, 36, 59, 60] (Supplementary Table 1). As six patients used in our expression validation cohort did not have neuroimaging measures, we initially re-evaluated our 203 probes on this subset of 46 patients. Accuracy, sensitivity, specificity, PPV, and NPV were all 69.6%, indicating a small drop in performance in this subgroup. However, when bootstrapping, the mean accuracy achieved was 76.0% accuracy (95% CI, 75.1–76.9%), with a sensitivity of 76.4% (95% CI, 75.1–77.7%) and specificity of 75.4% (95% CI, 74.1–76.7%) (Fig. 2D; Table 3A), values similar to that observed in the larger expression cohort (Fig. 2A; Table 3A) and demonstrating that bootstrapping is important for assessing the true variation in accuracy within a population. The sMRI measures correctly classified 39 people out of 46 tested (84.8% accuracy), corresponding to 20 out of 23 AD subjects correctly included (87.0% sensitivity), and 19 out of 23 control subjects correctly excluded (82.6% specificity) (Table 3A). The mean accuracy from bootstrapping was lower (82.0%;
531 95% CI, 81.3–82.7%), due to fewer AD subjects being
532 correctly classified over the 200 models (80.0% mean
533 sensitivity; 95% CI, 78.9–81.1%), although specificity
534 was slightly improved (84.0% specificity; 95% CI,
535 82.9–85.1%) (Fig. 2C; Table 3A). The Kappa coeffi-
536 cient was 0.31 (p-value <0.05) demonstrating good
537 agreement between the imaging and expression classi-
538 fiers.

539 Combining the expression and imaging measures
540 resulted in a small increase in mean accuracy following
541 bootstrapping (84.0; 95% CI, 83.3–84.7%) (Fig. 2B;
542 Table 3A). This was due to a small increase in the num-
543 ber of AD subjects being correctly classified (84.4%)
544 mean sensitivity; 95% CI, 83.4–85.4%), than achieved
545 with imaging or expression alone.

546 Performance of diagnostic classifier in MCI
547 subjects

548 To evaluate whether our diagnostic blood gene
549 expression classifier could be an early marker of cog-
550 nitive dysfunction, we tested it in 118 MCI subjects
551 to see how many subjects would be classified as “AD-
552 like” rather than as controls, and whether there was
553 any preference for subjects who subsequently received
554 an AD diagnosis within 2 years (MCL) compared to
555 those who had not received an AD diagnosis (MCLc) but
556 nevertheless were likely to have an AD endpoint. It
categorized the majority of MCI subjects as “AD-like”
(90/118 subjects, 76.3%) rather than as controls, which
corresponded to 32/41 MCLc (78.0%) and 58/77 MCInc
(75.3%) (Table 3B). When bootstrapped, the mean
accuracy was higher (86.0; 95% CI, 84.6–87.4%), with
a similar proportion of MCInc and MCLc being classi-
fied as AD-like (mean 87.2 and 86.5%, respectively)
(Fig. 3A; Table 3B).

557 Although there were only a limited number of MCI
558 subjects with sMRI scans available for analysis (only
19 subjects with imaging compared to 41 available with
expression), we assessed the performance of our diag-
nostic expression classifier in the MCI and MCLc
groups separately and compared this to the perfor-
mance with sMRI based models in 92 individuals for
whom sMRI and expression data were both available.

560 Of the 92 people with MCL 64 were classified as
AD-like (69.6%) using the expression classifier, cor-
responding to 48/73 MCLc (65.8%) and 16/19 MCLc
(84.2%). When the sample cohort was bootstrapped,
the mean accuracy was 70.4% (95% CI, 69.8–71.0%).
Compared to the whole expression cohort, accuracy in
this subpopulation was lower and while the mean sen-
sitivity in classifying MCLc was high (89.0%; 95% CI,
88.4–89.7%), the mean sensitivity to classify MCLc
as AD-like was far lower than in the whole popula-
tion analyzed (65.6%; 95% CI, 65.0–66.2%) (Fig. 3D;
Table 3B).

561 sMRI alone appeared less able to classify MCLc as
“AD-like” (63.2%) compared to the expression classi-
 fier (84.2%), classifying only 12 out of 19 patients as
AD-like, suggesting imaging is not as sensitive at iden-
tifying potential prodromal AD subjects compared to
the expression classifier, even when these individuals
were within two years of receiving an AD diagnosis. In
the MCLc group, the imaging classifier was no better
than chance (33/73; 45.2%) at classifying individuals
as “AD-like” or control, whereas the expression classi-
 fier identified more “AD-like” individuals in the MCLc
population (65.8%) (Table 3B).

562 DISCUSSION

563 We have shown that there may be potential in using
blood expression as a diagnostic classifier for AD.
Specifically, we have identified 48 genes (50 probes)
which can distinguish between AD and normal elderly
control subjects with an accuracy of 75% in a validation
cohort. This performance is consistent with previ-
ously reported accuracies using blood gene expression
measures [31–33] and is similar to other single or mul-
tivariate classifiers incorporating measures from CSF
and/or neuroimaging [64–68].

564 No marker would be expected to achieve perfect
accuracy when tested in a population of people clini-
cally diagnosed with AD as AD is a very heterogeneous
disease with symptoms similar to other dementias and
is therefore not always correctly diagnosed by a set
of clinical symptoms alone. Clinical diagnosis gener-
ally achieves 80–90% accuracy against a postmortem
AD diagnosis [54]. Furthermore, some of the patho-
logically relevant changes detected by a marker may
occur before symptoms emerge in apparently normal
people, leading to their possible in-life misclassifica-
tion. Amyloid deposition is not only possible to detect
in people with MCI, but is also present in 20–40% of
cognitively normal elderly people, up to 10 years
before AD diagnosis [11, 14, 15, 20, 69–75]. Some
of the normal elderly subjects in our study displayed
blood expression changes characteristic of people with
AD. Although speculative, this may not be misclassifi-
cation by the marker, but could instead represent subtle
peripheral changes occurring in pre-clinical disease.

565 Of the 39 genes included in the classifier with known
function, eight are associated with mitochondrial
Fig. 3. Bootstrap results for the AD-Control classifier in classifying MCI samples. The graphs demonstrate the mean accuracy of the classifier measured from bootstrapping (black dotted line) and the accuracy measured with the "out of bag" classifier in the validation cohort (red hashed line). Results are shown for (A) expression classifier alone, (B) expression and imaging classifiers combined, (C) imaging classifier alone, and (D) expression classifier alone in the subset of individuals with sMRI measures.

activity and oxidative phosphorylation including subunits of the electron transport chain, constituents of mitochondrial ribosomes and 12 are involved in translation. Changes in the expression of genes associated with these processes in AD blood was recently described in detail by us [76] and genes involved in these processes are enriched in other AD gene expression classifiers [32, 33]. These processes are also significantly altered in the brains of people with AD [77–80], which may represent a common response to the same disease-associated signal(s).

There are a number of limitations to this study, namely the sample size for validation and the lack of additional cohorts to further test the classifier. By performing power calculations using the MVPower R package, we have shown that our sample size has 80% power with a conservative effect size estimate of 0.2 and 100% with an effect size of 0.7 as derived from our data. We have addressed the second issue by applying a robust bootstrapping approach, considered by the community to be the most appropriate approach for evaluating a classifier when a second cohort is unavailable [61]. When new datasets become available to us we will be able to test the performance of our classifier in additional samples. We are confident that the findings will replicate, as the predictive genes are known to be AD-related and these processes have been previously identified in AD classifier studies, as described above.

In order to further explore the performance of the blood expression classifier we developed, we analyzed measures of cortical thickness and local
is in contrast to the performance of the imaging marker that classified far fewer MCI subjects overall as AD-like compared to the gene expression classifier, even those subjects who subsequently received an AD diagnosis within two years. This may suggest measures of structural brain changes have less sensitivity early in disease compared to the expression marker in blood we describe, but this will be uncertain until the MCI non-converters have been followed for a longer period.

Changes detected by sMRI appear to occur at a later stage of disease than changes in Aβ and tau [11, 12, 87–89], and it will therefore be important in the future to evaluate our blood expression classifier alongside measures of CSF or brain Aβ to see if the high classification of AD among the MCI subjects reflects early detection of prodromal disease and to map the dynamic onset of changes we observe.

Due to small sample numbers and therefore insufficient statistical power when we divided the MCI group into those who subsequently received an AD diagnosis within two years and those who did not, we were unable to develop a classifier able to distinguish these two groups. However, the majority of MCI subjects had peripheral blood gene expression changes in common with AD patients including those who did and did not receive an AD diagnosis within two years. This is a finding we have previously observed in a study of cell based proteins in blood comparing AD and MCI subjects [23] and is similar to the profile of reduced Aβ42 observed in the CSF in some control and many MCI subjects, which is believed to represent a pathological process that significantly departs from normality [37]. Two factors are likely to contribute to this observation; first, around half of the subjects in the MCI cohort were selected for analysis because we knew they had subsequently converted to a probable AD diagnosis. Second, our MCI sample population are drawn from specialist memory clinics and are therefore more likely to represent people with established cognitive problems resulting from prodromal AD than might be expected from a cohort drawn from a community population, as we previously reported from a meta-analysis of longitudinal studies of MCI [90].

Alternatively, our blood expression marker may represent an endophenotype shared by a group of diseases with related etiology. Our two year follow-up period is insufficient to confirm which of the normal elderly control or MCI nc subjects have prodromal AD and will therefore eventually receive a clinical diagnosis of AD. Pathological confirmation of an AD diagnosis will take longer still. We know of no existing studies with blood collected for RNA purposes where postmortem
significant neuronal loss has occurred. For example, a marker can assist in the development of drugs targeting relevant and common disease endophenotypes and does not necessarily specific to, or present in all individuals, has clinical relevance. Such a marker can assist in the development of drugs targeting relevant and common disease endophenotypes and does not necessarily unique disease association to achieve efficacy in a relevant disease population. It could also be useful as part of a staged diagnostic approach, providing a cost effective method of identifying individuals with a greater likelihood of developing disease who could subsequently be referred for more expensive diagnostic tests.

Blood is emerging as a very promising tissue in which to achieve a non-invasive and relatively inexpensive assay for detection of AD. In addition to changes in blood gene expression [31–33, 91], plasma protein panels have been described which accurately distinguish AD from normal elderly control people and/or MCI subjects who convert from those who remain stable, or to predict disease progression [92]. There are also many reports of individual proteins with altered abundance in plasma in AD and normal elderly control groups, suggesting blood is a realistic tissue in which to identify markers of AD [23, 93–102]. However, there are still technical issues to overcome as not all protein measures reliably change across studies [103]. In the future, it will be important to explore the performance of different combinations of markers to achieve the most accurate biomarker for diagnosis and make comparisons between blood markers and more established biomarkers linked to pathophysiology and establish their relative temporal patterns of change during disease progression in relation to these other biomarkers.

In conclusion, we have shown that peripheral blood shows promise as an AD-associated diagnostic blood gene expression marker that may be useful very early in pre-clinical disease. It performed similarly to using neuroimaging measures alone in AD and normal elderly control samples. Future work will evaluate this blood expression marker in subjects from which CSF measures of Aβ1-42 and/or PET amyloid have been measured in order to further explore the specificity and timing of these changes. The existence of such a marker in an accessible tissue such as blood would contribute significantly toward efforts to identify and treat people with AD very early in their disease before significant neuronal loss has occurred. For example, CSF Aβ1-42 and tau measurements to enrich for likely AD are predicted to reduce sample size by 67% and costs by 60% in a clinical trial compared to a trial with unselected MCI subjects [104]. Evaluation of the marker in related diseases will establish whether the changes we see are specific to AD or are shared across similar diseases. A lack of disease specificity would not diminish the value of a marker able to capture a common disease-associated endophenotype for which a specific treatment with real clinical value could be developed.

ACKNOWLEDGMENTS

We thank Rutina Lecing, Megan Pritchard, Belinda Martin, Kathryn Lord, Nicola Dunlop, and Catherine Tunnard for sample collection and neuropsychiatric assessments of the London cohort. We thank all participants and their families. This work was supported by ImoMed (Innovative Medicines in Europe), an Integrative Project funded by the European Union of the Sixth Framework program priority FP6-2004-LIFESPHEALTH-5, Alzheimer’s Research UK, The John and Lucille van Geest Foundation, the NIHR Biomedical Research Centre for Mental Health at the South London and Maudsley NHS Foundation Trust and Institute of Psychiatry Kings College London, and NIA/NH RC1 grant 1RC1AG035610 (to GC).


REFERENCES


K. Lunnon et al. / A Blood Gene Expression Marker of Early Alzheimer’s Disease


[28] Thambisetty M, Simmons A, Velayudhan L, Hye A, Campbel...


Hansson O, Zetterberg H, Buchhave P, Andreasson U, Lon

effect on cerebrospinal fluid beta-amyloid 42 in adults

Shaw LM, Vanderstichele H, Knopik-Czajka M, Clark CM,

Assen PS, Pfeifer RE, Blennow K, Sories H, Simon A, Lobwe

Tzouroukis, SD, Alzheimer’s Disease Neuroimaging Ini
tive (2009) Cerebrospinal fluid biomarker signature in Alz


Tapiola T, Alafuzoff I, Herukka SK, Parkkinen L, Har-

Frisoni GB, Fox NC, Jack CR, Scheltens P, Thompson PM

Manczak M, Anekonda T, Henson E, Park BS, Quinn J,

Ankarcrona M, Mangialasche F, Winblad B (2010) Rethink-

Performance of random forest when SNPs are in linkage


Hodges AJ, on behalf of the AddNeuroMed C (2012) Mito-

Hodges A, on behalf of the AddNeuroMed C (2012) Mitoch

F, Ozmen L, Bluethmann H, Drose S, Brandt U, Savaskan E, 

Chap G, Zeldar A (2009) Amyloid-beta and tau syn-

trophically impaired in the AddNeuroMed. Sci Ti

Fox NC, Scialli RL, Crow WR, Rossen MN (1999) Correla-

tion between rates of brain atrophy and cognitive decline in 

AD. Neurology 53, 1687-1693.


MR-based hippocampal volumetry in the diagnosis of 


Lantto R, Braam Z, Probst P, Loudauny A, New-

Hodges AJ, on behalf of the AddNeuroMed C (2012) Mitoch

Hodges A, on behalf of the AddNeuroMed C (2012) Mitoch

F, Ozmen L, Bluethmann H, Drose S, Brandt U, Savaskan E, 

Chap G, Zeldar A (2009) Amyloid-beta and tau syn-

trophically impaired in the AddNeuroMed. Sci Ti

Fox NC, Scialli RL, Crow WR, Rossen MN (1999) Correla-

tion between rates of brain atrophy and cognitive decline in 

AD. Neurology 53, 1687-1693.

Jack CR, Petersen RC, O'Brien PC, Tangdol EG (1992) MR-based hippocampal volumetry in the diagnosis of 


Lantto R, Braam Z, Probst P, Loudauny A, New-

Hodges AJ, on behalf of the AddNeuroMed C (2012) Mitoch

Hodges A, on behalf of the AddNeuroMed C (2012) Mitoch

F, Ozmen L, Bluethmann H, Drose S, Brandt U, Savaskan E, 

Chap G, Zeldar A (2009) Amyloid-beta and tau syn-

trophically impaired in the AddNeuroMed. Sci Ti

[104] K. Lunnon et al. / A Blood Gene Expression Marker of Early Alzheimer's Disease