Neurobiology of Aging xxx (2017) 1–13

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Multivariate analyses of peripheral blood leukocyte transcripts distinguish Alzheimer's, Parkinson's, control, and those at risk for developing Alzheimer's

Elaine Delvaux [a,b,c], Diego Mastroeni [a,b,c,d], Jennifer Nolz [a,b,c], Nienwen Chow [e], Marwan Sabbagh [f], Richard J. Caselli [g], Eric M. Reiman [h], Frederick J. Marshall [e], Paul D. Coleman [a,b,c,*]

[a] ASU-Banner Neurodegenerative Disease Research Center, Biodesign Institute, Arizona State University, Tempe, AZ, USA
[b] Arizona Alzheimer Consortium, Phoenix, AZ, USA
[c] Formerly at Banner Sun Health Research Institute, Sun City, AZ, USA
[d] Maastricht University, Medical Centre, Maastricht, The Netherlands
[e] University of Rochester Medical Center, Rochester, NY, USA
[f] Barrow Neurological Institute, Phoenix, AZ, USA
[g] Mayo Clinic, Scottsdale, AZ, USA
[h] Banner Alzheimer's Institute, Phoenix, AZ, USA

ARTICLE INFO

Article history:
Received 13 November 2016
Received in revised form 12 May 2017
Accepted 14 May 2017

Keywords:
Alzheimer's disease
Blood test
Prodromal detection
RNA

ABSTRACT

The need for a reliable, simple, and inexpensive blood test for Alzheimer's disease (AD) suitable for use in a primary care setting is widely recognized. This has led to a large number of publications describing blood tests for AD, which have, for the most part, not been replicable. We have chosen to examine transcripts expressed by the cellular, leukocyte compartment of blood. We have used hypothesis-based cDNA arrays and quantitative PCR to quantify the expression of selected sets of genes followed by multivariate analyses in multiple independent samples. Rather than a single study with no replicates, we chose an experimental design in which there were multiple replicates using different platforms and different sample populations. We have divided 177 blood samples and 27 brain samples into multiple replicates to demonstrate the ability to distinguish early clinical AD (Clinical Dementia Rating scale 0.5), Parkinson's disease (PD), and cognitively unimpaired APOE4 homozygotes, as well as to determine persons at risk for future cognitive impairment with significant accuracy. We assess our methods in a training/test set and also show that the variables we use distinguish AD, PD, and control brain. Importantly, we describe the variability of the weights assigned to individual transcripts in multivariate analyses in repeated studies and suggest that the variability we describe may be the cause of inability to repeat many earlier studies. Our data constitute a proof of principle that multivariate analysis of the transcriptome related to cell stress and inflammation of peripheral blood leukocytes has significant potential as a minimally invasive and inexpensive diagnostic tool for diagnosis and early detection of risk for AD.

1. Introduction

Dealing effectively with the Alzheimer epidemic requires a diagnostic method that is appropriate in primary care settings. A number of studies have quantified the accuracy of the clinical diagnosis of Alzheimer's disease (AD) in specialized Alzheimer centers. When the disease is already clinically diagnosed, comparison of clinical and neuropathologic diagnosis of AD showed specificity ranging from 44% to 70% with sensitivity ranging between 71% and 87% (Beach et al., 2012). In a primary care setting accuracy is significantly worse (Connolly et al., 2011). In addition, a wide variety of studies in brain imaging (e.g., Reiman, 2011), spinal fluids (e.g., Rosén et al., 2014), cognition (e.g., Kawas et al., 2003), and neuropathology (e.g., Braak and Braak, 1997) have established that AD has been affecting the brain and producing subtle cognitive symptoms decades before clinical diagnosis (see Edmonds et al., 2015 for review). Data such as these have led to the consensus

* Corresponding author at: ASU- Banner Neurodegenerative Disease Research Center, Biodesign Institute, Arizona State University, 727 E. Tyler St., Tempe, AZ 85287, USA. Tel.: +1-585-704-4391; fax: +1-480-727-8305.
E-mail address: paul.coleman@asu.edu (P.D. Coleman).

0197-4580/$ – see front matter © 2017 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2017.05.012

that optimally effective therapeutic intervention should be instituted as early as possible in the disease process. Early intervention requires a diagnostic that is simple to derive, minimally invasive, inexpensive, and that can be administered to a population in a primary care location. These requirements have led to intense activity directed at finding an Alzheimer biomarker and the literature is replete with purported biomarkers of AD. Some of the biomarkers that have been described include those that are invasive (e.g., spinal tap, Davidsson et al., 1997) or require expensive equipment and expertize (Killiany et al., 2002). Although these procedures are extremely useful in investigative studies, they do not offer promise for routine, large-scale diagnostic use in any care provider setting. Other tests have been described that draw on easily obtained peripheral samples from plasma (Mapstone et al., 2014; Soares et al., 2012; Williams et al., 2017), blood cells (Nagy et al., 2002; Padovani et al., 2002), skin (Ikeda et al., 2000), and urine (Pratico et al., 2002). Additional studies have described tests for AD that utilize responses to pharmacologic intervention (Scinto et al., 1999). Many of these studies directed at distinguishing AD from control samples on the basis of peripheral tissues have been successful at yielding statistically significant differences between AD and control samples, but, unfortunately, many do not include samples from any other neurodegenerative disease (for review see Laske et al., 2015; McGhee et al., 2014; Snyder et al., 2014). However, these studies have, for the most part, not survived further investigation (e.g., Casanova et al., 2016), thus leading to consideration of variables that may lead to inconsistent results (e.g., McGhee et al., 2014; O'Bryant et al., 2014). We here report data on multivariate biomarkers of AD based on the hypotheses that Alzheimer-related brain changes in markers of inflammation (Akiyama et al., 2000), cell stress (Lu et al., 2014) and cell cycle (Busser et al., 1998; Nagy et al., 1997), or epigenetics (Mastroeni et al., 2009) may be manifested in transcripts derived from peripheral blood leukocytes and be able to distinguish and predict AD. We also present data on the variability of these blood biomarker transcripts. Our data provide a proof of principle for the potential utility of biomarkers derived from peripheral blood leukocyte transcripts.

2. Methods

We extracted RNA from 177 blood and 27 brain samples. In our experimental design, we chose to use these samples as multiple replicates using different methods of analysis, samples from multiple sources and from persons at early clinical stages of probable AD, persons at risk, persons with another neurodegenerative disease as well as unaffected persons. We assert that this design can provide increased confidence in repeatability and utility. The data reported here result from univariate and multivariate analyses of selected transcripts derived from 177 probable AD, Parkinson's disease (PD), and age- and gender-matched control blood samples obtained from different sources at different times and analyzed in different ways. By intention, we made every effort to exclude mild cognitive impairment (MCI) cases, while including cases with very mild but clinically determined AD. Blood samples were obtained from 4 different sources: (1) Outpatient Geriatric Neurology and Psychiatry Clinic at Monroe Community Hospital (MCH), Rochester, NY, USA; (2) the Rochester, NY cohort of the Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT) Study; (3) the Alzheimer clinic at Banner Sun Health Research Institute (BSHRI), Sun City, AZ; and (4) the Mayo Clinic (Mayo), Scottsdale, AZ (see Table 1). All samples were drawn between 10AM and 3PM, and processed blind. All samples were collected with informed consent and under IRB-approved protocols. To examine relationships between blood data and the brain, 27 samples of superior frontal gyrus (SFG) were obtained from the brain bank at BSHRI. We chose to work with transcripts obtained from peripheral blood leukocytes for several reasons, among them: (1) relative ease of surveying multiple transcripts from 1 limited sample; (2) relative precision of working with nucleotides; and (3) relatively precise definition of the source of signals. Two different procedures were used for isolation of RNA from samples: (1) collection into EDTA tubes and subsequent isolation of RNA or (2) collection into PAXgene tubes and subsequent RNA isolation. Two different methods of generating RNA data were used; cDNA arrays or quantitative RT-PCR (qRT-PCR). Statistical analyses were conducted at the University of Rochester Department of Biostatistics and at BSHRI using 2 different algorithms. Table 1 illustrates the platforms and algorithms utilized.

2.1. Sources of samples

2.1.1. Patient recruitment from MCH, Rochester, NY

Entrance into this study was with informed consent. AD subjects were diagnosed with probable or possible AD on the basis of NINCDS (McKhann et al., 1984) and DSM IV criteria for AD. Examination by a neurologist was performed to confirm diagnosis and to measure disease severity. Disease severity was assessed using the Mini-Mental Status Examination (Folstein et al., 1975), the Clinical Dementia Rating scale (CDR; Morris, 1997), and the Blessed Dementia Rating Scale (Blessed et al., 1968). Control subjects included in the study scored above 27 on the Mini-Mental Status Examination, whereas AD cases scored below 22. The mean CDR of AD cases was 0.5 (on a 3-point scale) indicating very mild but clear dementia. We made every effort to exclude MCI cases while including cases with very mild but clinically determined AD. Since these were not autopsy-confirmed cases, AD cases should be considered under the conventional nomenclature as "probable Alzheimer's disease". Any

Table 1
Sample source, type and collection method, quantification platform, analysis, result, and figure reference

Source	Tissue collected	Platform	Analysis	Result	Figure(s)/table
UR-MCH Samples 1 and 2	Blood-EDTA	cDNA Array	SAS	Initial screen distinguishes AD, ND, and PD in 3 replicates	Figs. 1 and 2
UR-MCH Sample 3	Blood-PaxGene	qRT-PCR/TaqMan	GB-STAT	qRT-PCR validation of array data	Fig. 3
UR-ADAPT	Blood-PaxGene	qRT-PCR/TaqMan	GB-STAT	Distinguishes Not at Risk, At Risk, and Phenoconverters	Fig. 5
BSHRI Sample 1	Blood-PaxGene	qRT-PCR/TaqMan	GB-STAT	Epigenetic markers distinguish AD and ND	Fig. 4
BSHRI Sample 2	Blood-PaxGene	qRT-PCR/TaqMan	GB-STAT	Distinguishes AD and PD	Fig. 8
Mayo	Blood-PaxGene	qRT-PCR/TaqMan	GB-STAT	Distinguishes ApoE4$^{+/+}$ AD and ND	Figs. 6 and 7
BSHRI Sample 3	Blood-PaxGene	qRT-PCR/TaqMan	GB-STAT	Training/Test Sets	Table 4
All BSHRI Samples	Blood-PaxGene	qRT-PCR/TaqMan	GB-STAT	Variability study	Fig. 9
BSHRI Brain Bank Samples 1 and 2	Brain-Superior Frontal Gyrus	qRT-PCR/TaqMan	GB-STAT	Distinguishes AD, ND, and PD in brain	Figs. 10 and 11

A summary of the blood and brain samples contributing to the data of this manuscript noting how collected, quantified and analyzed, the outcome and figure references. Key: AD, Alzheimer's disease; ADAPT, Alzheimer's Disease Anti-Inflammatory Prevention Trial; BSHRI, Banner Sun Health Research Institute; Mayo, Mayo Clinic, Scottsdale, AZ; MCH, Monroe Community Hospital; ND, nondemented; PD, Parkinson's disease; UR, University of Rochester.