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

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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...
that optimally effective therapeutic intervention should be insti-
tuted as early as possible in the disease process. Early intervention
requires a diagnostic that is simple to derive, minimally invasive,
expensive, and that can be administered to a population in a
primary care location. These requirements have led to intense ac-
tivity 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 pe-
ripheral 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 in yielding statis-
tically 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 vari-
ables that may lead to inconsistent results (e.g., McGhee et al., 2014;
O’Bryant et al., 2014). We here report data on multivariate bio-
markers 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 man-
ifested 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 mul-
tiple sources and from persons at early clinical stages of probable
AD, persons at risk, persons with another neurodegenerative dis-
ease 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 be-
 tween 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 algo-
rithms. 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. Exam-
ination 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 De-
mentia Rating Scale (Blessed et al., 1968). Control subjects included
in the study scored above 27 on the Mini-Mental Status Examina-
tion, 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

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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.
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