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Drug treatment of Alzheimer's disease patients leads to expression changes in peripheral blood cells

Margaret A. Calciano[a], Weiyin Zhou[a], Peter J. Snyder[b], Richard Einstein[a,*]

[a]*ExonHit Therapeutics Inc, Gaithersburg, MD, USA*

[b]*Lifespan Affiliated Hospitals & Alpert Medical School of Brown University, Providence, RI, USA*

Abstract

Background: Increasing cholinergic activity has been the primary mechanism for treating dementia due to Alzheimer's disease. However, the effectiveness of cholinesterase inhibitors (ChEIs) is still widely debated. The identification of specific biomarkers capable of identifying patients more likely to respond to these treatments could potentially provide specific evidence to clearly address this controversy through patient stratification. The goal of this study was to determine the feasibility of discovering biomarkers specific for the treatment of Alzheimer's disease.

Methods: Peripheral blood was collected from a cohort of patients treated with different ChEIs. Total RNA was isolated and profiled on the human Genome-Wide SpliceArray (GWSA) to test the feasibility of discriminating the different treatment subgroups of subjects based on the expression patterns generated from the Genome-Wide SpliceArray.

Results: Specific expression differences were identified for the various treatment groups that lead to a clear separation between patients treated with ChEIs versus naïve patients when Principal Component Analysis was performed on probe sets selected for differential expression. In addition, specific probe sets were identified to be dependent on the inhibitor used among the treated patients.

Conclusions: Distinct separation between non-treated, galantamine, donepezil, and rivastigmine-treated patients was clearly identified based on small sets of expression probes. The ability to identify drug-specific treatment expression differences strengthens the potential for using peripheral gene signatures for the identification of individuals responding to drug treatment.

Keywords: Alzheimer's disease; Cholinesterase inhibitors; Acetylcholinesterase; Donepezil; Galantamine; Rivastigmine; Expression profiling; Microarray; Genome-wide SpliceArray; Pharmacogenomics; Peripheral blood

1. Introduction

Alzheimer's disease (AD) has proven to be an exceptionally difficult neurodegenerative disease to treat successfully, despite the massive efforts made over the past several decades. Work completed in the early 1980s established the relationship between AD and diminished availability of several neurotransmitters, including reductions in central cholinergic tone [1]. The consistent finding of reduced central nervous system cholinergic activity in AD led to the theory that preventing the breakdown of the existing acetylcholine could improve symptoms of the disease. Stemming from this concept, cholinesterase inhibitors (ChEIs) have been used to treat AD in an attempt to prolong the activity of acetylcholine by reducing its metabolism. The first ChEI to be approved by the Food and Drug Administration in 1993 was tacrine [2]; however, it is rarely used today because of its lack of clinical significance, toxicity, and the introduction of safer ChEIs [3]. Donepezil, galantamine, and rivastigmine are the more commonly used ChEIs which have all been shown clinically to stabilize or slow the cognitive decline of AD [3–5]. Donepezil and galantamine specifically inhibit acetylcholinesterase (AChE), and in addition galantamine also modulates nicotinic acetylcholine

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*Corresponding author. Tel.: 240-683-7073; Fax: 240-683-7060.

E-mail address: Richard.Einstein@exonhit.com

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receptors. In contrast, rivastigmine can inhibit both AChE and butyrylcholinesterase (BuChE) [6]. Although ChEIs are moderately effective in slowing the cognitive decline of AD patients [4] and appear to slow the decline in mild cognitive impairment for at least a short period of time [7], they do have different mechanisms of action. More recent reviews and comparative studies have noted subtle differences between the effectiveness of these drugs on cognition, function, behavior, and global change [5,6].

The ability to determine whether a patient with AD would respond to a select ChEI versus another would be of significant benefit and would provide a means of rational drug selection within this class of medications. Moreover, reaching this goal for ChEIs by relying on the new discipline of pharmacogenomics–the examination of the genetic variability between patients as a way to predict drug response and toxicity–would serve as a useful model for advancing personalized approaches to treatment selection for other classes of therapeutics to be developed for AD in the future. In addition to improving drug efficacy, pharmacogenomics holds the promise of targeting more appropriate populations for clinical trials requiring fewer trials which would result in lower drug development costs and ultimately more cost-effective drugs [8].

There have been several recent attempts to predict individual response to ChEI treatment based on individual genetic factors. For example, Ferris et al. [9] found that the sex and BuChE genotype of individual patients contribute to predicting treatment response with rivastigmine. Also, a synergistic effect has been seen between apolipoprotein E and BuChE genotype on cognitive decline [10]. Current studies in AD patients treated with ChEIs have focused on the effects of polymorphic variants of apolipoprotein E, BuChE, and cytochrome P450-related enzymes [11,12] on therapeutic response. The complex interaction between these genetic markers and other individual variables (e.g., educational attainment, other medical risk factors) for predicting age of onset and rate of decline for the disease are also being actively examined [13].

In an attempt toward a pharmacogenomic approach, we measured expression changes within the peripheral blood from a series of AD patients treated with different ChEIs to determine whether we could identify different treatment populations. The unique approach described here is that blood samples were profiled using the human Genome-Wide SpliceArray (GWSA) [14], a microarray platform designed to monitor alternatively spliced transcripts within the human genome through the use of a probe design targeted to exon bodies, exon-exon junctions, and exon-intron junctions [15,16]. Extensive alterations in transcripts resulting from alternative splicing produce structurally different products which can significantly impact gene function in biology, disease [17–20], as well as processes such as evolution [21,22]. Presented here is the comparative analysis of expression data generated from peripheral blood of differentially treated AD patients using the GWSA platform.

2. Methods

2.1. Samples

All samples were obtained from an ongoing and independent study which seeks to use the peripheral blood from a cohort of patients for the discovery of a blood based genomic signature to distinguish AD from non-demented control subjects. Of the available 40 AD patients from this larger study, 28 samples were identified who had either received no treatment for their dementia or, if they were medicated, only a single ChEI was used throughout their treatment of AD. Eight of 40 patients had been excluded because they were treated with a *N*-methyl-d-aspartic acid receptor antagonist in conjunction with a ChEI or independently. A total of three additional patients were removed because of the use of Premarin, a conjugated estrogen, as a potential treatment for AD, and a final patient was removed because of unclear drug treatment. Blood samples from each patient were provided through a Clinical Research Organization (PrecisionMed; San Diego, CA) after complying with full patient consent regulations. Patients who had a clinical diagnosis of probable AD according to Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) and National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria were included. In all cases, the clinical diagnosis was supported by brain imaging results, either by computed tomography-scan or magnetic resonance imaging, which were compatible with AD. Additional clinical examination was performed to rule out any other cognitive impairment and any clinically significant and uncontrolled medical conditions. Inclusion criteria allowed both men and women aged $\geq$40-years. The 28 AD patients included 12 men and 16 women with an average age of 75. Their mini-mental state exam (MMSE) scores ranged from 0 to 23, with an average of 16.1. Drug treatment duration varied among the patients; however, all patients treated with donepezil took a daily dose of 10 mg, whereas the daily dose varied for galantamine and rivastigmine treatment with a range of 8–24 mg and 6–12 mg, respectively (for individual patient details, see Supplemental Table S1).

2.2. Sample RNA

Blood samples from each patient were collected in PAXgene (Qiagen, Valencia, CA) tubes to preserve the integrity of the RNA. All samples were received as frozen PAXgene tubes, and total RNA was extracted following manufacturer's instructions. RNA quality and quantity were assessed using the RNA 6000 Nano LabChip kit with the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and by spectrophotometry. Total RNA passing quality control criteria were used for microarray analysis.

2.3. Microarray processing

Microarray processing details for the GWSA platform have been described previously [14]. Briefly, starting with