A Blood Based Gene Expression Test For The Early Detection Of Alzheimer’s Disease

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Summary
A new blood test, ADtect®, has been developed that can aid the early detection of Alzheimer’s Disease (AD). The test is based on measuring expression in selected genes in blood, also defined as the AD-specific gene signature. ADtect® comprises a low density array of 96 selected gene assays using RNA extracted from a blood sample (2.5µL), collected in an FDA approved PAXgene® tube. The performance of each of the 96 gene assays is calculated with an algorithm resulting in a prediction value indicating the presence or absence of AD.

Introduction
Although there are currently a lack of treatment options to arrest the more debilitating symptoms of Alzheimer’s disease (AD), early diagnosis and active management strategies can temporarily delay the onset. Diagnosis of AD today involves a detailed clinical interview, cognitive tests and imaging techniques. Despite the variety of testing approaches, there is still variability in diagnostic accuracy and it remains difficult to make an accurate diagnosis at an early stage of disease.

The DiaGenic ADtect™ Test: Basic Principles
• The primary diseased part of the body is not the only part responding to a disease.
• The disease also leaves a unique “signature” that include subtle systemic changes in gene expression, in other parts of the body. The response can be detected by measuring the amount of RNA for specific genes in peripheral blood.
• These signatures can be identified using gene expression technology.

Several independent papers have recently indicated that a peripheral blood based test could be used for diagnostic profiling in neurological diseases13. Indeed, our own previous studies based on a whole genome screening of more than 32000 genes have shown that AD patients could be identified with a blood-based gene expression test14. Moreover, AD patients could be accurately distinguished from another neurodegenerative disease, Parkinson Disease6.

Patient and control samples used in the development of the ADtect™ blood test

Whole Genome Array
• ABI 1700 platform
• >32000 gene probes

Gene Validation
• ABI 7900 platform
• TaqMan™ 384 format

Prototype Assay
• ABI 9700 platform
• TaqMan™ 96 format

ADtect™ Assay
• ABI 9700 platform
• TaqMan™ 96 format

Figure 1

We have designed a gene expression based assay that runs on a commercially available real time RT-PCR instrument, which utilizes low density arrays (LDA).

ADtect® Test Procedure

Sample Preparation
Whole blood is collected from individuals in PAXgene™ Blood RNA tubes and processed according to manufacturers instructions. Total RNA is extracted from blood samples using PAXgene™ Blood RNA kit and quality assessed by NanoDrop spectrophotometer and Agilent 2100 Bioanalyzer. CDNA is prepared using the high-capacity cDNA kit from Applied Biosystems and samples are applied to the TaqMan™ LDA.

Gene sets and expression analysis
The gene expression analysis is done on the ABI Prism 7900HT Fast System using TaqMan™ LDA with a 96-assay format, containing an AD-specific gene signature in a custom format, such that 4 individual samples can be run in parallel on each 384 LDA card (Figure 2). The genes were selected based on the performance characteristics from previous studies using an Applied Biosystems Whole Genome Array and TaqMan® LDA. In addition to the pre-selected AD-specific gene signature a range of other genes are included as technical controls. A software will be included to help analyze the real time RT-PCR data and for prediction.

Introduction

Results & Discussion
A set of 96 gene assays including relevant controls have been included in the ADtect™ test. The gene assays cover different biological processes as indicated below. It has been shown that -80% of the genes expressed in brain are also expressed in blood cells12. It has also been shown that several in brain genes have a comparable regulation in brain tissue and blood11. It is thus not surprising to find that some of the assays included in ADtect® represent genes that have a vital function in brain and in Alzheimer related processes. These are some of the genes represented in ADtect®.

Insulin-like growth factor-binding protein (IGFBP) 7 is a secreted protein and is expressed in both primary neurons and glial cells. It has been shown that IGFBP regulates cellular proliferation, adhesion, and angiogenesis2. A neuroprotective and neuromodulatory effect has also been suggested13.

Neuropagin is a CPEB binding protein and guides neural development by regulating the translation of CPE-containing mRNAs15. It binds to the CPEB isoform present in neurons that has been suggested to have a role in the formation of long-term memory is not clear.

TAR DNA-binding protein 43 (TDP-43) is an RNA-binding protein that functions in mammalian cells in transcriptional repression and exon skipping16. TDP-43 has been identified as the major pathological protein of FTLD with ubiquitin-immunoreactive inclusions (FTLD-U) with or without amyotrophic lateral sclerosis (ALS) and sporadic ALS13.

Phosphatidylinositolamine-binding protein (PEBP) is a calpain substrate both in vitro and in situ. Calpains are proteases whose dysregulation has been implicated in Alzheimer’s disease. In postmortem brain samples from the hippocampus of AD PEBP levels were approximately 20% increased17.

Conclusion
Included in the ADtect™ test are assays representing genes that have vital biological functions in brain and that has been reported to be implicated in Alzheimer’s disease pathophysiology. Our current findings indicate that the ADtect™ test is a relevant biomarker for early detection of AD.

References
9. Lønnborg A et al. 2007; P-044, AAC, Prevention of Dementia, Washington, DC, US.