ADNI Alzheimer's Disease Neuroimaging Initiative

Longitudinal Analysis of Cerebrospinal Fluid Visinin-like protein-1

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Summary

Recent preclinical biomarker studies in AD have shown that development of the pathological hallmarks of AD begins as early as 10-15 years before the development of cognitive decline[1-5]. Clinicians, researchers, and pharmaceutical companies have begun to appreciate possible intervention in the preclinical stages of AD as a patient's best option for the prevention of dementia and, in the shorter term, as the best opportunity to test proposed disease-modifying drugs currently in development. Cerebrospinal fluid (CSF) Visinin-like protein-1 (VILIP-1) shows utility in distinguishing AD from cognitively normal (CN) controls and in some cases from other dementias[6,7]. Importantly, it appears useful as a prognostic marker, particularly when used in combination with Aβ42. Further work, including in-depth cross-sectional studies across multiple cohorts and especially longitudinal follow-up of CN subjects, is needed to improve the ability of biomarkers to contribute to AD research and clinical trials. Adding VILIP-1 and other novel biomarkers to a panel including Aβ42, tau, and ptau181 may improve our diagnostic and prognostic capabilities. Better knowing the order of changes in biomarkers as preclinical AD progresses to symptomatic AD will enable clinical trials to select those participants who are most likely to benefit from drug intervention, as well as to reduce the number of participants needed to attain statistical relevance.

Method

Mouse anti-human VILIP-1, clone 3A8.1 and sheep anti-human VILIP-1 were provided to Singulex, Inc. (Alameda, CA) for development and manufacture of an Erenna® immunoassay assay kit (#03-0008-02) according to an agreement with Washington University. The proprietary custom order kit reagents included preparation of the monoclonal capture antibody for binding to Invitrogen (Carlsbad, CA) MyOne magnetic micro particles and Invitrogen Alexa fluor dye labeling of the sheep polyclonal detection antibody and all assay buffers.

A sandwich ELISA was developed using the Erenna® immunoassay system to measure VILIP-1 in CSF. Prior to the assay all samples were centrifuged (11,000 g x 3 minutes) to remove particulates. All assay steps were performed at room temperature unless otherwise indicated. A calibration curve was prepared using dilutions of recombinant human VILIP-1 ranging from 3.9 to 3000 pg/mL and , with each concentration assayed in triplicate. 15 µL standards or CSF were combined with 135 ul assay buffer and 50 µL antibody coated micro

particles for measurement of VILIP-1 in CSF. The assay plate was incubated for two hours on a plate shaker set to 525 revolutions per minute. Micro particles were then magnetically separated and washed one time using an Agilent (Santa Clara, CA) Bravo Automated Liquid Handling Platform using Singulex wash buffer. Fluorescent dye labeled detection antibody prepared by Singulex (20 µL per well) was added and incubated for one hour. After washing the magnetic micro particles 5 times, 20 µL per well of Singulex elution buffer was added for 10 minutes to separate detection antibody from the micro particles. Eluted antibodies were then transferred with the Bravo instrument to a clean 384 well plate for reading in the Erenna® immunoassay system.

All samples (first freeze/thaw cycle) were run in duplicate on a single lot number, and within-person longitudinal samples were run on the same assay plate to reduce inter- and intra-plate variability. Quality control (QC) included analysis of three internal standard CSF pools run on each plate. Each standard was averaged over all assays; plates with 2 or more standards greater than two standard deviations (SD) from the mean were reanalyzed. Further, any individual sample with a Coefficient of Variation (% CV) greater than 25% was reanalyzed. Samples that failed quality control (QC) were refrozen and stored at -80^{o}C for at least 48 hours before being reanalyzed. When samples were reanalyzed due to QC failure, all within-person longitudinal samples were reanalyzed as well, on the second freeze/thaw cycle.

Dataset Information

This methods document applies to the following dataset(s) available from the ADNI repository:

Dataset Name	**Date Submitted**
Fagan Lab – Longitudinal VILIP-1 CSF Analysis	27 February 2015

References

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