which were combined with the PD and DS samples. Support Vector Machine analysis was utilized to classify the 4 diagnostic groups using their overall biomarker profiles. The biomarker profiles yielded 100% accuracy in classifying all 4 diagnostic categories. When looking at individual proteins, pancreatic polypeptide was selectively dysregulated in PD, throrombopoeitin in DS, IL6 and TNFz in AD and IL7 in both AD and DS. Conclusions: To our knowledge, this study represents the first ever for a blood-based biomarker profile of AD to cross-validate on an independent assay platform. Additionally, our preliminary results suggest that the profile approach to analyzing multiple markers by disease states is a viable option for differential diagnosis between AD and non-AD neurological diseases. Additional analyses with larger samples of AD, PD and non-AD samples are underway.

PI-233 FURTHER EXPLORATION OF PLASMA BIOMARKERS FOR ALZHEIMER’S DISEASE USING ISOTOPIC TANDEM MASS TAGS AND A COMBINED TARGETED/NONTARGETED LC/MS/MS METHOD

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Background: There is an urgent need for biomarkers to aid the diagnosis of Alzheimer’s disease (AD) and following our previous report detailing the discovery of several protein candidates in plasma we developed a targeted assay to verify these observations in a large clinical cohort comprising of 1000 samples. We also conducted a further quantitative discovery study involving the analysis of a subset of 90 samples to determine if additional peptides would provide more scope for the reliable quantitation of our biomarker proteins. Methods: Samples were selected to create a balanced subset in terms of the center, gender and disease status. Male (n=5) and female (n=5) subjects classified as either AD, mild cognitive impairment (MCI) or elderly controls were chosen from each of three clinical centres. Each plasma sample was digested with trypsin and individually labelled with the TMT0 reagent. An aliquot of a reference pooled plasma digest labelled with a single TMT6 reagent was then added to each sample. Mass spectrometry data was acquired using the LTQ Orbitrap Velos using an inclusion list method but at elution times when none of the peptides in the inclusion list could be detected, the remaining precursors were selected for MS/MS based on intensity. The inclusion list method was created separately to contain a total of 95 peptides from a panel of nine existing AD biomarker candidates. Results: Analysis of TMT labelled peptides within undepleted, unfractionated plasma specimens has facilitated the quantitation of several leading biomarker candidates of AD. All 9 proteins represented within the include list were detected. Additional peptides were also detected and quantified, with some being statistically significant for the diagnosis of AD. Furthermore, a new set of peptides, not previously described as biomarkers for AD, have been shown to be differentially regulated in AD and MCI patients compared to the control subjects. Conclusions: These findings are encouraging and demonstrate the value of the combined targeted and data dependent approach. Although further work is required to define the panel of markers that afford the best clinical performance, these results add further support to the rationale that credible biomarker signals exist within plasma from AD patients.

PI-234 CIRCULATING MI RNA BIOMARKERS FOR ALZHEIMER’S DISEASE

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Background: A minimally invasive diagnostic assay for early detection of Alzheimer’s disease (AD) is required to select optimal patient groups in clinical trials, monitor disease progression and response to treatment, and to better plan patient clinical care. Blood is an attractive source for biomarkers due to minimal discomfort to the patient, encouraging greater compliance in clinical trials and frequent testing. MiRNAs belong to the class of non-coding regulatory RNA molecules of ¼ 22nt length and are now recognized to regulate ~60% of all known genes through post-transcriptional gene silencing (RNAi). They have great potential as clinical biomarkers because of their stability and ease of detection in blood. Circulating profiles of miRNAs have been shown to discriminate different tumor types, indicate staging and progression of the disease and to be useful as prognostic markers. Recently their role in neurodegenerative diseases, both in terms of diagnostic as well as disease etiology has come into focus. Methods: Our discovery cohort consisted of 11 AD and 20 age-matched control (NC) plasma samples. Global miRNA profiles were first generated by the Nanostring platform, followed by confirmation of candidate signature miRNAs using the TaqMan qPCR platform. The validation cohort, which was an independent set of 17 AD and 20 NC samples was then tested for the candidate signature miRNAs using TaqMan qPCR. Results: Here we report the discovery and validation of a unique circulating 7-miRNA signature (hsa-let-7d, hsa-let-7g, hsa-miR-15b, hsa-miR-142-3p, hsa-miR-191, hsa-miR-301a and hsa-545) in plasma, which could accurately distinguish AD patients from healthy controls with >95% accuracy (AUC of 0.953). There was a¼ 2 fold difference for all signature miRNAs between the AD and NC samples, with p-values <0.05. Conclusions: This miRNA signature-set is now being validated in a large Asian AD cohort. Prediction accuracies and further stratification potential will be presented. Pathway analysis, taking into account enriched target mRNAs for these signature miRNAs was also carried out, hinting that the disturbance of multiple enzymatic pathways including lipid metabolism could play a role in AD etiology.

PI-235 ASSOCIATION OF PLASMA BETA-AMYLOID LEVELS AND RISK OF DEMENTIA, ALZHEIMER’S DISEASE, BRAIN STRUCTURE AND COGNITIVE FUNCTIONS IN THE FRAMINGHAM HEART STUDY

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Background: Amyloid beta isoforms (Aβ 42 and Aβ 40) are known to play a role in the pathophysiology of Alzheimer’s disease (AD). Cerebrospinal fluid levels fall in parallel with increased brain deposition, detectable by amyloid imaging, as AD pathology accumulates. But lumbar puncture is invasive and amyloid imaging expensive. Hence there is considerable interest in examining whether circulating Aβ levels correlate with AD risk, but studies have been hampered by high intra- and inter-assay variability and published studies have conflicting results. We measured plasma Aβ 42 and Aβ 40 in a prospective, community-based, cohort under ongoing surveillance for AD using a second-generation amyloid assay. Methods: 4,039 Framingham Study dementia-free participants who attended the 23rd Original cohort examination or the 7th Offspring examination had plasma Aβ 42 and Aβ 40 measured using a fluorimetric immunosay from Innogenetics (Gent, Belgium). These levels were related to risk of incident dementia/AD in persons over age 60 (n=733 Original and 585 Offspring participants, mean age 72 ± 8; 56% women) and to baseline MRI brain volumes and cognitive functions in 2323 dementia-free young-old Offspring (mean age 62±4; 54% women). Models were adjusted for age and sex (and education for cognitive functions). Results: After 10 years of follow-up, 228 participants were diagnosed with dementia (189 had AD). Increased plasma amyloid beta levels were associated with lower dementia risk (risk associated with standard deviation increase: Aβ 40 HR = 0.90, 95% CI [0.80-1.01], p = 0.078; Aβ 42