ADNI Alzheimer's Disease Neuroimaging Initiative

Comparison of Plasma and CSF for Alzheimer's disease miRNA Biomarkers

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Introduction

Alzheimer's disease (AD) is the most common form of dementia in the United States. The development of diagnostic measures capable of detecting early brain changes prior to symptom onset is critical for AD disease management. The use of CSF-derived miRNAs that are detectable in plasma by quantitative PCR offers a precise and reliable method to observe discreet changes in RNA-based biomarkers that may discriminate patients with AD from neurologically normal controls.

Summary

This study utilized 160 participants (AD vs. healthy controls) who donated plasma and CSF collected under fasting conditions on the same day, for a total of 320 participant samples. The samples were obtained from ADNI, and analyzed using miRNA array cards as we have done previously for CSF [1-5] and plasma [6]. Total RNA was isolated from each biofluid and assayed by qPCR using a Custom TaqMan Low Density Array (TLDA) card in the 384-well format. Each custom TLDA card includes 64 miRNAs in triplicate (192 probes) and 2 participant samples are run per card (384 wells total). The biofluids were processed for RNA isolation between November – December 2022, then processed for qPCR between January – February 2023. Data analysis is performed by an OHSU biostatistician, and includes data quality control measures, normalization, calibration, and relative quantification. Comparison of each donor's plasma and CSF miRNA profiles will be used to elucidate the predictive power of candidate miRNAs at stratifying AD patients from healthy participants.

Methodology

RNA Isolation

Each RNA isolation batch consisted of 24 samples: 12 donor- and date-matched CSF and plasma samples, grouped to keep individual donor samples together to avoid any potential effect due to RNA isolation batch. Biofluid samples were thawed on ice and total RNA was isolated from 250 µL of CSF and 250 µL of plasma using the Urine miRNA Purification kit (Norgen, Thorold, ON) following the manufacturers protocol. Following addition of lysis buffer to the sample, an exogenous cel-miR-39-3p spike-in (Thermo Fisher, Waltham, MA) was added at a final

concentration of 3 pM. CSF and plasma RNA were eluted with 30 µL and 50 µL of nuclease free water, respectively. The miRNA concentration of each sample was measured using the Qubit miRNA Assay kit (Thermo Fisher) and read using a Qubit 4.0 fluorometer (Thermo Fisher).

RT-qPCR

MiRNA was converted to cDNA using the TaqMan Advanced miRNA cDNA Synthesis kit (A28007, Thermo Fisher). Briefly, 3 µL of RNA was 3' poly-adenylated, followed by a 5' adaptor ligation step and reverse transcription (RT). 5 µL of the RT mix was added to a 14 cycle universal miR-amplification step (miR-amp). The miR-amp reaction was diluted 1:10 with nuclease free water, then 110 µL of the diluted cDNA was mixed with 220 µL of TaqMan Fast Advanced master mix (4444556, Thermo Fisher) and 110 µL of nuclease free water. 100 µL of the qPCR mix from one sample was loaded into the first 4 ports of a 384-well custom TaqMan Advanced Human miRNA Card (Thermo Fisher), then 100 µL of the qPCR mix from a second sample was loaded into the last 4 ports. Each card was assayed on a QuantStudio 7 Flex (Thermo Fisher) by automatic loading of the cards using an Orbitor R2 robot to increase throughput and to ensure that each participants matching CSF and plasma were assayed on the same day.

Analysis

Expression data (quantification cycle, or Cq values) will be examined thoroughly for artifacts and subjected to rigorous quality control (QC) rules based on the performance of array card well-level QC statistics calculated and exported by the QuantStudio software as proxy measures for spurious amplification, PCR efficiency, and reverse transcription (RT) failures. We will also examine the performance of samples that contain no cDNA (no template) to verify absence of measurable expression for each miRNA of potential scientific interest. After QC checks and filtering of miRNAs with failed QC or sparse measurement coverage, we will normalize Cq values using both a positive control miRNA (cel-miR-39-3p spike-in) and a combination of endogenous normalization reference control miRNAs (minimally hsa-miR-16-5p, upon verification of full observation in all samples). Normalization will minimally comprise technical RNA isolate concentration adjustment, processing batch alignment, and normalization to endogenous reference expression levels measurable in all samples. Wells with acceptable QC but Cq > 34 will be considered censored values and given special handling using censoring-aware methods in the analysis.

Since Cq values from RT-qPCR may become unreliable at very low input concentrations (generally, at Cq > 34 or so) and cannot be trusted to give fully quantitative information in those ranges, the recorded Cq value represents an upper boundary on the expression (i.e. the true expression is no greater than the value represented by the observed Cq) and the result is actually an interval-censored measurement: We know that the true Cq must fall somewhere between the observed value and 40 (the final PCR cycle). Thus, we treat Cq = 40 as the zero-expression point and for any Cq value x > 34 we say that the true (unobserved) Cq falls within the interval [x,40]. We then use Bayesian interval-censored regression methods to estimate and contrast group (AD vs. healthy control) means with adjustment for donor sex and age, separately for each miRNA measured for each biofluid (CSF and plasma) sample set. Agreement between CSF and plasma results will be assessed by variance-scaled differences in association estimates (i.e. Welch's t-test) and compared visually using Bland-Altman plots and other methods as appropriate.

Bayesian prior distributions for the regression analysis will be vague but proper, selected for robustness of reference. MiRNAs will be ranked according to magnitude and confidence of group contrast, concordance between CSF and plasma estimates, and agreement with prior knowledge based on scientific literature and our own past studies of these miRNAs in AD vs. healthy control populations.

References

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