Novel use of aptamer libraries for prediction of amyloid-β PET status from blood serum

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NeoNeuro aptamer solutions

APMI ALZHEIMER PRECISION MEDICINE INITIATIVE

INSIGHT pre-AD study group

BACKGROUND

The development of effective disease-modifying treatments for Alzheimer's disease (AD) is constrained by the capacity to screen the right target population for clinical trials, broadly and cost-effectively. NeoNeuro has developed a novel, alternative approach in collaboration with the INSIGHT-preAD study involving the application of enriched aptamer libraries as a means of mapping multiple pathological epitopes in blood, both rapidly and cost-effectively.

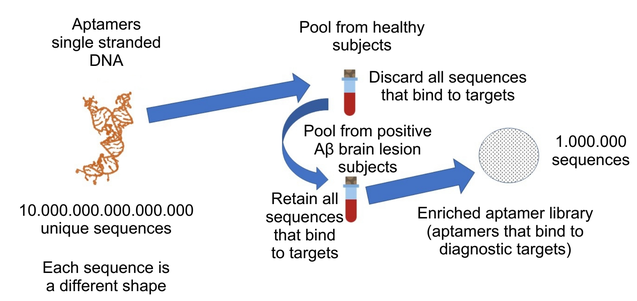
METHODS

STEP #1: We applied FRELEX[†] selection to a random aptamer library consisting of 10^{16} sequences against a pool of serum from six cognitively normal older individuals with subjective memory complaint (SMC) showing evidence of cerebral amyloidosis (SMC-A+), recruited from the INSIGHT-preAD cohort. FRELEX is a selection method that does not require immobilization or knowledge of the selection targets. Within the INSIGHT-preAD cohort, a threshold of 0.79 for SUVR is used as the threshold between negative and positive brain Aβ deposition status. A pool of serum from six SMC individuals without cerebral amyloidosis (SMC-A-) from the same cohort was used for counter selection with the library in each selection round after the first one.

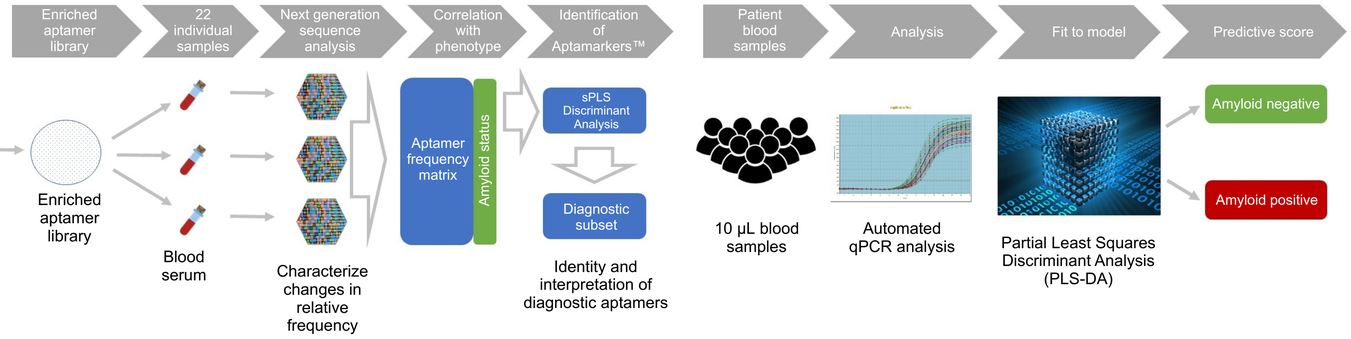
STEP #2: After ten rounds of selection, aliquots of the enriched library were applied for a single round of positive selection against individual serum samples of 22 SMC subjects (11 SMC-A+ and 11 SMC-A-). Each of these 22 selected libraries of aptamers was characterized by next-generation sequencing (NGS) analysis. The relative frequencies of the top 1,000 sequences in terms of copy number were correlated with Aβ status using sparse partial least squares discriminant analysis (sPLS-DA). Based on this analysis a subset of 21 aptamers (defined as Aptamarkers™) was defined as sufficient to obtain sensitivity and specificity of 1.0 on the 22 subjects with PLS-DA analysis and cross validation.

STEP #3: These aptamers were divided into two subsets of 13 and 10 aptamers each with two aptamers being in common between the subsets. Both subsets were applied in a single round of FRELEX against serum from 42 SMC subjects from the INSIGHT-preAD cohort (25 SMC-A+, 17 SMC-A). The effect of the selection process on aptamer frequency was determined by qPCR analysis with specific primers for each aptamer.

Step #1: Selection of enriched aptamer library against blood serum



Step #2: Correlating aptamer frequency with Aβ brain lesions status.



Step #3: Diagostic analysis

RESULTS

Figure 1: Distribution of 42 individual samples by dimensional contrast based on aptamer frequencies.

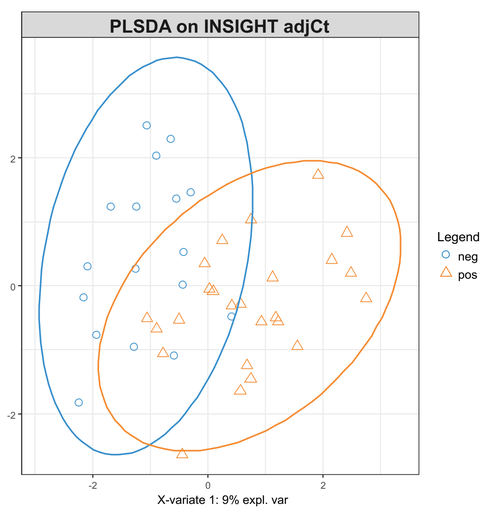


Figure 1 provides the distribution of the 42 individual samples based on aptamer frequencies as weighted by the PLS-DA model.

Figure 2: Areas for Aβ brain lesion prediction for 42 samples with 21 Aptamarkers™.

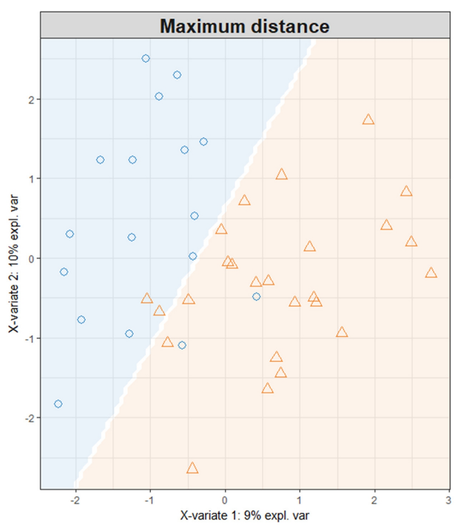


Figure 2 provides the area predicted for each of the classes of Aβ status considered, with the locations of each of the 42 samples within these prediction areas. These predicted areas are not subject to the cross-validation analysis, only the first two dimensions are illustrated.

Figure 3: ROC AUC over three dimensions with Aptamarker™ test of 42 subjects

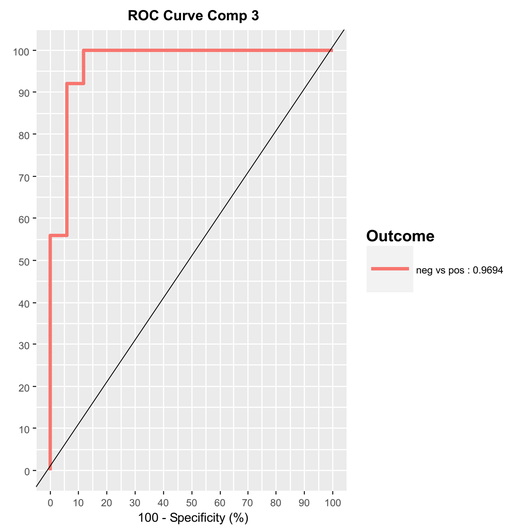


Figure 3 provides the ROC curve following cross-validation analysis with 100 replications and 10 folds. The calculations are based on multivariate prediction methods with a threshold based on distance. The values provided are based on analysis across three principal components.

Based on the cross-validation analysis:

Sensitivity = 0.80
Specificity = 0.88
Accuracy = 0.83

AUC = 0.9694 in three dimensions

CONCLUSIONS

Aptamers were identified that bound to target molecules that were present at higher concentrations in the serum of SMC subjects with elevated levels of Aβ brain lesion accumulation. The binding of these aptamers to their targets was evaluated by characterizing the effect of selection in individual serum samples on individual aptamer frequencies. To our knowledge, this is the first time that aptamer frequency has been used as a biomarker for any disease.
The results reported within this study are comparable to results with known biomarkers with cognitively normal subjects. There are several advantages to this approach.

1.) The ability to identify Aptamarkers™ within SMC-A+ subjects at different time stages prior to the onset of cognitive dysfunction.
2.) The low cost and scalability of the analysis.
3.) The ability to combine Aptamarkers™ from different trained libraries in one simultaneous analysis.
4.) The implicit capacity to continually improve the diagnostic process as the number of subjects analyzed increases through stratification of subgroups.
5.) The capacity to improve predictive power by using non-conforming subjects as a basis for the selection of additional diagnostic Aptamarkers™.

We envision the application of this platform in synergy with other diagnostic tools including assays for known blood-based biomarkers and molecular genetic analyses.

REFERENCES

[†]Aptamers as biomarkers for neurological disorders. Proof of concept in transgenic mice Soizic Lecocq, Katia Spinella, Bruno Dubois, Simone Lista, Harald Hampel, Gregory Penner

05 Jan 2018 PLOS ONE, https://doi.org/10.1371/journal.pone.0190212

The Aptamarker™ process is the subject of patents filed by NeoNeuro.

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