Combined Use of Aptamer Libraries and Known Biomarkers for Prediction of A-β Brain Lesion Accumulation from Blood Plasma

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BACKGROUND

We have previously described the development of a novel platform involving the use of aptamers directly as biomarkers against unknown molecular targets in blood as a means of predicting Aβ deposition in cognitively normal individuals[1]. Forty-one samples used in that study were the same as samples analyzed with known biomarkers[2], thus enabling a combined analysis with both approaches.

OBJECTIVES

1). To compare the predictive performance of brain Aβ of known biomarkers with Aptamarkers in plasma.
2). To assess whether the Aptamarkers are identifying similar or different aspects of the pathology.

METHODS

Figure 1A: FRELEX process

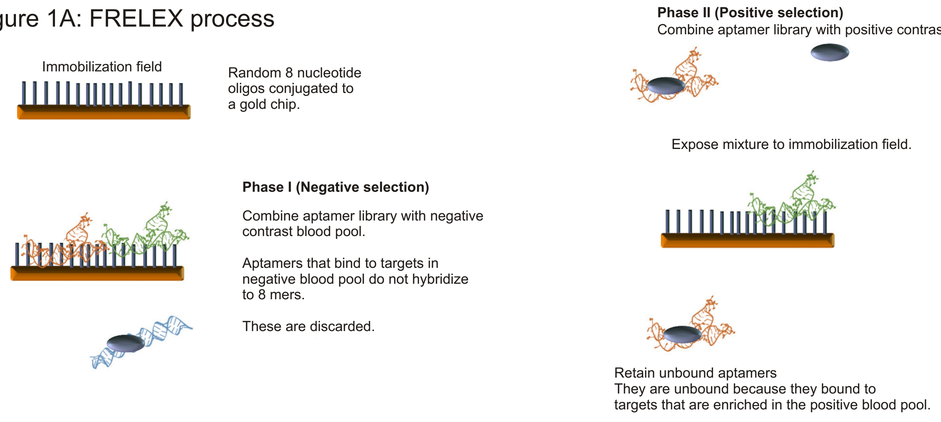


Figure 1B: Step #1 - Create an enriched aptamer library against diagnostic targets

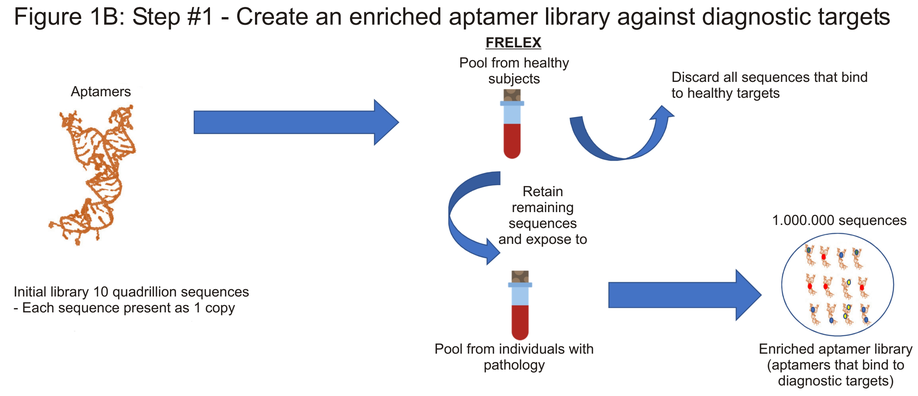


Figure 1C: Step #2 - Correlating aptamer frequency and disease state

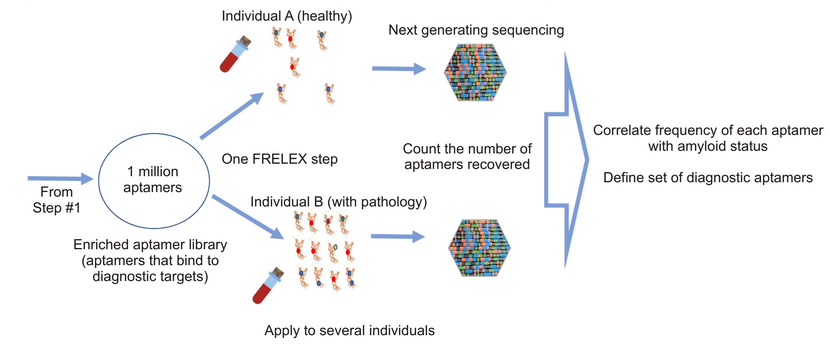
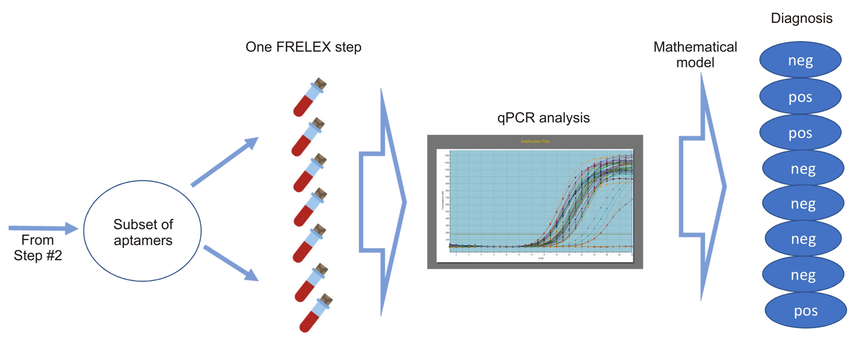


Figure 1D: Step #3 - Diagnostic application of Aptamarkers



We applied FRELEX[3] selection to a random aptamer library consisting of 10^{16} sequences against a pool of plasma from six cognitively normal older individuals with subjective memory complaint (SMC) showing evidence of cerebral amyloidosis (SMC-A+), recruited from the INSIGHT-preAD cohort[4]. FRELEX is a selection method that does not require immobilization or knowledge of the selection targets (Figure 1A). 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 plasma 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 (Figure 1B). This process was repeated for ten selection rounds. The enriched aptamer library was applied for a single round of positive FRELEX selection against individual samples of plasma from 22 individuals (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 10,000 sequences in terms of copy number were correlated with Aβ status using sparse Partial Least Squares Discriminant Analysis (sPLS-DA). Based on this analysis as well as analysis of enrichment trajectories, 44 aptamers were defined as candidates as biomarkers (Aptamarkers) (Figure 1C). These Aptamarkers were synthesized and applied in a single round of positive FRELEX selection in three separate subsets of 13, 10 and 23 Aptamarkers each (with two Aptamarkers being included in both of the first two subsets against individual plasma samples from each of the 41 SMC subjects (INSIGHT-preAD study group) that were also analyzed with known biomarkers. A specific reverse primer was designed for each Aptamarker and the amount of Aptamarker bound to an unknown target was determined by qPCR analysis (Figure 1D). The known biomarkers used were, YKL40, NFL, Tau, Ab 42, Ab 40, the ratio between these peptides and BACE1[5,6,7]. Predictive values were determined by excluding from the training set any samples that exhibited SUVr values within 0.05 units of the threshold value used of 0.79. The test set used was the full data set subjected to 100 k-folds with a fold size of 5.

CONCLUSIONS

The discovery of Aptamarkers is agnostic, in that no hypotheses need to be formulated prior to the identification of aptamers that serve as biomarkers. In this study, Aptamarkers exhibited better predictive capacity across the 41 individuals tested than known biomarkers. The addition of the known biomarkers to the Aptamarker dataset did not result in a significant improvement in predictive capacity over the Aptamarkers alone.

General Conclusions:

1.) Aptamarkers provide a rapid, simple method for the prediction of brain Aβ deposition in the cognitively normal individuals through plasma analysis.

2.) Certain of the molecular targets characterized by the Aptamrkers are not known biomarkers.

3.) Aptamarkers appear to provide an im proved method of predicting brain Aβ deposition in cognitively normal individuals compared to a panel of known biomarkers.

4.) The Aptamarker platform can be applied to any pathology and any aspect of any pathology.

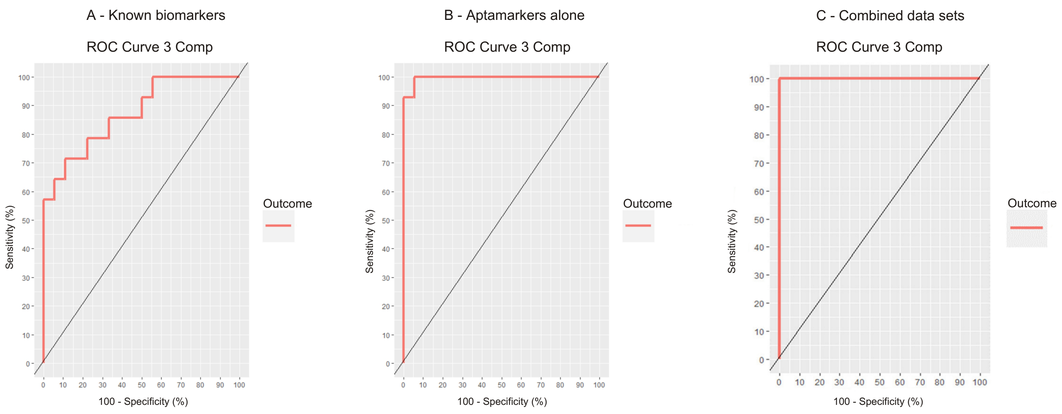
RESULTS

Table 1 - Predicted Values

	Sensitivity	Specificity	Accuracy	AUC
Known biomarkers	0.88	0.59	0.76	0.873
Aptamarkers	0.88	0.88	0.88	0.996
Combined	0.88	0.88	0.88	1.000

Aptamarkers outperformed the known biomarkers in terms of specificity and accuracy (Table 1) with the sPLS-DA for each of the three data sets. The combination of known biomarkers and Aptamarkers did not improve predictive capacity.

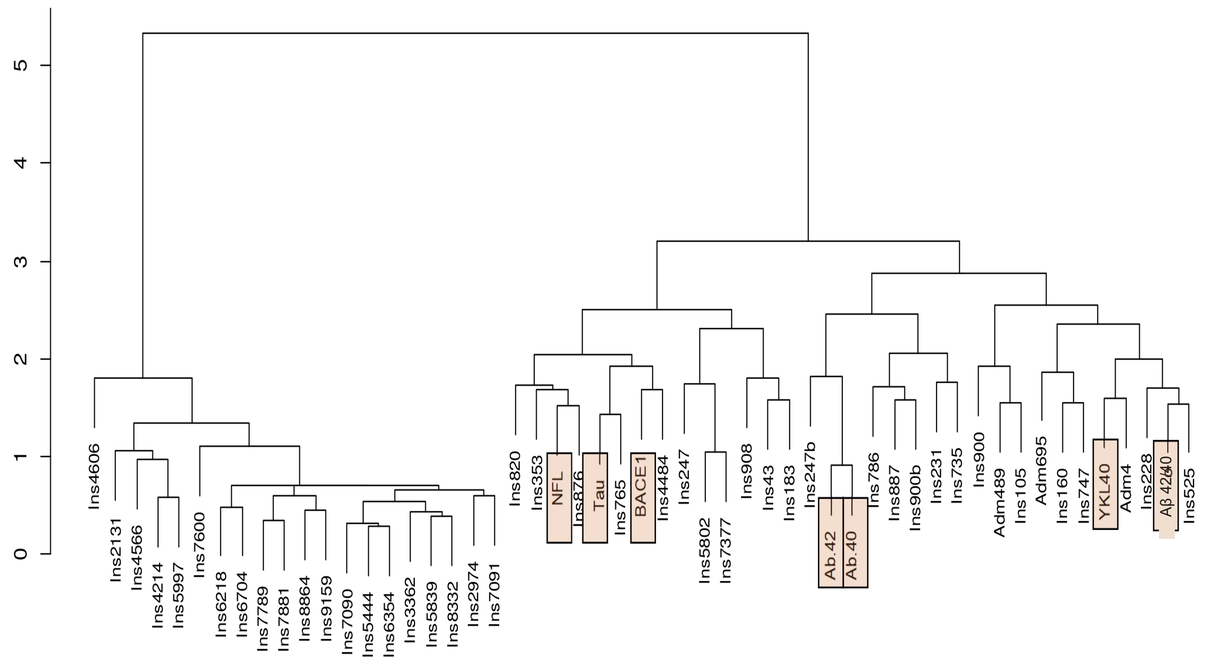
Figure 2: ROC-AUC curves



The combination of Aptamarkers and known biomarkers together did not result in an improvement in ROC-AUC (Figure 2) The application of sPLS-DA to each of these datasets resulted in clarification that for the known biomarkers only the ratio between Aβ peptides meaningfully explained the variation. The analysis with the ratio alone resulted in similar predictive capacity as the combined set of known biomarkers. Then the Aptamarkers were combined with the known biomarkers and subjected to sPLA-DA. The ratio of the Aβ peptides was the only variable retained for the explanation of variation in the first dimension, while all other variables were represented in the second and third dimension, with the exception of Aβ 40.

We also performed a covariance analysis between Aptamarkers and the known biomarkers (Figure 4) The Aβ peptide ratio 42/40 did not co-vary with any of theother variables. This is in accordance with he observation that the ratio was the only variable included in the first dimension of the combined mathematical model. Several of the Aptamarkers did co-vary with known biomarkers except for the Aβ peptides.

Figure 4: Covariance analysis of Aptamarkers with known biomarkers



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The Aptamarker process is the subject of patents filed by NeoNeuro.
www.neoneuro-aptamers.com