

# 6-miRNA panel predicts amyloid status in cognitively normal older adults

Joanna Williams<sup>a</sup>, Diane Guévremont<sup>a</sup>, Chris Frampton<sup>a</sup>, Margaret Ryan<sup>a</sup>, Chris Fowler<sup>b,c</sup>, Colin Masters<sup>c,d</sup>, Ralph Martins<sup>c,e</sup>, Leslie Shaw<sup>f,g</sup>, Wickliffe Abraham<sup>a</sup>, Warren Tate<sup>a</sup>, Nicholas Cutfield<sup>a</sup> University of Otago (New Zealand)<sup>a</sup>, The Florey Institute (Australia)<sup>b</sup>, Macquarie University (Australia)<sup>c</sup>, University of Western Australia (Australia)<sup>d</sup>, Edith Cowan University (Australia)<sup>e</sup>, University of Pennsylvania (USA)<sup>f</sup>, Alzheimer’s Disease Neuroimaging Initiative<sup>g</sup>.



MicroRNA || Regulate AD relevant genes || Stable within blood || Easily detected using RT-qPCR || Alter with disease

➤ Enhanced molecular diagnosis of Alzheimer’s disease.

### SUMMARY

**BACKGROUND:** miRNA control neuronal functions dysregulated in Alzheimer’s disease (AD) and are highly stable in blood plasma. Previously, we have shown that plasma miRNA levels vary with the progression of AD (Guévremont *et al.*, 2022).

**AIM:** To identify miRNA capable of distinguishing cognitively normal older adults based on amyloid status.

**STUDY SUMMARY:** Plasma neurodegeneration-related microRNA levels were assessed using TaqMan arrays in cognitively normal older adults who were Aβ+ or Aβ-.

In the AIBL discovery cohort, stepwise logistic regression identified a 6-miRNA panel that accurately differentiated cognitively normal, Aβ+ and Aβ- individuals (AUC = 0.990). The model was validated in ADNI (AUC=0.981) and KARVIAH (AUC=0.988) and distinguished amyloid converters (AUC 0.928) in a longitudinal AIBL cohort. Using a two-threshold approach, only 9% of samples fell within the intermediate classification zone (95% sensitivity and specificity).

### 1.DISCOVERY

#### 6-miRNA panel discovered in AIBL

| Model | AUC   | 95% CI    | SP (%) | SE (%) | Accuracy (%) | NPV | PPV | Cohort Proportion (%) |       |      |
|-------|-------|-----------|--------|--------|--------------|-----|-----|-----------------------|-------|------|
|       |       |           |        |        |              |     |     | Low                   | Inter | High |
| 1     | 0.754 | 0.68-0.82 | 72     | 57     | 34.1         | 64  | 65  | 28                    | 62    | 10   |
| 2     | 0.855 | 0.80-0.91 | 78     | 74     | 51.7         | 77  | 78  | 38                    | 41    | 21   |
| 3     | 0.909 | 0.87-0.95 | 85     | 81     | 61.9         | 81  | 80  | 40                    | 36    | 24   |
| 4     | 0.961 | 0.94-0.99 | 86     | 89     | 78.4         | 89  | 87  | 43                    | 21    | 36   |
| 5     | 0.974 | 0.96-0.99 | 92     | 94     | 83.0         | 94  | 88  | 47                    | 17    | 36   |
| 6     | 0.990 | 0.98-1.00 | 96     | 94     | 94.3         | 95  | 95  | 49                    | 9     | 42   |
| 7     | 0.995 | 0.99-1.00 | 98     | 97     | 97.2         | 97  | 98  | 51                    | 4     | 45   |
| 8     | 0.997 | 0.99-1.00 | 97     | 99     | 94.3         | 99  | 97  | 51                    | 5     | 44   |

**AIBL:** Aβ+ n = 84; Aβ- n = 93

**Univariable logistic regression:** association between individual differentially expressed miRNA and amyloid status → **Multivariable logistic regressions:** identified the combination of miRNA most able to predict group membership (9-miRNA; AUC 0.93) → **Stepwise logistic regression** identified a 6-miRNA panel.

**Fig. 1 Stepwise logistic regression models identify a high preforming 6-miRNA panel**  
(a) Performance of miRNA combinations used to derive the final 6-miRNA panel in the AIBL cohort.  
(b) Violin plot of predicted probabilities from Model 6 and risk stratification. Each point represents an individual participant. Dotted lines indicate 95% specificity (SP) and 95% sensitivity (SE) thresholds, showing clear separation between groups and reduced intermediate zone.

### 2. VALIDATION

#### Validated ADNI and KARVIAH

**ADNI:** Aβ+ n = 84; Aβ- n = 101; **KARVIAH:** Aβ+ n = 31; Aβ- n = 61

Model generalisability was demonstrated through external validation (ADNI and KARVIAH cohorts) using fixed discovery-model regression coefficients without re-estimation or variable selection.

**Fig. 2 Evaluation of 6-miRNA panel in ADNI and KARVIAH cohorts.**  
(a) Heatmap: 6-miRNA differential expression patterns in AIBL, ADNI and KARVIAH. FC = Fold change, 2-tailed unpaired Mann-Whitney U test, \* p < 0.05.  
(b) ADNI: Violin plot of the predicted probabilities from Model 6 and risk stratification.

### Nonredundant

Principal component analysis pattern matrix demonstrated that the 6-miRNA were distributed across 3 components which explained 79% of the total variance: PC1 (35%), PC2 (25%), PC3 (19%).

### Model consistency

Bootstrap resampling (1000 iterations) within the AIBL cohort supports internal consistency of the 6-miRNA model.

### ApoE-ε4 independent

4-group classification framework based on amyloid (Aβ- vs. Aβ+) and ApoE-ε4 status (carriers vs. non-carriers) showed the 6-miRNA panel works independently of ApoE genotype.

### CONTACT

Joanna Williams  
joanna.williams@otago.ac.nz

Alex Tickle  
alexandra.tickle@otagoinnovation.com

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### 3. LONGITUDINAL

#### Levels increase with amyloid load

**AIBL:** Aβ- → Aβ+ (n = 33 pairs)

**Fig.3 Box and whisker plots**  
Generalized estimating equations (\*p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001). Median values from the cross-sectional AIBL CN-Aβ- (black hashed line) CN-Aβ+ (pink hashed line) groups are shown for reference and were not included in the GEE.

### 4. BIOLOGICAL RELEVANCE

#### AD relevant pathways

**Fig.4 Most enriched Reactome pathways** targeted by at least three miRNA. Numbers represent miRNA.

### 5. TECHNICAL VALIDATION

#### Linear amplification

**No effect of freeze-thaw**

ANOVA table with Dunnett's multiple comparison tests

### 6. CONCLUSION

The 6-miRNA panel is a promising standalone biomarker of amyloid status which could enhance molecular diagnosis of Alzheimer’s disease.