A blood-based 7-metabolite signature for the early diagnosis of Alzheimer´s disease

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Summary

Accurate blood-based biomarkers of Alzheimer´s disease (AD) could provide a simple, inexpensive and non-invasive means of diagnosing AD and monitoring disease progression over time, circumventing the many problems associated with the analysis of CSF, the current diagnostic sample of choice. We sought to develop a robust AD biomarker panel by identifying alterations in plasma metabolites that persist throughout the continuum of AD pathophysiology. Using a multicenter, cross-sectional study design, we based our analysis on metabolites whose levels were altered both in AD patients and in patients with amnestic mild cognitive impairment (aMCI), the earliest clinical manifestation of AD. The resulting 7-metabolite biomarker panel accurately distinguishes AD and aMCI patients from normal cognition controls, and thus constitutes a potentially useful tool for the early diagnosis of AD.

Materials and Methods

Ultra performance liquid cromatography coupled to mass spectrometry (UPLC-MS) was used to independently compare the levels of 495 plasma metabolites in aMCI (n=58) and AD (n=100) patients with those of normal cognition controls (NC, n=93). Three separate UPLC-MS platforms were used to ensure optimal metabolite profiling (Fig. 1). Metabolites whose levels were altered in both AD and aMCI patients with respect to NC were selected and used to generate a logistic regression model that accurately distinguished AD from NC patients.

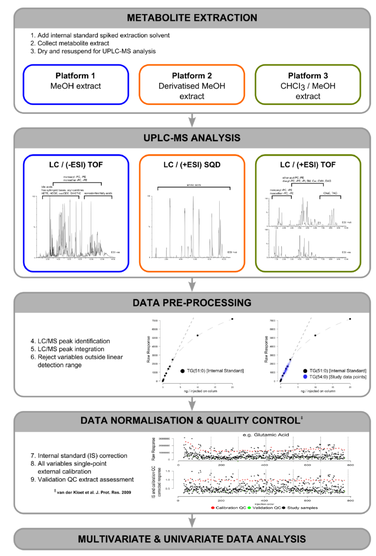
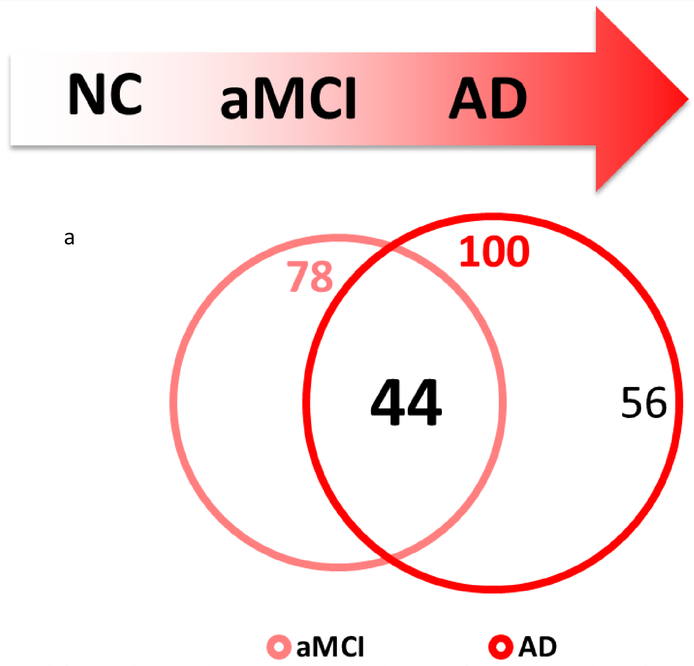


Figure 1. Metabolic profiling workflow

Results



b

NC *vs* aMCI		METABOLITE	NC *vs* AD	
p-value	AUC		p-value	AUC
0.001	0.68	↓ Glutamic acid	<0.001	0.79
0.008	0.61	Alanine ↑	<0.001	0.64
0.008	0.68	↓ Aspartic acid	<0.001	0.71
0.046	0.58	Deoxycholic acid ↑	<0.001	0.64
0.004	0.66	↓ NEFA 22:6n-3 (DHA)	<0.001	0.67
0.030	0.61	PE(36:4) ↑	0.006	0.63
0.032	0.57	↓ SM(39:1)	<0.001	0.64

c

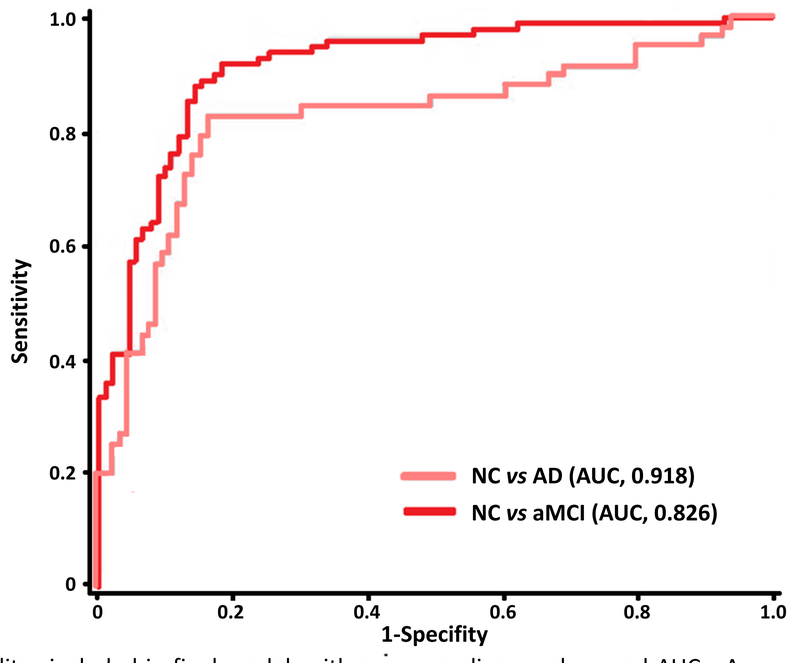


Figure 2. (a) Significant alterations in the levels of 44 metabolites (p <0.05) were detected in both the aMCI and AD groups. (b) The seven metabolites included in final model, with corresponding p-values and AUCs. Arrows indicate direction of change, which was the same in both groups for all metabolites. (c) Performance of the final model when applied to the NC *vs* AD and the NC *vs* aMCI comparisons, based on the full population of each group.

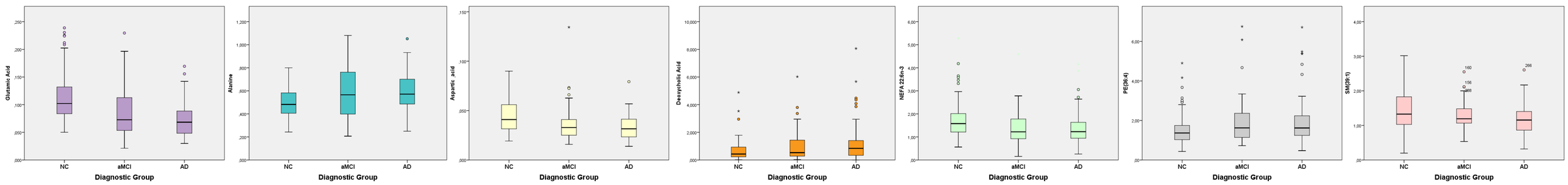


Figure 3. Box plots show comparative levels of each of the seven metabolites included in the final model. Values are expressed relative to batch-averaged quality-control plasma samples (arbitrarily set at 1).

We identified 44 metabolites whose levels were significantly altered (p <0.05) in both aMCI and AD patients (Fig. 2a). These metabolites were used as independent variables to build a multivariate diagnostic algorithm based on a logistic regression model using cross-validation of data. We next developed a classification rule to distinguish the NC from the AD group, the more homogeneous and better diagnosed of the two patient groups. Iterative logistic regression models assuming stepwise selection were used to generate the final 7-metabolite model, which consisted of three amino acids (glutamic acid, alanine and aspartic acid), one non-esterified fatty acid (22:6n-3, DHA), one bile acid (deoxycholic acid), one phosphatidylethanolamine [PE(36:4)] and one sphingomyelin [SM(39:1)] (Fig. 2b). The final model accurately distinguished AD patients from NC controls (AUC, 0.918). Importantly, the model also distinguished aMCI patients from NC controls (AUC, 0.826), supporting its potential diagnostic utility in early disease stages (Fig. 2c). Detailed analysis ruled out the influence of potential confounding variables, including comorbidities and treatments, on each of the seven biomarkers included in the final model (data not shown).

Conclusions

Our findings describe a robust and sensitive biomarker panel that accurately distinguishes AD patients from normal cognition controls, even in early disease stages. This panel constitutes an important tool for the early identification of AD pathophysiology through the analysis of plasma samples.

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