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Exploring Changes in Primary Metabolites in Alzheimer's Disease using Targeted LC-MS/MS

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Introduction

High-throughput and unbiased global profiling metabolomic approaches provide a suitable platform to analyse variations in metabolite levels, offering insights into mechanisms of disease through the discovery of novel biomarkers. Targeted approaches, however, offer a more specific, sensitive and quantitative measure for selected compounds. Here we investigate biochemical changes in enzymatic and metabolic processes at the cellular level in patients with Alzheimer's Disease (AD) using a LC-MS/MS for primary metabolites in plasma. A method using a pentafluorophenylproyl (PFPP) column enables the comprehensive analysis of 97 hydrophilic and hydrophobic primary metabolites. Using this approach the simultaneous analysis of amino acids, organic acids, nucleotides, nucleosides and co-enzymes was performed on 33 plasma samples from AD patients and 43 age- and sex-matched controls.

Methods

Human plasma (20 µL) was prepared using a two-phase extraction with methyl tert-butyl ether (MTBE) and methanol/water. The methanol/water phase was injected directly into the LC-MS/MS. Data were processed using LabSolutions and Traverse™ MS Multivariate Analysis Software for MRM Data (Shimadzu Corporation, Japan), SIMCA P 14.0 (Umetrics, Sweden) and R packages for further statistical analysis.

Liquid chromatography

LC system	: Shimadzu Nexera X2
Column	: Discovery HS F5-3 (150 x 2.1 mm, 3 µm)
Mobile phase A	: 0.1 % formic acid in water
Mobile phase B	: 0.1 % formic acid in acetonitrile
Flow rate	: 0.25 mL/min
Column temperature	: 40 °C
Injection volume	: 0.5 µL
Binary gradient	:

Time (mins)	%B	Time (mins)	%B
0.0	0	15.0	95
2.0	0	20.0	95
5.0	25	20.1	0
11.0	35	25.0	0

Mass spectrometry

MS system	: Shimadzu LCMS-8060
Ionisation	: Heated ESI (positive/negative)
Dwell time	: 5 msec
Pause time	: 1 msec
Polarity switching	: 5 msec
Source temperatures	: Interface: 350 °C
	Heat block: 300 °C
	Desolvation line: 150 °C
Gas flows	: Heated gas (air): 10 L/min
	Drying gas (N2): 10 L/min
	Nebulising gas (N2): 3 L/min

Results and discussion

Figure 1 shows a representative MRM chromatogram of the from a pooled quality control (QC) of all healthy controls and AD patient samples considered in this study. Peak area measurements for 62 metabolites were detected with CV < 30% across QC samples, used to determine the level of metabolic change between control and AD patients. A number of these metabolites exhibited significant changes between control and AD samples by applying a t-test, with a summary of the most significant findings shown in Figure 2.

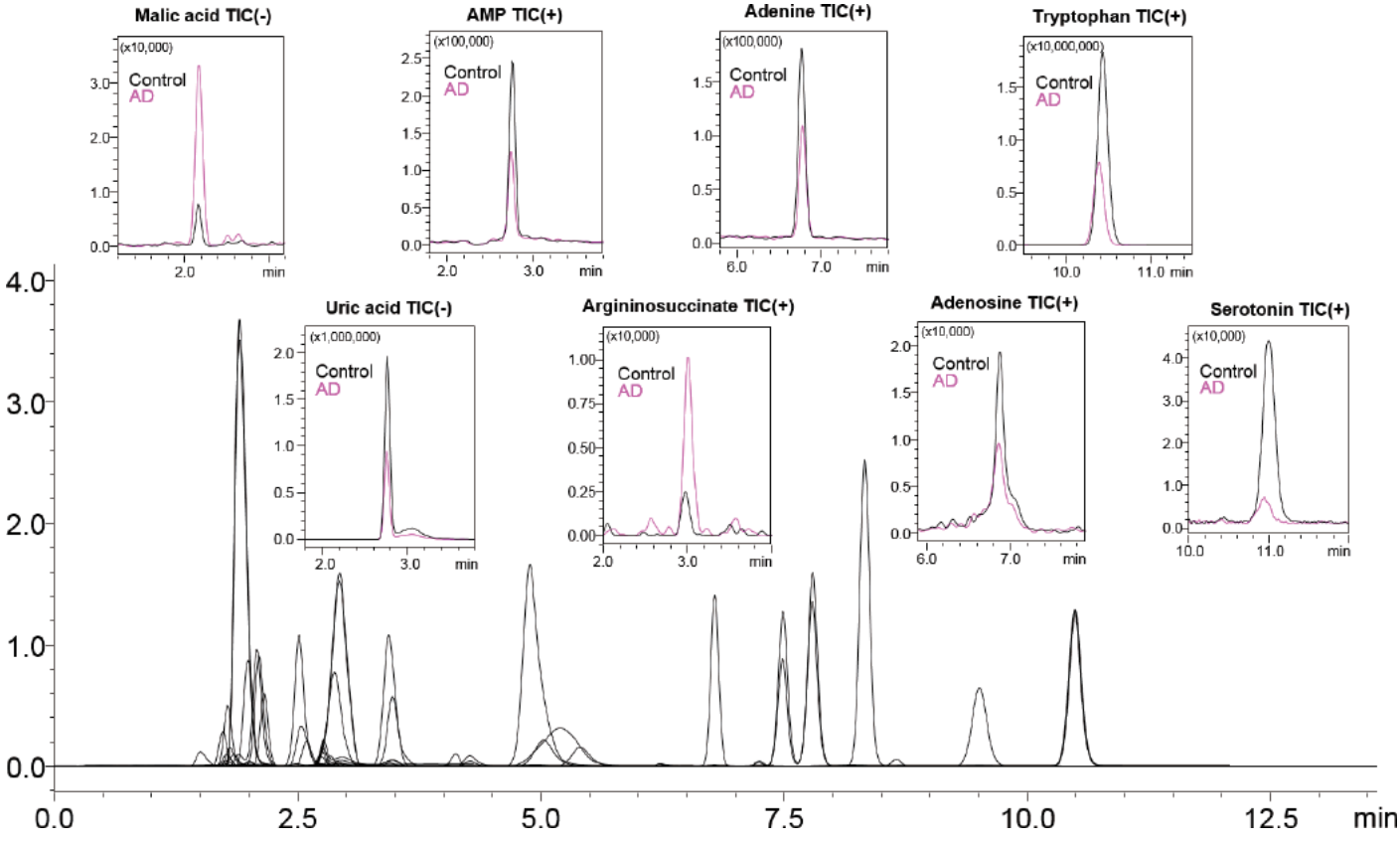

Figure 1 – Chromatogram of 62 metabolites detected in human plasma extract and overlaid metabolites exhibiting significant change between control and AD samples.

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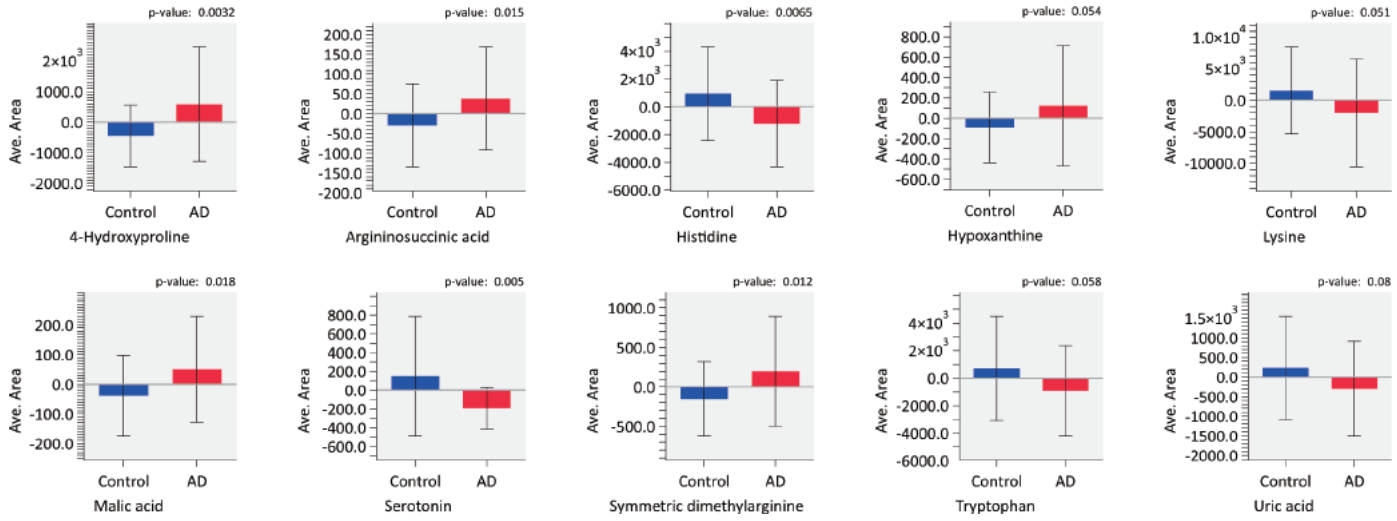

Figure 2 – Pareto scaled plots for metabolites with most significant change between control and AD samples.

Of particular interest were the changes in the expression of neurotransmitters. As an example, the box plots in Figure 3 show the variation in metabolic changes in the serotonin synthesis pathway. From these results, we can see the decrease of tryptophan and serotonin in the AD plasma (Figure 3A and B), which complement similar findings from measurements made in a separate study of brain tissue (Figure 3C and D).

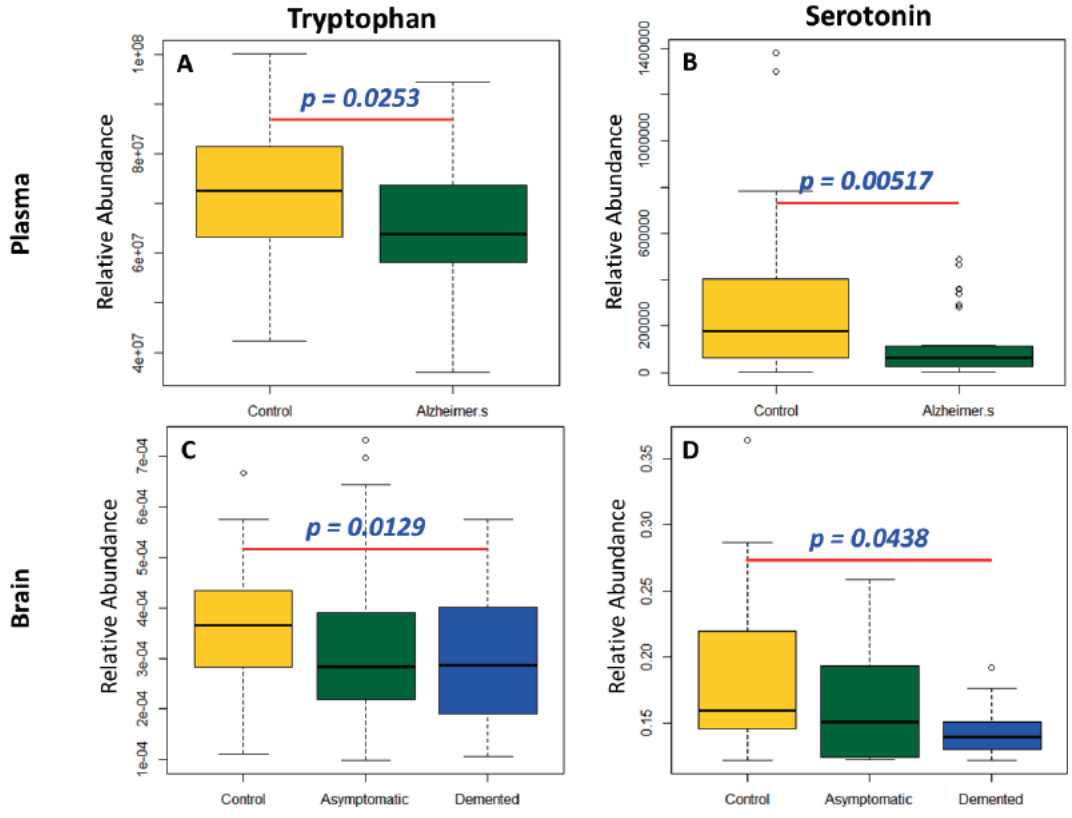

Figure 3 – Box plots highlighting changes in tryptophan and serotonin levels between control and disease groups in plasma (A and B) and in brain tissue (C and D). Brain tissue analysis as part of a separate study; data not shown here.

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A reduction in tryptophan, coupled with an increase in the abundance of the aromatic amino acid decarboxylase (AAAD) inhibitor alpha-synuclein in the brain of AD patients, suggests a mechanism for the reduction of serotonin in the brain. The reduction in the abundance of this monoamine neurotransmitter would result in impaired signal transmission in the brain of AD patients, leading to cognitive function decline (Figure 4).

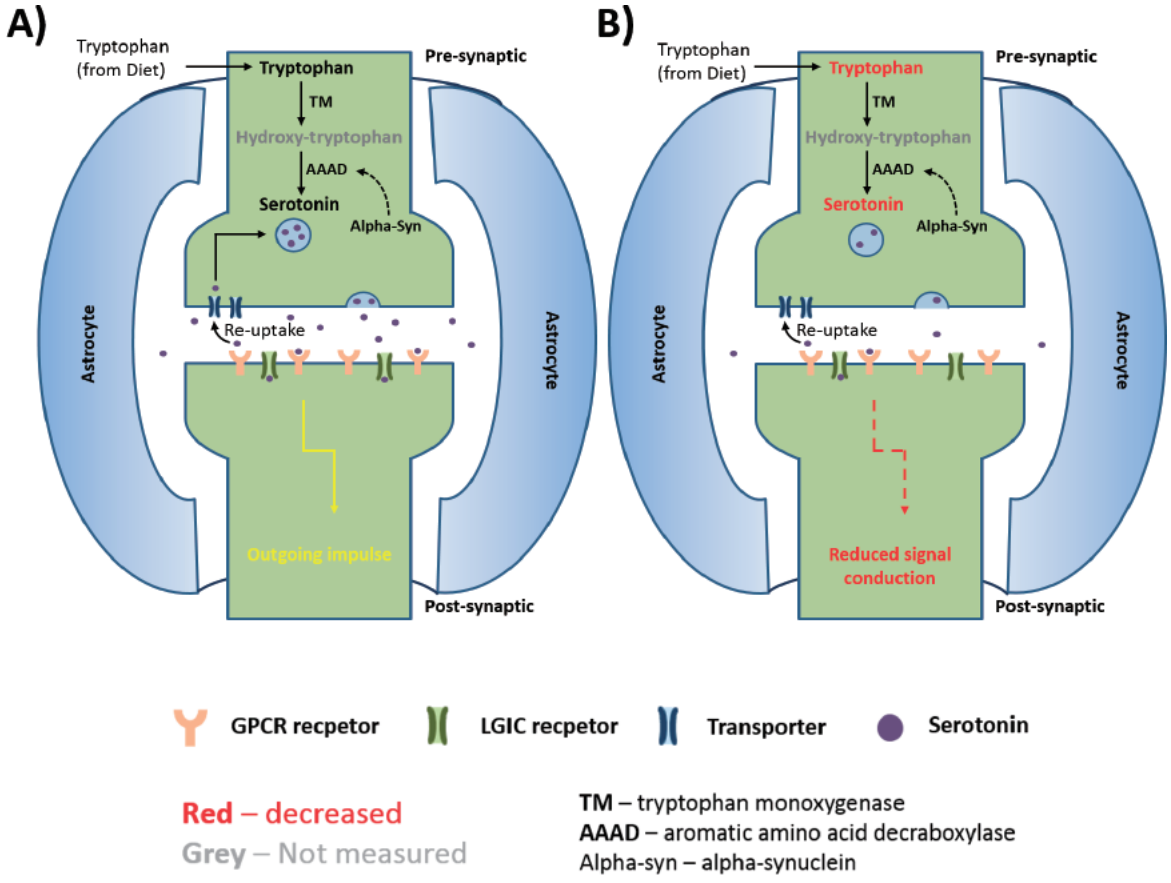

Figure 4 – Illustration of serotonin synaptic transmission with normal (A) and reduced (B) serotonin levels

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Conclusions

- In this presentation we have shown the comprehensive analysis of primary metabolites in human plasma, which revealed metabolic changes between control and AD patients
- This targeted approach focused on primary metabolites provides a sensitive and selective way of exploring biochemical changes leading to AD
- Variation in a number of metabolites suggest perturbations in purine metabolism, the urea/TCA cycle and serotonin metabolism in AD
- Reduced plasma tryptophan and serotonin suggest a disturbance in neurotransmission, changes to which may result in reduced signal transduction in AD patients
- Acetylcholinesterase inhibitors are well established treatments for AD, and the results of this study suggest the need to consider the abundance of other neurotransmitters.

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