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Application of a metabolomic multiplatform to investigate Alzheimer's disease pathogenesis

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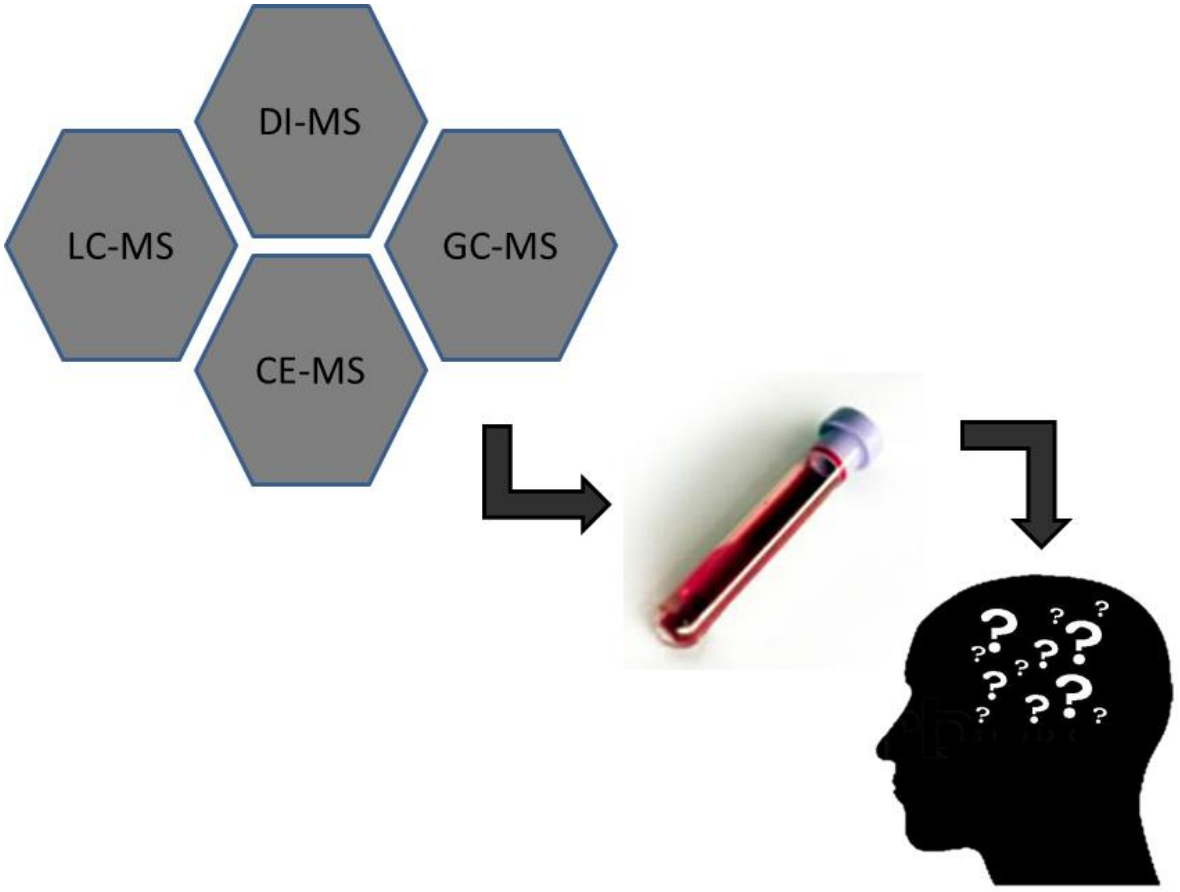
Application of a metabolomic multiplatform to investigate Alzheimer's disease pathogenesis

Graphical Abstract



Abstract:
Alzheimer's disease (AD) is the most common neurodegenerative disorder, but nowadays there is no cure mainly because its etiology is still unclear. With the aim to get a comprehensive overview of pathological mechanisms associated with AD, complementary metabolomic platforms were developed, including screening procedures based on direct mass spectrometry analysis and hyphenated approaches with orthogonal separation mechanisms such as liquid chromatography, gas chromatography and capillary electrophoresis. The application of these techniques to serum samples from patients suffering from Alzheimer's disease and mild cognitive impairment enabled the identification of numerous metabolic alterations linked to pathogenesis of this disorder and its progression from pre-clinical stages, including abnormalities in the composition of membrane lipids, deficits in energy metabolism and neurotransmission, and oxidative stress, among others. In turn, these metabolomics perturbations were also observed in multiple biological compartments from the APP/PS1 model, including serum, brain, liver, kidney, spleen and thymus, thus demonstrating the utility of these transgenic mice to model Alzheimer's disease. The comparison of different brain regions evidenced that the most affected areas are hippocampus and cortex, but other regions were also significantly perturbed to a lesser extent. Furthermore, alterations detected in peripheral organs confirm the systemic nature of this neurodegenerative disorder.

Keywords: metabolomics; mass spectrometry; Alzheimer's disease

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Introduction

Alzheimer's disease (AD): most common neurodegenerative disorder among older people

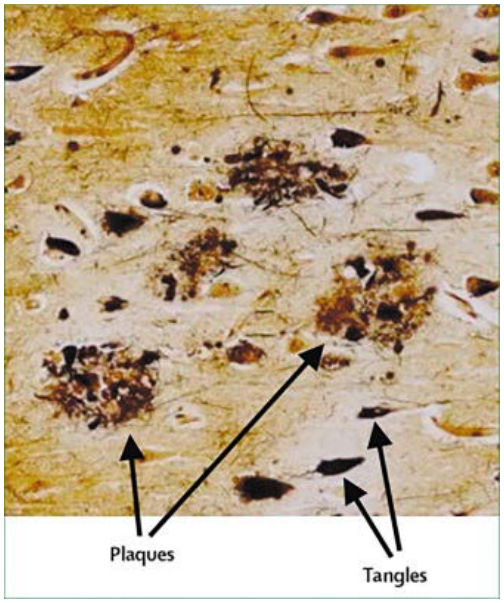
- Insidious onset
- Progressive decline of cognitive functions

Mild cognitive impairment (MCI)

- Incipient dementia
- Preclinical phase of AD

Etiology of Alzheimer`s disease

- Loss of neurons and synapses
- Unknown causes
- Multifactorial pathology
- Systemic disease



- ➢ Genetic factors
- ➢ Inflammation
- ➢ Oxidative stress
- ➢ Metal homeostasis
- ➢ Amyloid hypothesis
- ➢ Tau hypothesis

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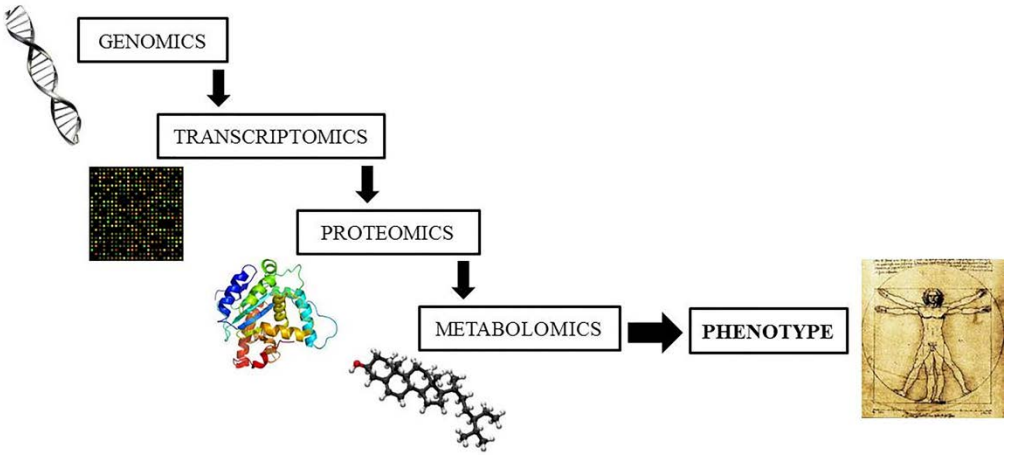
Introduction

Diagnosis *via* **clinical criteria** (NINCDS-ADRDA*)

Limitations

- Definitive diagnosis requires post-mortem confirmation
- Early detection is not possible
- Low specificity and sensitivity



Discovery of new diagnostic **biomarkers**

Systems biology (omic sciences)

*NINCDS-ADRDA: *National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association*

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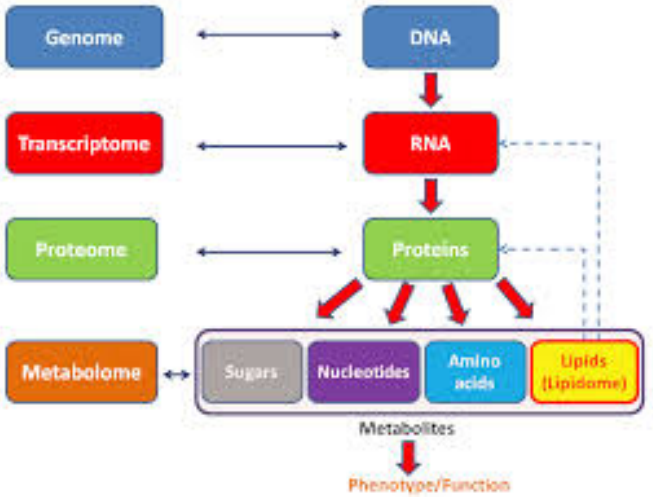
Introduction

Metabolomics: comprehensive study of the entire set of metabolites from a biological system as well as of metabolic changes produced in response to a genetic or environmental perturbation

- Chemical heterogeneity
- Wide range of concentrations
- Temporal and inter-individual variability



Analytical Techniques in Metabolomics

- Sensitivity
- Versatility

Mass Spectrometry

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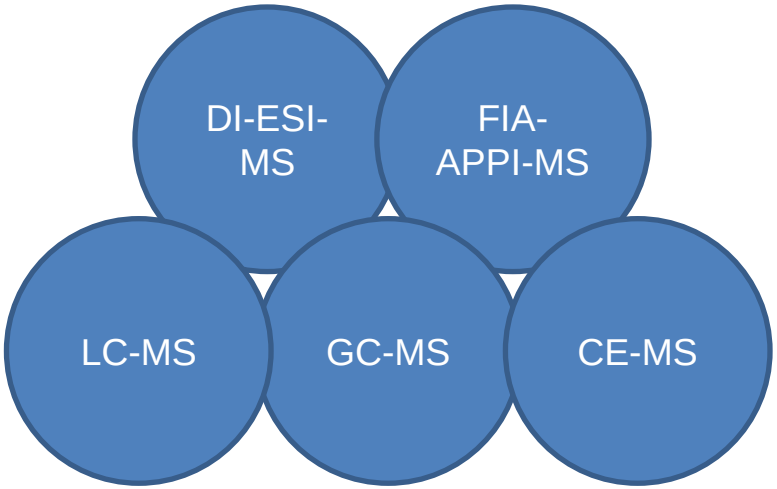
Results and discussion

Complementary Metabolomic Platforms



Workset 1

Aged individuals (N=137)

- Alzheimer's disease (AD)
- Mild cognitive impairment (MCI)
- Healthy controls

Workset 2

APP/PS1 and wild type mice (N=60)

- blood serum
- brain
  - cortex
  - hippocampus
  - cerebellum
  - striatum
  - Olfactory bulbs
- metabolically active organs
  - liver
  - kidney
- immune system organs
  - spleen
  - thymus

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Results and discussion

Sample Treatment

Serum Extraction

100 µl serum + 400 µl MeOH:EtOH 50%

Supernatant **POLAR EXTRACT**

Precipitate + 400 µl $MeOH:CHCl_3$ 50%

LIPOPHILIC EXTRACT

Tissue Extraction

30 mg tissue + 300 µl MeOH, FA 0.1%

Supernatant **POLAR EXTRACT**

Precipitate + 300 µl $MeOH:CHCl_3$ 2:1, NH_4F 10 mM

LIPOPHILIC EXTRACT

Derivatization (GC-MS)

50 µl polar extract + 50 µl MeOX 20 mg/mL 80ºC, 15 min → + 50 µl MSTFA 80ºC, 15 min → GC-MS

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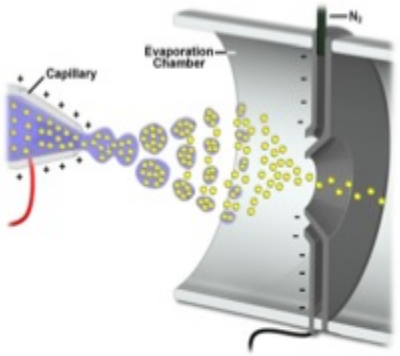
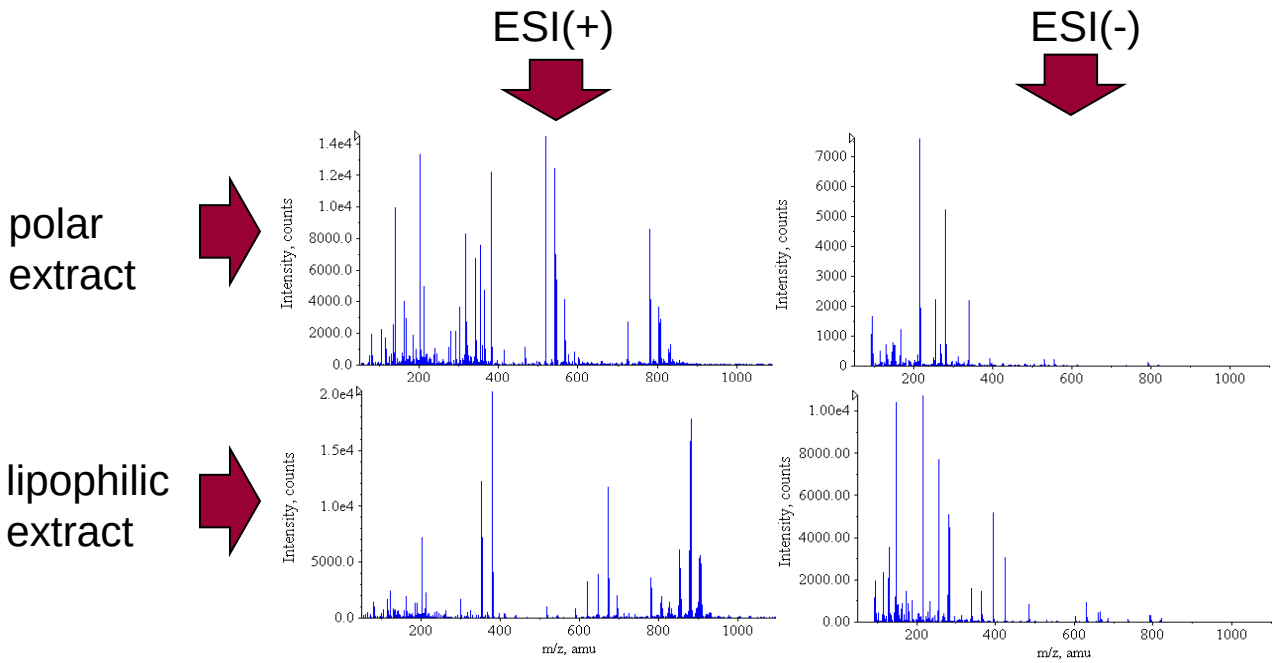
Results and discussion

DI-ESI(+/-)-QTOF-MS



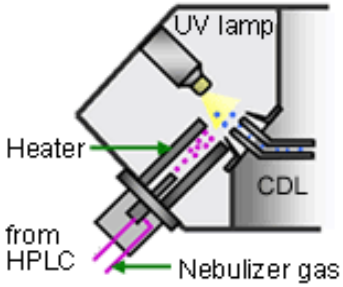
	ESI(+)	ESI(-)
ion spray voltage	3300 V	-4000 V
declustering potential	60 V	-100 V
focusing potential	250 V	-250 V
curtain gas (N_2)	1.13 L/min	
nebulizer gas (N_2)	1.56 L/min	
heater gas (N_2)	0	
source temperature	60 ºC	
m/z range	50-1100	
flow rate	5 μL/Min	



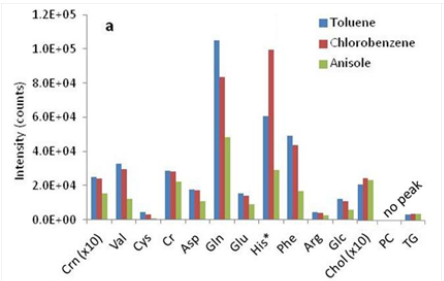
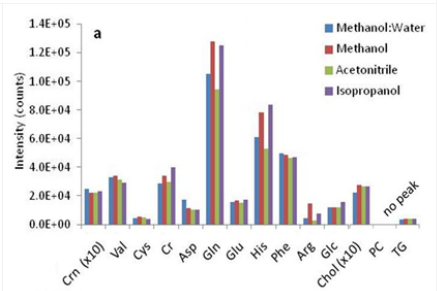
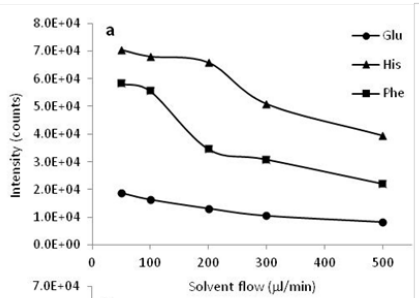
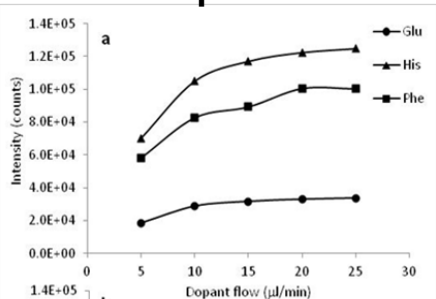
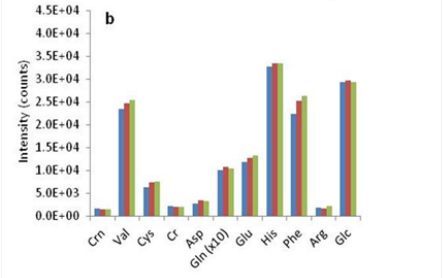
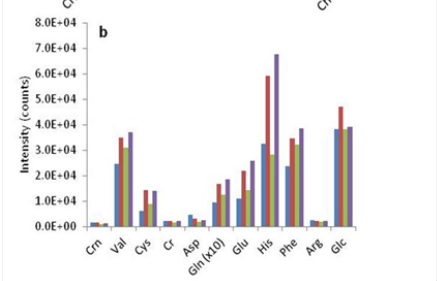
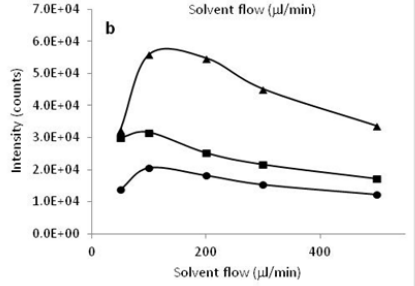
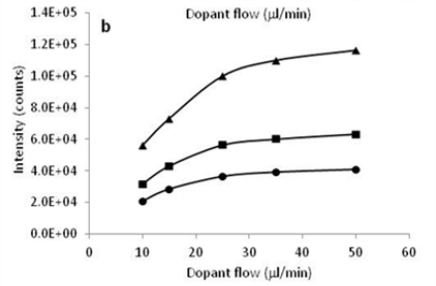
Anal Bioanal Chem 2014, **406**:7137-7148

Results and discussion

FIA-APPI(+/-)-QTOF-MS



$$D + h\nu \rightarrow D^{\bullet+}$$
$$D^{\bullet+} + AB \rightarrow D + AB^{\bullet+}$$
$$D^{\bullet+} + MP \rightarrow [D - H]^{\bullet} + [MP + H]^{+}$$
$$[MP + H]^{+} + AB \rightarrow MP + [AB + H]^{+}$$

	dopant	carrier solvent	carrier flow	dopant flow
APPI(+)				
APPI(-)				

Talanta 2015, **131**:480-489

Results and discussion

Direct Mass Spectrometry Analysis

Advantages

- Short Analysis Time
- Non-discriminant character
- Instrumental simplicity

Disadvantages

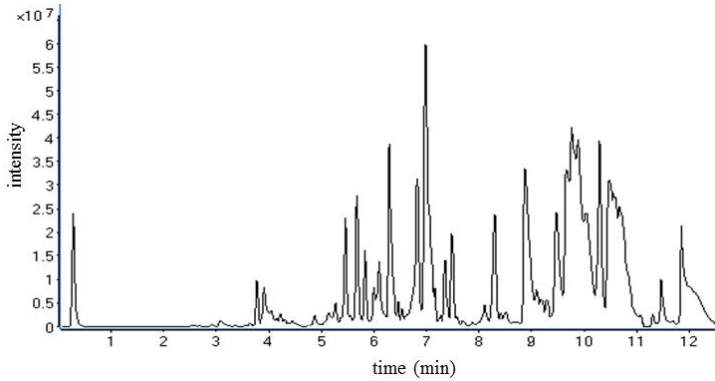
- Ion suppression
- Isomers

***Screening* techniques**

UPLC/GC/CE-MS

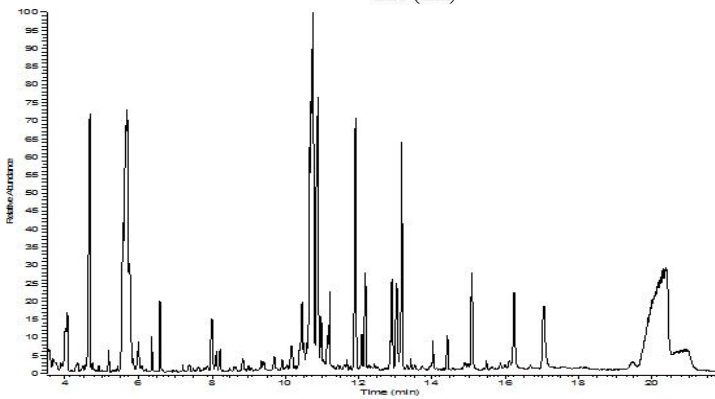
RP-UHPLC-MS

Curr Alzheimer Res 2016, **13**:641-653



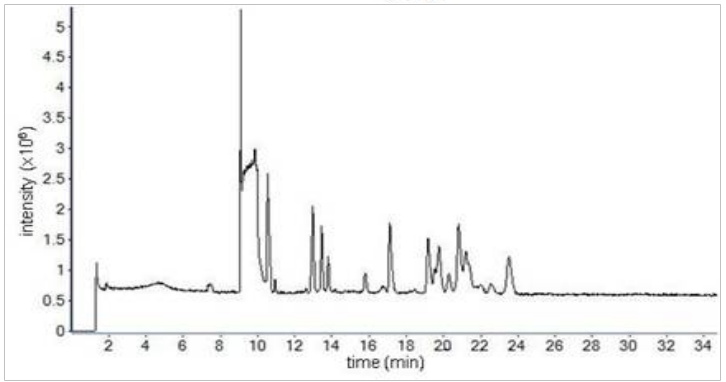
GC-MS

J Pharm Biomed Anal 2015, **107**:75-81



CE-MS

Electrophoresis 2014, **35**:3321-3330



Results and discussion

- **Serum**: similar metabolic alterations between AD patients and APP/PS1 mice
- **Brain**: hippocampus and cortex are the most affected regions
- **Peripheral organs**: systemic nature of AD

J Pharm Biomed Anal 2015, **107**:378-385

Biochimie 2015, **110**:119-128

J Pharm Biomed Anal 2015, **102**:425-435.

Biochim Biophys Acta 2014, **1842**:2395-2402

Electrophoresis 2015, **36**:2237-2249

Mol Biosystems 2015, **11**:2429-2440

Electrophoresis 2015, **36**:577-587

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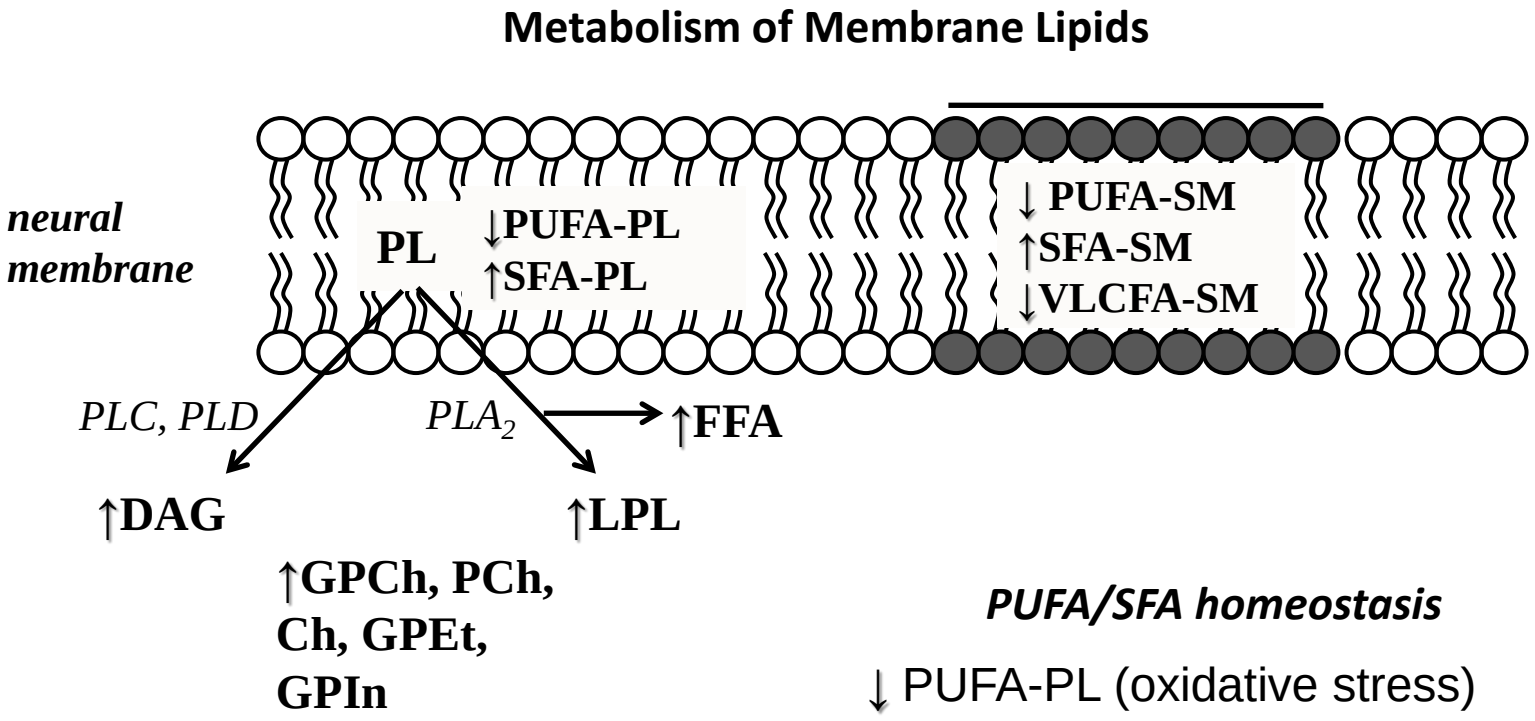
Results and discussion



PUFA/SFA homeostasis

↓ PUFA-PL (oxidative stress)

SFA/PUFA-PL imbalance

Neuronal membrane breakdown

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Results and discussion

Oxidative stress

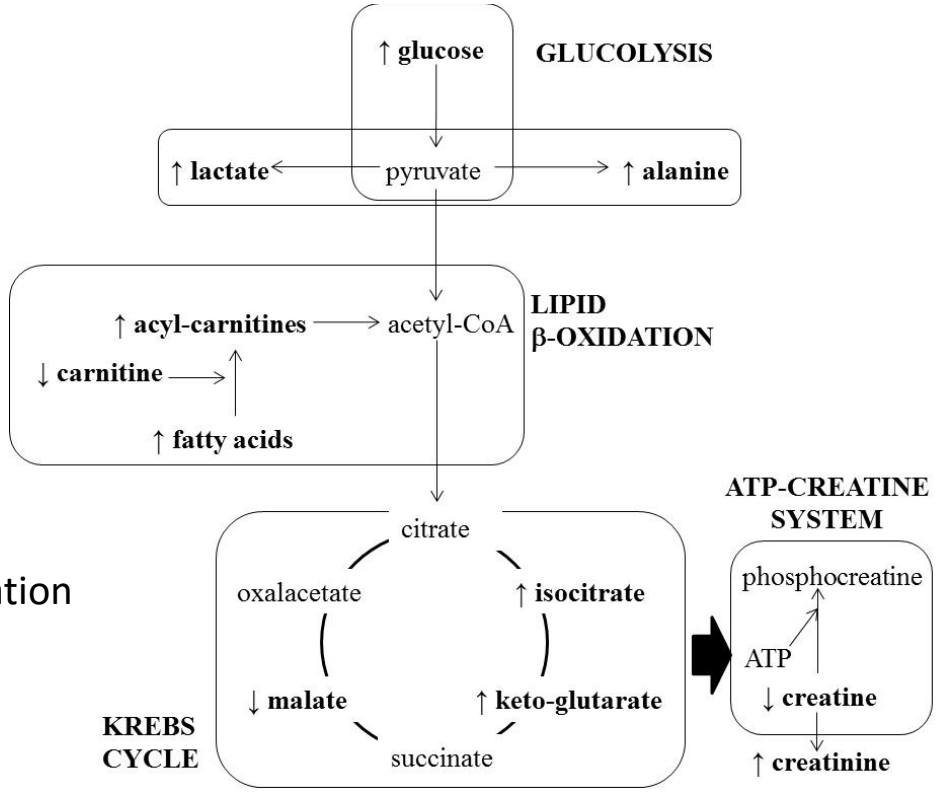
↓ antioxidants

- Uric acid
- Histidine & imidazole ➡ carnosine
- Cystine & pyroglutamic acid ➡ glutathione

↑ oxidation products

- Eicosanoids (prostaglandins, LTB4) ➡ lipid peroxidation
- Adenosine ➡ nucleotides oxidation

Energy Metabolism



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Results and discussion

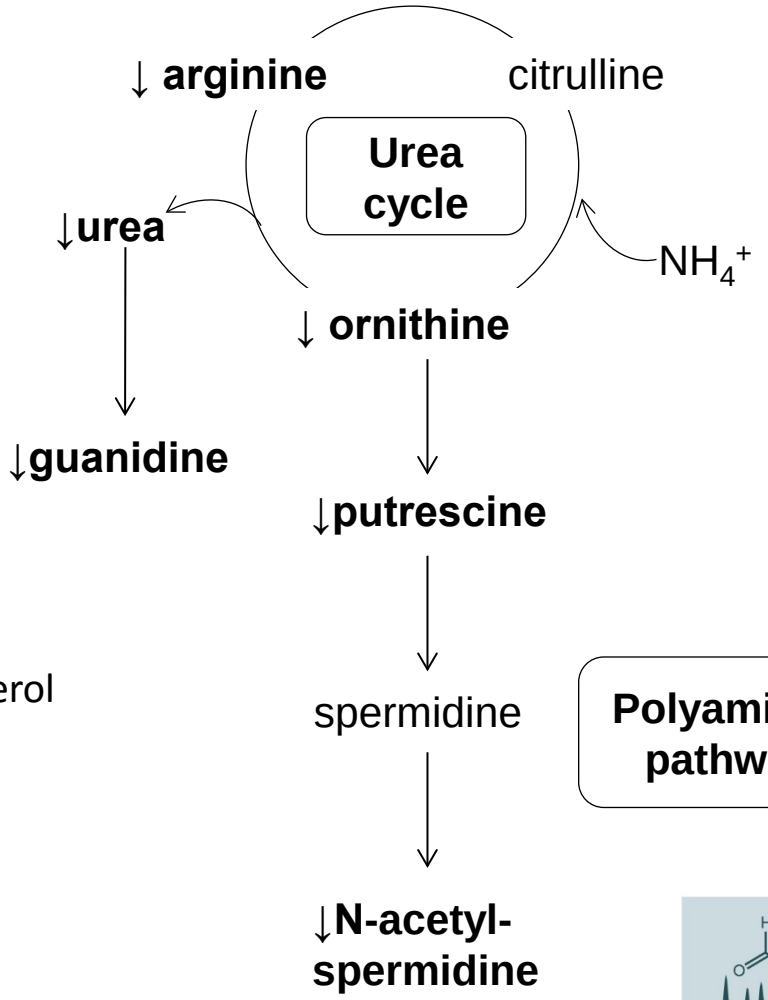
Neurotransmission

↓ dopamine, serotonin, Tyr, Trp, Phe

↓ Glu, Gln, N-Ac-Gln

↓ MAG, FAA

Hyperammonemia



Vascular Risk Factors

- hyperlipidemia → ↑ TAG, cholesterol
- NO synthetase inhibition → ↑ ADMA
- hyper-homocysteinemia → ↑ HCys, ↓ Met
- renin-angiotensin system → ↑ Phe-Phe

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Conclusions

- Metabolomic platforms based on **direct mass spectrometry analysis** (DI-ESI-MS, FIA-APPI-MS) show a great potential in order to perform a first metabolic **screening** due to its wide metabolome coverage, reduced analysis time and instrumental simplicity

- The combination of **orthogonal separation techniques** allows performing a more comprehensive investigation of the entire metabolome

RP-UHPLC-MS GC-MS CE-MS

- The application of these metabolomic approaches enabled the detection of numerous **metabolic alterations** associated to AD **pathogenesis** and **progression** from MCI (abnormal metabolism of membrane lipids, failures in neurotransmission, deficit in energy metabolism, oxidative stress)

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Conclusions

- Metabolomic study of the **APP/PS1** transgenic mouse allowed performing a holistic investigation about pathological mechanisms associated with the development of Alzheimer's disease in multiple biological compartments

- **Serum** metabolomic profiles from patients and APP/PS1 mice showed great similarities, thus demonstrating the potential of this transgenic mouse to model AD

- Comparative analysis of different brain regions showed that the most affected areas by the characteristic neuropathology of Alzheimer's disease in the APP/PS1 mouse were **hippocampus** and **cortex**, although other regions were also disrupted to a lesser extent, including the striatum, cerebellum and olfactory bulbs.

- Metabolomic profiles of **liver, kidney, spleen and thymus** showed significant changes in levels of multiple metabolites, common to those previously described in serum and brain, corroborating the systemic nature of this neurodegenerative disorder

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Acknowledgments

European Union