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The combination of complementary metabolomic platforms to unravel Alzheimer's disease pathogenesis

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Abstract: *Alzheimer's disease (AD) is the most common neurodegenerative disorder among older people, characterized by an insidious onset and a progressive decline of cognitive functions. Nowadays, there is no cure for AD mainly because its etiology is still unclear and current diagnostic tests show great limitations, including low sensitivity and specificity, as well as the impossibility to detect characteristic symptoms at early stages of disease. Thus, the main objective of this work was the optimization of complementary metabolomic approaches based on mass spectrometry in order to investigate AD pathogenesis and discover potential biomarkers for diagnosis. With the aim to get a comprehensive metabolome coverage, multiple analytical 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. Accordingly, it could be concluded that the combination of complementary metabolomic platforms allows studying etiology associated with Alzheimer's disease in a deeper manner.*

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Graphical Abstract:
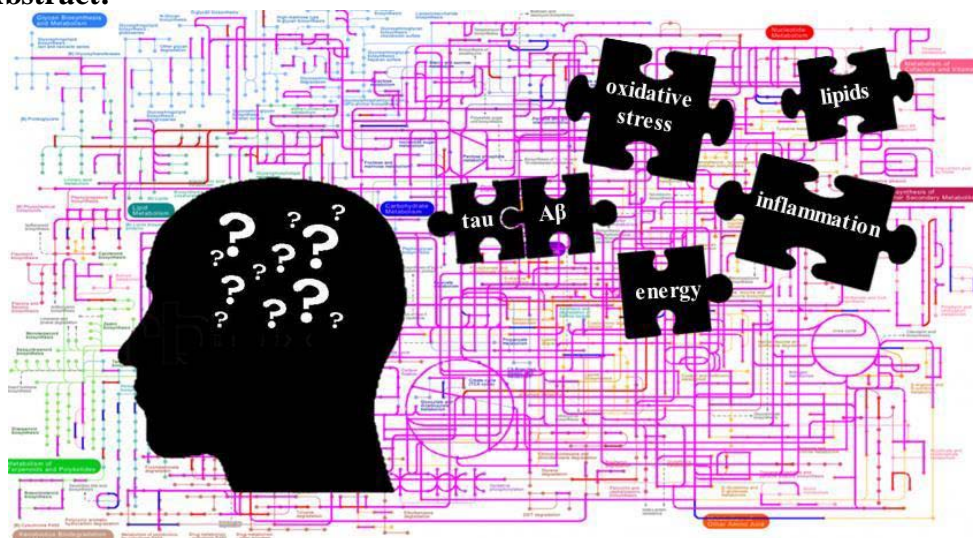
Introduction:

Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by a multifactorial pathogenesis, still not completely understood, in which numerous pathological processes are involved, including the deposition of β-amyloid plaques and neurofibrillary tangles, inflammation, oxidative stress, and abnormal metal homeostasis, among many others. Furthermore, current diagnostic tests for AD show great limitations, including low sensitivity and specificity, as well as the impossibility to detect characteristic symptoms at early stages of disease. For these reasons, the discovery of novel AD biomarkers is crucial in order to identify key features of the underlying pathology and develop accurate diagnostic methods. In this context, metabolomics plays a prominent role because of its potential to provide a global overview of altered biochemical pathways in response to genetic or environmental factors.

Materials and Methods:

Blood serum samples from AD patients and healthy controls were subjected to multiple complementary metabolomic platforms based on mass spectrometry, including direct infusion electrospray mass spectrometry (DI-ESI-MS) [1], flow injection atmospheric pressure photoionization mass spectrometry (FI-APPI-MS) [2], reversed phase ultra-high performance liquid chromatography mass spectrometry (RP-UHPLC-MS) [3], gas chromatography mass spectrometry (GC-MS) [4] and capillary electrophoresis mass spectrometry (CE-MS) [5].

Data were then submitted to multivariate and univariate statistical analysis in order to find significant metabolic perturbations between groups.

Results and Discussion:

Metabolomics based on direct mass spectrometry analysis showed a great potential to perform a preliminary metabolic screening due to its reduced analysis time and instrumental simplicity. For this purpose, serum samples were treated according to a two-step extraction protocol and then analyzed by high resolution mass spectrometry with combined electrospray (ESI) and atmospheric pressure photoionization (APPI) sources, in both positive and negative ionization modes, in order to maximize metabolome coverage [1,2]. Complementarily, these samples were also fingerprinted with three complementary metabolomic platforms based on the coupling of orthogonal separation techniques with mass spectrometry. Analysis by RP-UHPLC-MS revealed significant alterations in serum levels of numerous lipids, such as phospholipids, sphingolipids, acyl-glycerides and acyl-carnitines, thus demonstrating the potential of this platform for the investigation of low polarity compounds [3]. Alternatively, the use of GC-MS enabled the detection of several low molecular weight metabolite classes, including carbohydrates, amino acids, fatty acids and organic acids [4]. To conclude, CE-MS was proposed for studying the polar fraction of the serum metabolome [5]. The application of these metabolomic tools to serum samples from patients with Alzheimer's disease and healthy

controls allowed the identification of numerous pathological mechanisms associated with the pathogenesis of this disorder. Thus, some of the most important findings of this study were the detection of significant changes in the composition of membrane lipids, deficits in energy metabolism and neurotransmitter systems, oxidative stress, hyperammonemia or hyperlipidemia, among others.

Conclusions:
The combination of multiple complementary metabolomic approaches allows investigating in depth the etiology associated with Alzheimer's disease, as well as the discovery of potential biomarkers for diagnosis.

Conflicts of Interest:
The author declares no conflict of interest

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