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Research paper

Deciphering metabolic abnormalities associated with Alzheimer's disease in the APP/PS1 mouse model using integrated metabolomic approaches

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ABSTRACT

The transgenic mouse APP/PS1 is widely employed by neuroscientists because reproduces well some of the neuropathological and cognitive deficits observed in human Alzheimer's disease. In this study, serum samples from APP/PS1 mice (n = 30) and wild-type controls (n = 30) were analyzed using a metabolomic multiplatform based on the combination of gas chromatography-mass spectrometry and ultra-high performance liquid chromatography-mass spectrometry, in order to obtain wide information about serum metabolome. Metabolic profiles showed significant differences between the groups of study, and numerous metabolites were identified as potential players in the development of Alzheimer-type disorders in this transgenic model. Pathway analysis revealed the involvement of multiple metabolic networks in the underlying pathology, such as deficiencies in energy metabolism, altered amino acid homeostasis, abnormal membrane lipid metabolism, and other impairments related to the integrity of the central nervous system. It is noteworthy that some of these metabolomic markers are in accordance with pathological alterations observed in human Alzheimer's disease, while others have not been previously described. Therefore, these results demonstrate the potential of metabolomics and the use of transgenic animal models to understand the pathogenesis of Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD), the most common neurodegenerative disorder worldwide, is characterized by an insidious onset and a progressive decline of cognitive functions. It is recognized that genetics plays an important role in AD, principally due to mutations in the genes of amyloid precursor protein (APP) and presenilin 1 and 2 (PS1 and PS2), which lead to the accumulation of Aβ peptides [1]. However, the exact etiology and pathogenesis of this disorder is not clear, given that its study is hindered by the complex underlying biochemistry, the long presymptomatic period, the inability to study microscopic morphological changes in tissues until the final stage of the disease and the variability of clinical expression of symptoms. Thereby, nowadays AD can only be detected at

Abbreviations: AD, Alzheimer's disease; GC–MS, gas chromatography mass spectrometry; UHPLC-MS, ultra-high performance liquid chromatography mass spectrometry; TG, transgenic; WT, wild type; QC, quality control; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; VIP, variable importance in the projection; HEPE, hydroxyl-eicosapentaenoic acid; LPC, lyso-phosphocholine; LPI, lyso-phosphoinositol; LPE, lyso-phosphoethanolamine; PI, phosphoinositol; PC, phosphocholine; PE, phosphoethanolamine; PPE, plasmenylethanolamine; PPC, plasmenylcholine; SM, sphingomyelin.

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advanced stages of disease by exclusion of other pathologies based on clinical criteria defined by the NINCDS-ADRDA, and a definitive diagnosis is only made when there is histopathological confirmation [2]. Therefore, early preclinical detection has become a primary focus of AD research. Numerous transgenic mouse models that mimic the main features of Alzheimer's disease have been developed to better understand the pathogenic mechanism of neurodegeneration and to test potential therapies [3]. In most of these models, the transgenic animals over-express mutated forms of human amyloid precursor protein, developing amyloid plaques with aging predominantly in the hippocampus and cortex. However, it has been demonstrated that co-expression of mutated human presenilin 1 or presenilin 2 in the model accelerates amyloid deposition. In this sense, the double mutant transgenic line APP/PS1 has been widely used in neuroscience studies given that reproduce some of the neuropathological and cognitive deficits observed in AD, with a phenotype characterized by early amyloid deposits and behavioral deficits [4].

Metabolomics presents a high potential in health survey and biomarkers discovery, because changes in specific groups of metabolites may be sensitive to pathogenically relevant factors. Thus, metabolomic profiling is emerging as a powerful tool for the characterization of complex phenotypes affected by both genetic and environmental factors [5]. Particularly, metabolomics plays a prominent role in biomarker identification of multifunctional disorders such as Alzheimer's disease, in which many heterogeneous cellular processes are involved. Numerous studies have been performed to assess the pathophysiological status of transgenic animals by postmortem analysis of brain tissue samples [6–9]. However, the use of peripheral biofluids has been only scarcely considered, although the discovery of a well-established peripheral biomarker easily accessible is of primary importance when considering the prevalence of this disease [10]. Jiang et al. found significant differences in endogenous metabolites from serum of the senescence-accelerated mouse, suggesting perturbed glucose and lipid metabolisms, and attenuated protective function of inosine [11]. In other studies, the metabolic profiles of both brain and plasma of transgenic mice were characterized and compared to those of wild-type mice [12,13]. Lower levels of metabolites were found in plasma samples, and they fluctuate more between the two groups than brain metabolites. However, the statistical models built using plasma metabolite profiles were more accurate than brain tissue despite the smaller number of factors. Finally, metabolomics has been also applied to examine changes in urinary metabolites of transgenic AD mice, demonstrating the utility of a very simple and easily available sample in clinical laboratory as is urine for the search of potential biomarkers [14,15].

In the study presented here, serum samples from APP/PS1 transgenic mice were analyzed by a metabolomic multiplatform combining gas chromatography-mass spectrometry (GC–MS) and ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS). No single analytical technique covers the entire spectrum of the metabolome, so the application of combined LC-MS and GC–MS technologies is becoming the most relevant tool for biomarkers discovery due to their complementarity. Gas chromatography-mass spectrometry has been traditionally employed for the profiling of low molecular weight metabolites with high sensitivity, peak resolution and reproducibility [16]. On the other hand, reversed-phase ultra-high performance liquid chromatography-mass spectrometry has become the main workhorse in metabolomics due to its high resolution and sensitivity, fast analysis and good potential for biomarker identification, which provides efficient retention and separation of relatively nonpolar metabolites across a large molecular weight range [17]. Thus, the application of this global metabolomic approach using two complementary platforms allowed a broad analytical coverage of serum metabolites, from small polar metabolites to lipids. Then, multivariate statistical analysis was used to differentiate serum metabolomic profiles of APP/PS1 mice from wild-type controls, and to identify perturbations in biochemical pathways related to pathological processes.

2. Material and methods

2.1. Animal handling

Transgenic APP/PS1 mice (C57BL/6 background) were generated as previously described by Jankowsky et al., expressing the Swedish mutation of APP together with PS1 deleted in exon 9 [18]. On the other hand, age-matched wild-type mice of the same genetic background (C57BL/6) were purchased from Charles River Laboratory for their use as controls. In this study, male and female animals at 6 months of age were used for experiments (TG: N = 30, male/female 13/17; WT: N = 30, male/female 15/15). Animals were acclimated for 3 days after reception in rooms with a 12-h light/dark cycle at 20–25 °C, with water and food available *ad libitum*. Then, mice were anesthetized by isoflurane inhalation and blood was extracted by cardiac puncture. Blood samples were immediately cooled and protected from light for 30 min to allow clot retraction, and then centrifuged at 3500 rpm for 10 min at 4 °C. Serum was aliquoted in Eppendorf tubes and frozen at −80 °C until analysis. Animals were handled according to the directive 2010/63/EU stipulated by the European Community, and the study was approved by the Ethical Committee of University of Huelva.

2.2. Serum samples preparation

For the extraction of metabolites, 100 μL of serum were mixed with 400 μL of methanol/ethanol (50% v/v) and vortexed for 5 min. Then, samples were centrifuged at 4000 rpm for 10 min at 4 °C, and the supernatant was transferred to another tube to be dried under nitrogen stream. Finally, the resulting residue was reconstituted with methanol/water (80:20 v/v) containing 0.1% formic acid. An aliquot of this extract (50 μl) was split for derivatization before GC–MS fingerprinting, and the rest of the sample was transferred to the injection vial for LC-MS analysis. Derivatization was carried out according to the two step methodology proposed by Begley et al. [19]. For this, 50 μl of extracts were dried under nitrogen stream and redissolved in 50 μL of 20 mg mL^{-1} methoxyamine in pyridine for protection of carbonyl groups by methoximation. After briefly vortexing, samples were incubated at 80 °C for 15 min in a water bath. Then silylation was performed by adding 50 μL of MSTFA (N-methyl-N-trimethylsilyl trifluoroacetamide) and incubating at 80 °C for a further 15 min. Finally, extracts were centrifuged at 4000 rpm for 1 min and supernatant was collected for analysis.

2.3. Metabolomic profiling by GC–MS

Analyses were performed in a Trace GC ULTRA gas chromatograph coupled to an ion trap mass spectrometer detector ITQ 900 (Thermo Fisher Scientific), using a Factor Four capillary column VF-5MS 30 m × 0.25 mm ID, with 0.25 μm of film thickness (Varian). The GC column temperature was set to 100 °C for 0.5 min, and programmed to reach 320 °C at a rate of 15 °C per minute. Finally, this temperature was maintained for other 2.8 min, being the total time of analysis 18 min. The injector temperature was kept at 280 °C, and helium was used as carrier gas at a constant flow rate of 1 ml min^{-1}. For mass spectrometry detection, ionization was carried out by electron ionization (EI) using a voltage of 70 eV, and the