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Blood-Based Biomarkers

The ratio of phosphatidylcholines to lysophosphatidylcholines in plasma differentiates healthy controls from patients with Alzheimer's disease and mild cognitive impairment

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Abstract

Background: Metabolomic processes have been identified as being strongly linked to the development of Alzheimer's disease (AD). Thus, lipid metabolites appear to be highly useful as diagnostic substrates for the diagnosis of AD and mild cognitive impairment (MCI) in plasma.
Methods: We analyzed plasma samples from controls (n = 35), MCI (n = 33), and AD patients (n = 43) using the AbsoluteIDQ p180 Kit (Biocrates Life Sciences), which included quantitative analysis of 40 acylcarnitines, 21 amino acids, 19 biogenic amines, 15 sphingolipids, 90 glycerophospholipids, and sum of hexoses.
Results: We found that individual lipid metabolites can differentiate controls from MCI and AD with relevant significance. However, the ratio between PC aa C34:4 and lysoPC a C18:2 differentiates controls from MCI ($P = .0000007$) and from AD ($P = .0000009$) with greater significance.
Conclusions: The results provide evidence that the ratio of these two lipid metabolites is useful for diagnosing MCI and AD with an accuracy of 82%–85%.

Keywords: Alzheimer's disease; Mild cognitive impairment; Diagnosis; Plasma; Metabolomics

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease of the brain characterized by extracellular β-amyloid plaques, intraneuronal neurofibrillary tau tangles, inflammation and glial responses, vascular dysfunction, cholinergic neurodegeneration, and synapse loss that directly correlates with cognitive decline and memory loss. The causes of AD are not known but the most prominent hypothesis is the β-amyloid cascade hypothesis [1]. However, dysfunctions in tau phosphorylation may also play a role independent of β-amyloid [2] but recent evidence indicates that both proteins interact [3]. More and more clinical and basic data show that a vascular risk factors may play a role in the development of AD [4] and that a dysfunction of the blood-brain barrier may also account for dysregulated clearing of β-amyloid from the brain [5].

The diagnosis of possible or probable AD is made on the basis of a time-consuming psychological test and clinical examination by excluding other psychiatric and neurologic diseases. Brain imaging and the analysis of cerebrospinal fluid (CSF) samples are important but expensive tools for verifying the diagnosis. Due to the invasive nature of CSF collection, blood biomarkers need to be found to allow screening and multiple analyses of patients, especially those with mild cognitive impairment (MCI). There is more and more evidence to show that a single biomarker cannot yield enough sensitivity and specificity to diagnose AD [6,7].

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Thus, multiple analyses and the generation of a patient-specific signature are state-of-the-art.

In 2007, Ray et al. [8] claimed to diagnose AD from plasma using a panel of 18 biomarkers. However, several groups including ours failed to reproduce this finding [9,10]. Recently, Mapstone et al. [11] demonstrated that a set of 10 endogenous lipids from peripheral blood can predict phenoconversion to either amnestic MCI or AD within a 2–3 year time frame with over 90% accuracy. In fact, there are clear indications that metabolic processes are linked to the development and pathology of AD [12,13] and metabolomics is turning out to be a novel fascinating method for analyzing a large panel of lipid metabolites [14]. Two recent articles clearly demonstrate that plasma lipidomics is associated with AD [15] and that a blood-based 7-metabolite signature may diagnose early AD [16].

The aim of the present study was to analyze the metabolome in plasma samples of controls, MCI, and AD patients. We quantitatively analyzed 40 acylcarnitine metabolites, 21 amino acids, 19 biogenic amines, 15 sphingolipids, and 90 glycerophospholipids using the AbsoluteIDQ p180 Kit (Biocrates Life Sciences AG, Innsbruck, Austria). We here show that several lipids are altered in MCI and AD EDTA plasma and that two lipids or their ratio provide a potent biomarker for distinguishing MCI and AD from controls.

2. Methods

2.1. Patients

A total of 111 samples (healthy controls, AD, and MCI) were included in this study during the sample collection period 2004–2012. All patients were >70 years and were recruited from the memory clinics at the Department of Psychiatry of Innsbruck Medical University and Hall in Tirol State Hospital, both in Austria. Healthy subjects, mainly healthy caregivers and volunteers without any cognitive impairment, were also recruited at these sites. Psychiatrists clinically examined all subjects, performed a standardized psychiatric and neurologic examination, reviewed medical records, and all subjects underwent a neuropsychological assessment (mini mental state examination [MMSE] and geriatric depression scale [GDS]). Exclusion criteria for healthy subjects and patients suffering from MCI or AD included other psychiatric or neurologic diseases or diseases including cancer, vascular diseases, or other diseases with clinically significant hepatic, renal, pulmonary, metabolic or endocrine disturbances, and inflammation. Participants underwent continuous statin or ezetimibe treatment for at least 3 months before study entry. No patient had a cholesterol level >240 mg/dL that was not treated with a statin or ezetimibe. The procedure for diagnosis has been described by us in detail elsewhere [9,17]. The study was approved by the Local Ethics Committee of Innsbruck Medical University and was performed in accordance with the Helsinki Declaration. All subjects gave written informed consent.

2.2. Blood collection

Blood samples were taken between 9:30 and 11:00 AM. Participants had a fasting time ranging from 1 to 3 hours. Breakfast foods taken by the participants were not noted. After a patient was assigned to a group, 10 mL of EDTA blood was collected and processed. The samples were centrifuged (400 × *g*, 30 min), and the upper plasma phase was immediately frozen at −80°C. Blood processing time was 4.3 ± 0.2 hours; the blood from 26% of the patients was processed the next day. Thus, mean processing time was 10.3 ± 1.8 hours (controls), 8.8 ± 1.4 hours (MCI), and 10.3 ± 1.5 hours (AD). Processing times did not differ between groups. To test the stability of the metabolites, EDTA blood was taken from non–cognitively impaired volunteers and AD patients and processed immediately (t = 0) or it was left at room temperature for 1, 2, or 3 days and then processed. To test stability over 2 years at −20°C, blood was taken from a volunteer, processed and analyzed immediately or stored at −20°C for 2 years, and then analyzed.

2.3. Metabolomic analysis

The endogenous metabolites were analyzed with a targeted quantitative and quality controlled metabolomics approach using the AbsoluteIDQ p180 Kit (Biocrates Life Science AG) as described recently by us [18]. This validated assay allows the comprehensive identification and the quantification of 186 endogenous metabolites including 21 amino acids, 19 biogenic amine, 40 acylcarnitines, 76 phosphatidylcholines (PCs), 14 lysophosphatidylcholines (lysoPCs), 15 sphingomyelins, and sum of hexoses. Analyzed glycerophospholipids are differentiated according to the presence of ester and ether bonds in the glycerol moiety. The "aa" indicates that fatty acids are at the sn-1 and the sn-2 position bound to the glycerol backbone via ester bonds, whereas "ae" denotes that fatty acid at the sn-1 position is bound via ether bond. Total number of carbon atoms and double bonds present in lipid fatty acid chains are denoted as "C x:y," where x indicates the number of carbons and y the number of double bonds. Sample preparation was performed according to the user manual. Samples were randomized, and multiple quality control samples were included in the measurement sequence. Intra-assay variation was 3.8 ± 0.8% (n = 32) and inter-assay variation 4.4 ± 1.1% (n = 32).

2.4. Statistical analysis

Plasma metabolites were checked for deviations from a normal distribution using the Shapiro-Wilk test [19]. Metabolites with a nonnormal distribution were log-transformed before analysis. One-way analysis of variance was used to compare the three diagnostic groups (healthy controls, MCI, and AD) with respect to the plasma levels. Post hoc pairwise comparisons were performed with Fisher's least-significant difference method. In the case of the three groups,