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Blood-Based Biomarkers

Blood-based metabolic signatures in Alzheimer's disease

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Abstract

Introduction: Identification of blood-based metabolic changes might provide early and easy-to-obtain biomarkers.

Methods: We included 127 Alzheimer's disease (AD) patients and 121 control subjects with cerebrospinal fluid biomarker-confirmed diagnosis (cutoff tau/amyloid β peptide 42: 0.52). Mass spectrometry platforms determined the concentrations of 53 amine compounds, 22 organic acid compounds, 120 lipid compounds, and 40 oxidative stress compounds. Multiple signatures were assessed: differential expression (nested linear models), classification (logistic regression), and regulatory (network extraction).

Results: Twenty-six metabolites were differentially expressed. Metabolites improved the classification performance of clinical variables from 74% to 79%. Network models identified five hubs of metabolic dysregulation: tyrosine, glycylglycine, glutamine, lysophosphatic acid C18:2, and platelet-activating factor C16:0. The metabolite network for apolipoprotein E (*APOE*) ε4 negative AD patients was less cohesive compared with the network for *APOE* ε4 positive AD patients.

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Discussion: Multiple signatures point to various promising peripheral markers for further validation. The network differences in AD patients according to *APOE* genotype may reflect different pathways to AD.

Keywords: Alzheimer's disease; Amino acids; Biomarkers; Graphical modeling; Metabolomics; Oxidative stress

1. Introduction

Accumulation of amyloid and tau proteins is considered the core pathologic hallmark for Alzheimer's disease (AD) [1], but other factors such as genetic liability, oxidative stress, inflammation, and lifestyle contribute to the complex mechanism of this disease [1–5]. Noninvasive measurement of disease-specific biochemical changes in living patients is difficult but may have value in terms of prognosis and identification of patients at risk for AD.

The metabolome, that is, the collection of small molecules that result from metabolic processes, is organized in biochemical pathways and is influenced by many internal and external factors, including genetics [6]. Metabolomics refers to the collective quantification of these metabolites [7]. Analytical methods have improved tremendously, with (targeted) mass spectrometry (MS) platforms now available for most compound classes. In AD, metabolomics seems of utmost importance because various alterations in metabolism, for example, higher levels of insulin and insulin resistance, are associated with an increased risk of AD [8]. Moreover, the epsilon 4 (ε4) allele of the apolipoprotein E (*APOE*) gene is not only an important risk factor for AD but is also related to alterations in lipid metabolism [9,10]. Previous metabolomics studies in AD have reported alterations in lipid, antioxidant, and amino acid metabolism. However, results are not always unequivocal [11–15]. This is most likely due to differences in (analytical) methods, cohort selection, or context of use [16].

We aim to study AD-related metabolic change from various perspectives with the use of multiple signatures to generate hypotheses regarding dysregulated metabolic events. First, we evaluate shifts in the expression of individual metabolites using nested linear models. Afterward, we assess the classification performance of the metabolites in demarcating AD from control subjects. Finally, we use state-of-the-art graphical modeling to explore metabolic dysregulation from a network perspective. In addition, we evaluate metabolic network changes according to *APOE* status, to study the hypothesis that metabolic pathways are differentially dysregulated according to the genotype.

2. Methods

2.1. Patients

We selected 150 AD patients and 150 control subjects with available plasma from the Amsterdam Dementia Cohort [17]. All subjects underwent standard cognitive screening including medical history assessment; physical, neurologic, and cognitive examination; blood sampling; lumbar puncturing; and magnetic resonance imaging. Diagnoses were made in a multidisciplinary consensus meeting. Until 2012, the diagnosis "probable AD" was based on the clinical criteria formulated by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association [18]. From 2012 onward the criteria of the National Institute on Aging-Alzheimer's Association were used [19]. Subjects with subjective cognitive decline were used as control subjects. These subjects presented with memory complaints at the VUmc memory clinic, but performed normal on cognitive testing, that is, criteria for mild cognitive impairment, dementia, or psychiatric diagnosis were not fulfilled. Clinical characteristics are provided in Table 1. All subjects gave written informed consent to use their clinical data for research purposes and to collect their blood samples for biobanking.

2.2. Cerebrospinal fluid biomarkers

Amyloid β peptide 42 ($A\beta_{42}$) and total tau (t-tau) were, for all subjects, measured in cerebrospinal fluid (CSF) using commercially available enzyme-linked immunosorbent assays (Innotest $A\beta_{42}$ and Innotest hTAU-Ag; Innogenetics, Ghent, Belgium) [20]. The cutoff for pathologic biomarker status was defined as t-tau/$A\beta_{42} > 0.52$ [21]. Of the 300 subjects included, 263 (136 AD patients and 127 controls) had a biomarker status in concordance with their clinical diagnosis, that is, t-tau/$A\beta_{42} > 0.52$ for AD and t-tau/$A\beta_{42} \leq 0.52$ for controls. These subjects were included for further analysis.

2.3. APOE genotyping

DNA was isolated from 7 to 10 mL ethylenediaminetetraacetic acid (EDTA) blood. Subsequently, samples were subjected to polymerase chain reaction. A QIAxcel DNA Fast Analysis kit (Qiagen, Venlo, The Netherlands) was used to check for size. Sequencing was performed using Sanger sequencing on an ABI130XL.

2.4. Metabolic profiling

Nonfasting EDTA plasma samples were, within 2 hours of collection, centrifuged at 1800*g* for 10 minutes at room temperature and stored at −80°C in polypropylene tubes (Sarstedt, Nurmberg, Germany). Metabolic profiling of the