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Serum lipidomic analysis for the discovery of biomarkers for major depressive disorder in drug-free patients

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ABSTRACT

Lipidomic analysis can be used to efficiently identify hundreds of lipid molecular species in biological materials and has been recently established as an important tool for biomarker discovery in various neuropsychiatric disorders including major depressive disorder (MDD). In this study, quantitative targeted serum lipidomic profiling was performed on female subjects using liquid chromatography–mass spectrometry. Global lipid profiling of pooled serum samples from 10 patients currently with MDD (cMDD), 10 patients with remitted MDD (rMDD), and 10 healthy controls revealed 37 differentially regulated lipids (DRLs). DRLs were further verified using multiple-reaction monitoring (MRM) in each of the 25 samples from the three groups of independent cohorts. Using multivariate analysis and MRM data we identified serum biomarker panels of discriminatory lipids that differentiated between pairs of groups: lysophosphatidic acid (LPA)(16:1), triglycerides (TG)(44:0), and TG(54:8) distinguished cMDD from controls with 76% accuracy; lysophosphatidylcholines(16:1), TG(44:0), TG(46:0), and TG(50:1) distinguished between cMDD and rMDD at 65% accuracy; and LPA(16:1), TG(52:6), TG(54:8), and TG(58:10) distinguished between rMDD and controls with 60% accuracy. Our lipidomic analysis identified peripheral lipid signatures of MDD, which thereby provides providing important biomarker candidates for MDD.

1. Introduction

Recent studies have suggested that changes in blood lipid profile may be related to the pathogenesis of some important neuropsychiatric diseases such as depression, schizophrenia, and Alzheimer's disease (Demirkan et al., 2013; Kakefuda et al., 2010; Liu et al., 2015; McEvoy et al., 2013; Sato et al., 2012). Lipids constitute approximately half of the dry weight of the brain and are implicated in important brain functions including membrane composition, signal transduction, and biological messenger functions, in addition to physiological processes such as energy metabolism and neuroendocrine function (Fonteh et al., 2006). Thus, any perturbation of these biological pathways is expected to alter the abundance and/or composition of the lipid pool of those pathways (Yang and Han, 2011). Major depressive disorder (MDD) is a serious brain disorder characterized by complex interactions between genetic predisposition (McGuffin and Katz, 1989) and disturbances in key molecular pathways including neurotransmitter systems, synaptic plasticity, and immune and neuroendocrinological regulation (Harrison, 2002). Lipid analysis may therefore play an important role in the discovery of biomarkers for MDD (Muller et al., 2015).

The relationship between serum lipids and mental illness, including depression, has been documented for over a century (Cruickshank and Tisdall, 1916). Numerous studies have shown an association between abnormal peripheral lipid metabolism and depression and with related symptoms such as suicide (Huang et al., 2003; Papakostas et al., 2003; Sagud et al., 2009; Sahebzamani et al., 2013; van Reedt Dortland et al., 2009). However, most studies have focused on lipoprotein fractions including total cholesterol, very-low-density lipoprotein (VLDL), low-

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density lipoprotein (LDL), high-density protein (HDL), and triglycerides (TGs) that are measurable in the clinical setting (da Graça Cantarelli et al., 2015; da Graça Cantarelli et al., 2014; Papakostas et al., 2003; van Reedt Dortland et al., 2009; You et al., 2013). However, these approaches do not quantify changes in lipid species, and there is currently only indirect and limited understanding of the role of lipids in the pathophysiology of depression. Over the last decade, technological advances in liquid chromatography–mass spectrometry (LC/MS) based lipidomic analysis have allowed for efficient and comprehensive identification of hundreds to thousands of lipid molecular species in tissues and body fluids (Gao et al., 2012; Pellegrino et al., 2014; Rhee et al., 2011; Sarafian et al., 2014). Lipidomics has recently become established as one of the most important and pivotal tools for biomarker discovery for the diagnosis of psychiatric illnesses (Sethi et al., 2016).

However, there have been few lipidomic studies using peripheral blood of patients with MDD. Demirkan et al. (2013) assessed the relationships between 148 different plasma phospholipid and sphingolipid species with depression/anxiety symptoms in 742 people recruited to a Dutch family-based study, and found that levels of plasma sphingomyelins (SM) and phosphatidylcholines (PC) were inversely correlated with depressive symptom scores, suggesting that plasma SMs and ether phospholipids could be used as potential biomarkers (Demirkan et al., 2013). Another recent metabolomic study has shown that PCs, phosphatidylethanolamines (PEs), lysophosphatidylcholines (LPCs), lysophosphatidylethanolamines (LPEs) and ether phospholipids in plasma were altered in patients with MDD (Liu et al., 2015). However, to date there have been no studies providing comprehensive coverage of diverse lipid classes in clinically depressed patients.

In this study, we analyzed serum samples from patients with MDD (currently affected and remitted) and healthy controls to identify novel peripheral markers of depression using comprehensive serum lipidome profiling based on multiple reaction monitoring (MRM). Differentially regulated lipids (DRLs) identified from a quantitative serum lipidome profiling experiment were validated using MRM, a highly sensitive and reproducible verification platform that enables the quantification of a large number of candidate lipids in a single experiment with high throughput (Surinova et al., 2011). We further demonstrated the utility of MRM for the development of a multiparametric biomarker panel using multivariate statistical analysis.

2. Methods

2.1. Study subjects

The study subjects consisted of 25 female patients with MDD with a current major depressive episode, 25 female patients with MDD in a state of remission, and 25 age- and sex-matched healthy controls at Seoul National University. The patients met the criteria for MDD according to the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders, as diagnosed by an experienced psychiatric nurse and a psychiatrist using the Mini-International-Neuropsychiatric Interview (MINI). Patients who were diagnosed with other comorbid psychiatric diseases or who had taken psychotropic medications (including anxiolytics, antidepressants, antipsychotic medications, and anticonvulsants) during the past 8 weeks were excluded from the study. Healthy controls had no current or past diagnosis of MDD, and no family history of any psychiatric disorders. None of the subjects were taking medications that might alter blood molecule levels, such as non-steroidal anti-inflammatory agents or steroids, and none suffered from chronic or acute diseases such as cardiovascular disease, pulmonary disease, hypertension, endocrine abnormalities, rheumatic diseases, or cerebrovascular disease. Subjects who were pregnant, nursing, or menstruating were also excluded. The protocol was approved by the Ethics Committee of Seoul National University Hospital, and the study was conducted in accordance with the latest version of the Declaration of Helsinki. Written informed consent was obtained from each patient prior to enrollment.

2.2. Clinical assessment of affective symptoms

The objective severity of depression was measured using the Hamilton Rating Scale for Depression (HAMD) (Hamilton, 1960), for which the total score ranges from 0 to 52 and higher scores reflect more severe depressive symptoms. The Inventory of Depressive Symptomatology–Self Report (IDS-SR) was used as a self-rating instrument for depressive symptoms (Rush et al., 1996); this instrument is composed of 30 items with total scores ranging from 0 to 84 (only an increase or decrease is scored for appetite and weight).

2.3. Experimental procedures

2.3.1. Sample collection

Blood samples were obtained from all subjects after an overnight fast (at least 12 h). The sampling time window was from 9:30 to 11:30 AM. The serum was separated within 1 h of collection and aliquoted serum samples were stored at −78 °C until analysis.

2.3.2. Reagents

HPLC-grade methanol, acetonitrile, water, and 2-propanol were purchased from J.T. Baker (Avantor Performance Material, Inc., Center Valley, PA, USA). HPLC-grade formic acid was purchased from Fluka Analytical (Sigma Aldrich Chemie GmbH, Steinheim, Germany). Chloroform and ammonium formate were purchased from Sigma-Aldrich (St. Louis, MO, USA). The lipid standards used in this study were as follows: PC (10:0–10:0), PC (12:0–12:0), PE (10:0–10:0), phosphatidylserine (PS) (10:0–10:0), phosphatidylglycerol (PG) (10:0–10:0), phosphatidylinositol (PI) (8:0–8:0), phosphatidic acid (PA) (10:0–10:0), LPC (13:0), LPE (14:0), lysophosphatidylserine (LPS) (17:1), lysophosphatidylglycerol (LPG) (14:0), lysophosphatidylinositol (LPI) (13:0), lysophosphatidic acid (LPA) (14:0), SM (d18:1–12:0), and ceramide (Cer) (d18:1–12:0) were purchased from Avanti Polar Lipids, Inc., Alabaster, AL, USA. TG (11:1–11:1–11:1), diacylglycerol (DG) (8:0–8:0), cholesterylester (ChE) (10:0), and cholesterol were purchased from Larodan Fine Chemicals AB, Malmö, Sweden.

2.3.3. Sample preparation

Each lipid standard was dissolved in chloroform/methanol (1:1, v/v) and stored at –30 °C. Serum lipids were extracted using a two-step extraction as follows: first, the 10 μL aliquots of the serum were added to 150 μL of chloroform/methanol (1:2, v/v) and 10 μL of lipid standards (1 μg/mL), which included TG (11:1–11:1–11:1), DG (8:0–8:0), ChE (10:0), PC (10:0–10:0), PE (10:0–10:0), PS (10:0–10:0), PG (10:0–10:0), PI (8:0–8:0), PA (10:0–10:0), LPC (13:0), LPE (14:0), LPS (17:1), LPG (14:0), LPI (13:0), LPA (14:0), SM (d18:1–12:0), and Cer (d18:1–12:0) as the internal standard (IS). The sample was then incubated for 10 min on ice. After centrifugation (13,800 × *g*, 2 min at 4 °C), 150 μL of supernatant was transferred to a new Eppendorf tube. Next, the remaining pellet was resuspended in 150 μL chloroform/methanol/37% (1 N) HCl (40:80:1, v/v/v) and incubated for 15 min on ice. Finally, 50 μL of cold chloroform and 90 L of cold 0.1 M HCl was added, followed by 1 min of vortexing and centrifugation (6500 × *g*, 2 min at 4 °C). The lower organic phase was then transferred to a new tube (Haag et al., 2012).

2.3.4. TMSD methylation

A solution of TMSD (2 mol/L) in hexane (60 μL) was added to the lipid extracts (200 μL) from the serum and the lipid standard samples dissolved in methanol to obtain yellow-colored solutions. After vortexing for 30 s, methylation was performed at 37 °C for 15 min. The addition of glacial acetic acid (6 μL) quenched the methylation and provided colorless samples. These samples were then analyzed using LC/MS (Lee et al., 2013).

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