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Lipidomic profiling of plasma samples from patients with mitochondrial disease

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ABSTRACT

Mitochondrial disease (MD) is a rare mitochondrial respiratory chain disorder with a high mortality and extremely challenging to treat. Although genomic, transcriptomic, and proteomic analyses have been performed to investigate the pathogenesis of MD, the role of metabolomics in MD, particularly of lipidomics remains unclear. This study was undertaken to identify potential lipid biomarkers of MD. An untargeted lipidomic approach was used to compare the plasma lipid metabolites in 20 MD patients and 20 controls through Liquid Chromatography coupled to Mass Spectrometry. Volcano plot analysis was performed to identify the different metabolites. Receiver operating characteristic (ROC) curves were constructed and the area under the ROC curves (AUC) was calculated to determine the potentially sensitive and specific biomarkers. A total of 41 lipids were significantly different in MD patients and controls. ROC curve analysis showed the top 5 AUC values of lipids (phosphatidylinositols 38:6, lysoPC 20:0, 19:0, 18:0, 17:0) are more than 0.99. Multivariate ROC curve based exploratory analysis showed the AUC of combination of top 5 lipids is 1, indicating they may be potentially sensitive and specific biomarkers for MD. We propose combination of these lipid species may be more valuable in predicting the development and progression of MD, and this will have important implications for the diagnosis and treatment of MD.

1. Introduction

Mitochondrial disease (MD) refers to a rare primary mitochondrial disorder that results in inadequate energy production. The most often affected tissues and organs are those with the highest energy demands, for example skeletal muscle, brain or heart. MD is a result of mutations either in the nuclear or mitochondrial DNA (mtDNA) [1]. The lowest prevalence of MD in adults is ~12.5 per 100 000, and ~4.7 per 100 000 in children [2]. Clinical symptoms can arise in childhood or later in life, and can affect one organ in isolation or present as multisystem disease [3]. Many genetically-characterized mitochondrial disorders present with a combination of clinical features which are characteristic of distinct MD syndromes, such as mitochondrial encephalomyopathy with lactic acidosis and stroke like episodes (MELAS) [4], Kearns-Sayre syndrome (KSS) [5], chronic progressive external ophthalmoplegia (CPEO) [6], mitochondrial limb girdle myopathy (MLGM), isolated mitochondrial myopathy (MM) [7], myoclonic epilepsy with ragged red fibres (MERRF) [8] and so on. Diagnosing MD is a challenge because of its genetic heterogeneity, diversity of clinical phenotypes [9]. In addition, significant difficulties may also be encountered in the management of MD. Despite progress in current understanding of the pathophysiology and genetics of MD, no effective cure for mitochondrial disorders has been found [10]. Apart from supportive therapy, a variety of therapeutic approaches have been evaluated in randomized clinical trials, but unfortunately none of these has delivered breakthrough results [11,12]. Therefore,

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increased understanding of MD characteristics is needed to facilitate diagnosis and treatment of MD.

Lipids are crucial components of cellular membranes and lipid particles such as lipoproteins. Lipids play many essential roles in cellular functions, including cellular barriers, membrane matrices, signaling, and energy depots [13]. Lipidomics has led to identification of new signaling molecules, motivated discovery of potential biomarkers for early diagnosis and treatment of disease, supported screening of drug targets and/or test drug efficiency, and allowed personalization of medical treatment [14].

So far there is no report on the lipidomics study in MD patients yet. Lipid droplet accumulation has been observed in the skeletal muscle of patients with MD with transmission electron microscopy [15] and magnetic resonance spectroscopy (MRS) [16]. This led us to suppose altered lipid metabolism occurs in MD. In this study, we aimed to examine plasma lipidomic metabolites with Liquid Chromatography coupled with Mass Spectrometry (LC-MS) to identify potential MD biomarkers, which may provide fundamental information which is of benefit for diagnosis and treatment of MD.

2. Materials and methods

2.1. Patients

Twenty patients with MD (10 MELAS, 3 KSS, 6 CPEO and 1 MERRF) and 20 healthy control subjects were recruited for untargeted lipidomic analysis (Table S1). The control subjects in whom MD was excluded by examination were recruited from hospital and laboratory personnel, and were age and sex matched with the study patients. Diagnosis of MD was undertaken based on the clinical phenotype, muscle pathology, and genetic criteria. None of these patients were taking any medication except for treatment of MD. All subjects gave informed consent for inclusion before participation in this study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Peking University First Hospital (2012-542) (Supplementary material 1).

2.2. Chemicals and reagents

Formic acid, HPLC grade methanol, acetonitrile (ACN) and isopropanol (IPA) were obtained from Fisher Scientific. Chloroform was obtained from Tong Guang Fine Chemicals Company (Beijing, China). Ultra-pure water was supplied by a Millipore system (Millipore, Billerica, MA, USA).

2.3. Sample preparations for untargeted lipidomic profiling

Lipids were extracted from plasma samples with a modified Folch method [17]. Typically, 100 μL of plasma were aliquoted into a 0.6 ml Eppendorf tube and mixed with 400 μL of chloroform/methanol (2:1, V/V) containing 20 μg/ml of free fatty acid 19:0 as an internal standard. After vortexing for 10 min, the mixture was centrifuged at 13000 rpm at 4 °C for 20 min. The lower lipid containing chloroform phase was evaporated with a speed vacuum, and the residue was stored at −80 °C for further analysis. All samples were processed in the same laboratory to avoid bias.

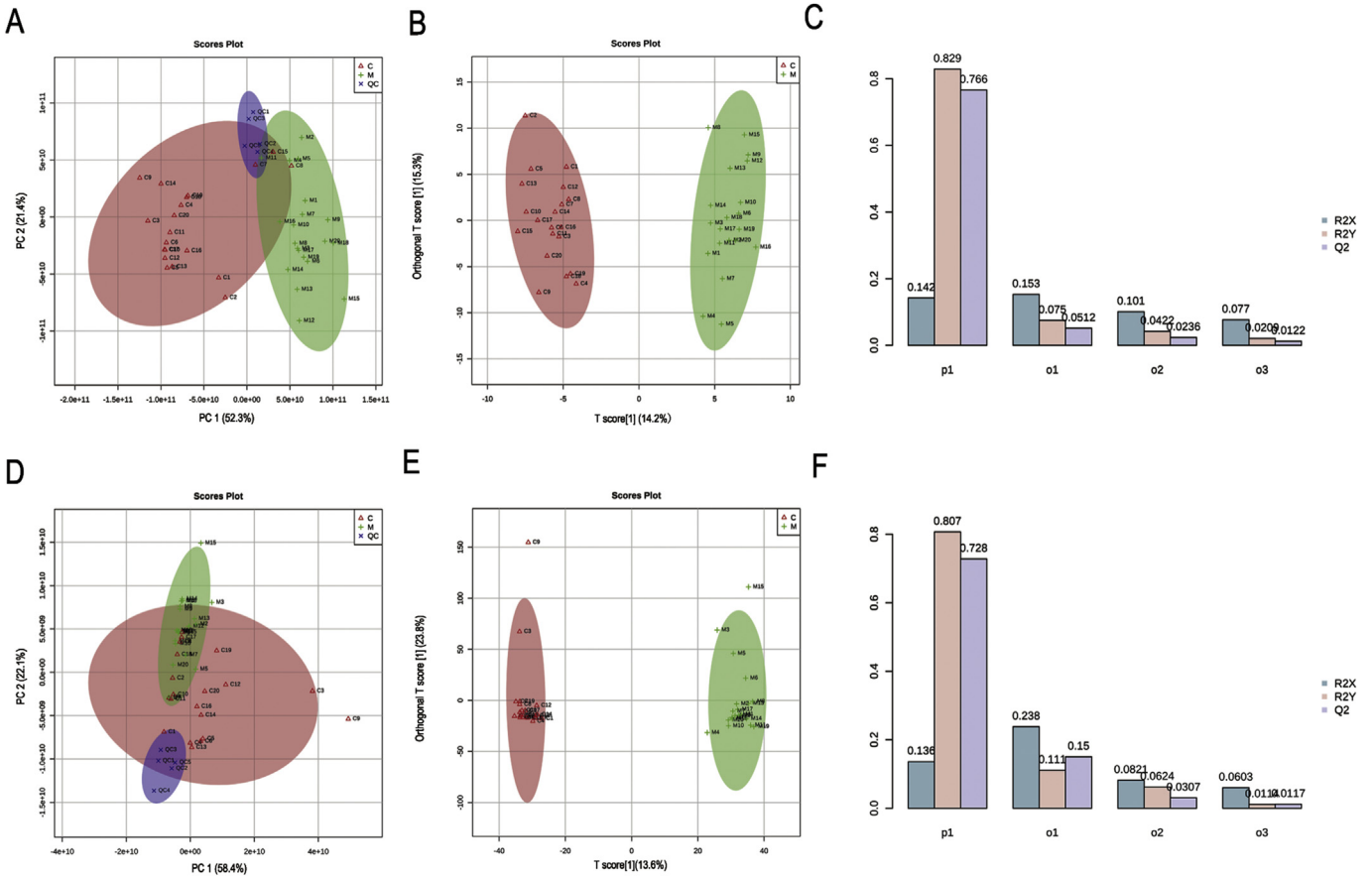

Fig. 1. PCA (A) and orthoPLS-DA (B) score plots of MD patients based on metabolomics data in positive ion mode. PCA (D) and orthoPLS-DA (E) score plots of MD patients based on metabolomics data in negative ion mode. Performance statistics of orthoPLS-DA in positive ion mode (C) and negative ion mode (F).

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