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Research report

Differential urinary metabolites related with the severity of major depressive disorder

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ABSTRACT

Major depressive disorder (MDD) is a common mental disorder that affects a person's general health. However, there is still no objective laboratory test for diagnosing MDD. Here, an integrated analysis of data from our previous studies was performed to identify the differential metabolites in the urine of moderate and severe MDD patients. A dual platform approach (NMR spectroscopy and GC–MS) was used. Consequently, 14 and 22 differential metabolites responsible for separating moderate and severe MDD patients, respectively, from their respective healthy controls (HCs) were identified. Meanwhile, the moderate MDD-specific panel (N-Methylnicotinamide, Acetone, Choline, Citrate, vanillic acid and azelaic acid) and severe MDD-specific panel (indoxyl sulphate, Taurine, Citrate, 3-hydroxyphenylacetic acid, palmitic acid and Lactate) could discriminate moderate and severe MDD patients, respectively, from their respective HCs with high accuracy. Moreover, the differential metabolites in severe MDD were significantly involved in three metabolic pathways and some biofunctions. These results showed that there were divergent urinary metabolic phenotypes in moderate and severe MDD patients, and the identified potential urinary biomarkers might be useful for future developing objective diagnostic tests for MDD diagnosis. Our results could also be helpful for researchers to study the pathogenesis of MDD.

1. Introduction

As a debilitating mental disease, major depressive disorder (MDD) could cause huge economic burden for individuals, families and society each year. MDD has a pronounced and negative impact on the quality of life of many people. Previous study reported that it affected about 350 million diagnosed patients in the worldwide [1], and 20% of the total population was with lifetime MDD [2]. However, up to now, the prevention and treatment of MDD still faces a tremendous challenge. Nowadays, a lot of MDD patients do not respond sufficiently to any recommended first-line treatment modalities [3–6]. Meanwhile, there is no objective diagnostic laboratory test for MDD, which might be caused by the unclear pathogenesis of MDD. In clinical practice, the subjective identification of symptom clusters is still the commonly method to diagnose MDD.

But considering the considerable error rate of this method [7], researchers have devoted themselves to develop objective methods for MDD diagnosis. In recent decades, metabolomics has become a powerful tool to identify potential biomarkers for many neuropsychiatric disorders and provide new insights to study their pathogenesis, such as bipolar disorder and schizophrenia [8–10]. Our group and other researchers have used metabolomics technologies to investigate the metabolic changes in MDD patients, and identify some potential biomarkers and key pathways [11–14]. However, none of these studies have considered whether there were different metabolic phenotypes in different severity of MDD patients and whether the different diagnostic methods were needed to diagnose the severity of MDD. Actually, the metabolic perturbations in biosamples are different in various disease

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states. And our earlier study found that the metabolic phenotypes in plasma of different severity of MDD patients were different [15]. Therefore, we hypothesized that there were different urinary metabolic phenotypes in different severity of MDD patients.

Meanwhile, previous studies indicated that the different severity of MDD patients might need the different treatment modalities [16,17]. But depending on a structured or semistructured interview to diagnose the severity of MDD is usually unduly time-consuming and sometimes highly trustless. Therefore, it is meaningful and needed to develop an objective method to diagnose the severity of MDD. Here, the combined application of nuclear magnetic resonance (NMR)- and gas chromatography-mass spectrometry (GC–MS)-based metabolomics were used to analyze the urinary metabolites in patients with moderate and severe MDD and preliminarily identify potential biomarkers for diagnosing moderate and severe MDD. Our findings could also provide new insights of the biological understanding of MDD pathways. The data were extracted from our previous metabolomics studies [12–14]. The candidates must have metabolites data identified using both NMR and GC–MS.

2. Methods

2.1. Subject recruitment

The healthy controls (HCs) were recruited from the Medical Examination Center of Chongqing Medical University (Chongqing, China). The MDD patients were recruited from the Psychiatric Center of the First Affiliated Hospital. The senior psychiatrists that studied and treated MDD for many years were responsible for recruiting MDD patients. Here, the fourth Diagnostic and Statistical Manual of Mental Disorders criteria for MDD (DSM-IV) were used to diagnose MDD patients [18]. The Hamilton Depression Scale (HAMD) score of MDD patients has to be more than 17. Our study was approved by the Ethical Committee of Chongqing Medical University, and all methods were performed in accordance with the relevant guidelines and regulations. All the recruited subjects known the purpose of this study and provided the written informed consent.

Patients with HAMD score ranged from 18 to 24 were assigned into moderate MDD group, and patients with HAMD score greater than 24 were assigned into severe MDD group [19]. At last, 59 moderate MDD patients and 82 age-, gender- and body mass index-matched HCs were recruited. Similarly, 34 severe MDD patients and 41 HCs were recruited. In order to exclude the possible effects of drugs, all the recruited MDD patients in this study were the first-episode drug-naïve MDD patients. The detailed clinical information of the recruited subjects was described in Table 1.

2.2. Sample processing

After overnight fasting, morning midstream urine samples (9:00-10:00 am) from the recruited subjects were collected with a sterile cup, and then quickly transferred to the sterile tubes. Subsequently, the urine samples will be brought into the laboratory under low temperature. In the laboratory, all the samples were immediately centrifuged for 10 min at 1500*g*. Then, we stored the supernatants at −80 °C in equal aliquots for subsequent processing. The metabolic profiling data acquisition (NMR and GC–MS) were detailed described in our previous studies [13,14].

2.3. Data analysis

SPSS 12.0 was used to conduct the student's T test and chi-square test to analyze the clinical information. The levels of metabolites were first normalized using the creatinine concentration, and then scaled to have unit variance before multivariate analysis. Afterwards, the orthogonal partial least-squares discriminant analysis (OPLS-DA) was conducted using the SIMCA-P software 14.0. The quality of the built model was assessed by R^2X, R^2Y and Q^2. The former two parameters were used to assess the goodness-of-fit, and the last one was used to assess the predictability of the model [20]. Meanwhile, the 199-iteration permutation test was conducted to assess whether there was overfitting in the built model [21]. The model was robust if the values of R^2 and Q^2 of the original model were higher than the values of the permutation test [21].

The coefficient loading plot of the built model was used to identify the metabolites responsible for the discrimination between MDD patients and HCs [22]. According to the number of subjects that were used to build the model, the correlation coefficients of $|r| > 0.254$ (equivalent to p-value < 0.05) in moderate MDD model and $|r| > 0.339$ (equivalent to p-value < 0.05) in severe MDD model were viewed as the cut-off values for identifying the differential metabolites. The Cytoscape software 3.2.1 was used to build the correlation network between these differential metabolites and the severity of MDD. The MetaboAnalyst 3.0 was used to identify the metabolic pathways significantly affected by these metabolites. The Ingenuity pathway analysis (IPA 9.0) was used to conduct metabolomics analysis. Furthermore, these differential metabolites were used to build the logistical regression model, and the Akaike's information criterion (AIC) was used to optimize the metabolite biomarker combination. At last, the receiver-operating characteristic (ROC) curve analysis was conducted to further assess the diagnostic performance of the identified combination.

3. Results

3.1. OPLS-DA model

The urinary metabolites from the HC and first-episode drug-naïve MDD patients (59 moderate MDD patients vs. 82 HCs; 34 severe MDD patients vs. 41HCs) were used to build OPLS-DA models. The OPLS-DA score plot showed that the moderate MDD patients could be distinctly separated from HCs with little overlap (SIMCA-P 14.0, Fig. 1A, R^2Y cum = 0.581, Q^2 = 0.357). The similar results were observed between the severe MDD patients and HCs (SIMCA-P 14.0, Fig. 1C, R^2Y cum = 0.595, Q^2 = 0.429). The positive R^2Y and Q^2 values in the both

Table 1
Clinical detail of HCs and MDD patients.

Variables	moderate MDD	HCs	P^a	severe MDD	HCs	P^a
Sample Size	59	82	–	34	41	–
Female/Male	20/39	28/54	0.98	23/11	20/21	0.10
Age (years)[b]	31.5 ± 10.2	33.0 ± 10.0	0.40	34.1 ± 10.3	31.6 ± 11.3	0.33
BMI[b]	21.6 ± 2.8	21.8 ± 2.8	0.62	21.4 ± 2.6	20.6 ± 2.2	0.14
HAMD scores[b]	19.9 ± 1.8	–	–	28.2 ± 2.6	–	–

Abbreviations: HCs: healthy controls; MDD: major depressive disorder; BMI: body mass. index; HAMD: Hamilton Depression Scale.

[a] Two-tailed student *t*-test.

[b] Values expressed as means ± SDs.