DOCTORAL THESIS

Mass spectrometry based metabolomics for biomarkers of Parkinson's disease

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ABSTRACT

Increasing evidence has shown that abnormal metabolic phenotypes in body fluids reflect the pathogenesis and pathophysiology of Parkinson's disease (PD). However, the relationship between metabolic phenotypes and PD is not fully understood. Mass spectrometry (MS) based metabolomics is a powerful technique, which was frequently used for the sensitive and reproducible detection of hundreds to thousands of metabolites in biofluid samples.

Here we developed and performed MS-based metabolomics studies involving hundreds of human urine samples with data acquired from multiple analytical batches for surveying potential biomarkers of PD. A new software *statTarget* was developed and introduced. Protocols for liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) were developed, including sample preparation, data acquisition, quality controls, quality assurance and data analysis. Urinary metabolites from a total of 401 clinical urine samples collected from 106 idiopathic PD patients and 104 normal control subjects were profiled by using LC-MS. Quality control (QC) strategy has been performed in MS-based metabolomics for high reproducibility and accuracy of MS data. GC-MS with methyl chloroformate (MCF) derivatization was used for profiling highly polar metabolites in patients with early-, middle- and advanced-stage PD. Our study revealed the significant correlation between clinical phenotypes and urinary metabolite profiles. Comprehensive metabolomics was successfully developed with the goal of identifying urinary metabolite markers that can be used for evaluating the development of PD. A group of 18 metabolites have shown not

only a high discriminating ability for the early-stage PD patients but also accurately distinguished the middle- and advanced- stages patients from control subjects. For the evaluation of PD, 18 metabolites showed good potential as metabolite markers with related metabolic pathway variations observed in branched chain amino acid metabolism, glycine derivation, steroid hormone biosynthesis, tryptophan metabolism, and phenylalanine metabolism.

We have further performed targeted analysis of potential biomarkers by using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and GC-MS. The UPLC-MS/MS method was developed and optimized for detecting the concentration variation of metabolites in tryptophan metabolism for alpha-synuclein over-expressed flies (Parkinson's disease model). The altered tryptophan metabolism was proved as one of the common metabolite signatures between PD patients and alpha-synuclein over-expressed fly model of PD, and thus may be used for developing potential markers of the disease and evaluating the efficacy of novel therapeutic agents. An asymmetric labeling strategy and positive chemical ionization gas chromatography-tandem mass spectrometry (PCI-GC-MS-MS) approach was developed for the determination of non-amino organic acids and amino acids, as well as short chain fatty acids. Carboxylic and amino groups could be selectively labelled by propyl and ethyl groups, respectively. The specific neutral losses of C_3H_8O (60 Da), $C_3H_5O_2$ (74 Da) and $C_4H_8O_2$ (88 Da) were useful in the selective identification for qualitative analysis of organic acids and amino acid derivatives. The developed PCI-GC-MS/MS method showed good reproducibility and linear range.

In summary, metabolomics study has its inherent advantage in the characterization of biomarkers for the development of PD and may bring new scientific knowledge as well as impact on the progression of PD and other related neurodegenerative diseases.

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