A metabonomic analysis of serum from Wilson′s disease rats using ^{1}H NMR spectroscopy and pattern recognition

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Introduction

Wilson's disease (WD), characterized by hepatic and neurological abnormity, is a rare autosomal recessive genetic disorder of copper metabolism. It was first described as a syndrome by Wilson in 1912 [1]. Its morbidity is 1/30 000 - 1/100 000 on human individuals. Nowadays, the early diagnosis of WD still puzzles clinical doctors and WD patients are often unable to obtain timely and effective treatments. In this abstract, metabonomics based on nuclear magnetic resonance (NMR) technique was applied to study the serum from WD rats to determine the characteristic metabolites. This would be helpful for the early diagnose of WD.

Materials and Methods

A total of 22 male Wistar rats (180±20g) were divided into control (n=11) and WD (n=11) groups, and housed individually in metabolism cages. The animals were allowed to acclimate for 6 days under controlled conditions (temperature, humidity, and a 12h light-dark cycle) and provided with food and water *at libitum*. The WD group was fed with copper overloaded diet (1g/kg $CuSO_4$ and free access to 1 ppm $CuSO_4$ dissolved in the tap water) while the control group was fed with normal diet. All rats were fed for 60 days with free access to food and water.

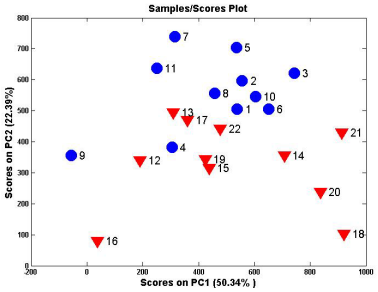

Fig. 1 PCA score plot of ^{1}H NMR spectra of serum from control (1-11, ●) and WD (12-22,▼) groups.

Serum samples from the sacrificed rats were collected by ultrafiltration and centrifugation (3000rpm, 10min at 4°C) and were stored frozen at -20°C until NMR spectroscopic analysis. Phosphate buffer solution (0.2M Na_2HPO_4/NaH_2PO_4, pH 7.4, 99.9% D_2O) was added to minimize pH variations in the serum samples and DSS (2,2-dimethyl-2-silapentane-5-sulfonic acid) was used as an internal reference standard at δ 0.00. ^{1}H NMR spectra of serum samples were acquired on Varian NMR System 500MHz spectrometer at temperature 298K using the CPMG pulse sequence combined with presaturation. All NMR spectra were phased, baseline corrected, and segmented into regions of 0.02 ppm width in the region of 0.2 ~ 4.6 ppm. The region of 4.6 ~ 10.0 ppm was excluded prior to principle component analysis (PCA). The data were normalized to the sum of the spectral integral to account for the differences of concentrations.

Results and Discussion

The PCA score plot (Fig. 1) shows that the WD and control groups are separated despite of a slight overlap of data points. The endogenous metabolites responsible for the classification are listed in Table 1. ^{1}H NMR spectra and pattern recognition analysis show increased levels of lactate, glycoprotein, glutamine, creatine, creatinine, arginine and decreased levels of glucose, TMAO, betaine, lipid and choline. The most remarkable difference of the WD rats from the control ones is the increased level of lactate which may result from acute liver injury. The elevated level of creatinine in blood suggests a dysfunction in renal glomerular filtration, indicating the disturbance of energy metabolism and oxidative stress induced by copper. Choline and phosphatidylcholine, the major membrane constituents, together with lipids, are decreased in serum, which denotes the disruption of membrane fluidity caused by lipid peroxidation [2]. The decreased level of phosphatidylcholine might also imply the breakdown of cell membrane. The increase of glutamine may arise from neuronal cell death. The markedly decrease of glucose indicates the disturbed carbohydrate metabolism.

Table 1 Endogenous serum metabolites responsible for classification of WD and control rats

Metabolites	δ (^{1}H, ppm)	Amount for WD rats (relative to control)
lactate	1.32, 4.10	↑↑
glycoprotein	2.04	↑↑
glutamine	2.12	↑↑
creatine+ creatinine	3.04	↑
arginine	1.64, 1.71	↑
glucose	3.23-3.80	↓↓
TMAO+ betaine	3.26	↓↓
Lipid (mainly LDL/LDL)	1.29-1.30	↓↓
choline	3.19	↓
phosphorylcholine	3.21	↓

Note: "↑" and "↓" denote higher or lower amount relative to the control group, and "↑↑" and "↓↓" denote much higher or much lower amount.

Conclusion

The metabolic profiling of serum from Wilson's disease rats was investigated. A clear separation of WD and control groups was observed in PCA score plot and the characteristic metabolites for WD rats were identified and analyzed. The results may further our understanding of the disease.

Acknowledgement

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References

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