UNTARGETED METABOLOMICS APPROACH IN THE STUDY OF POSTTRAUMATIC STRESS DISORDER

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Introduction

Posttraumatic stress disorder (PTSD) is a common, prevalent, severe, disabling and debilitating mental disorder developing after traumatic exposure. The exact biological factors, associated with vulnerability or resilience to develop PTSD after traumatic exposure, are still not well known. Additionally, there are no valid and reliable biomarkers that would help in setting the diagnosis of PTSD or in predicting the vulnerability to develop PTSD. This study translates the basic science advances into biomedical application by examining plasma metabolome changes in PTSD in order to define biological mechanisms in the development of PTSD and new, reliable, biomarkers of this complicated mental disorder.

Aim

In this research we sought to investigate a metabolic profile of patients with combat related PTSD and compare it with metabolic profile of healthy control subjects.

Results

Statistical analyses indicated 25 significant compounds for positive and 35 compounds for negative LC-MS mode (Fig. 3). In order to identify those compounds a tandem LC-MS experiment was done using the same chromatographic conditions as applied for the primary analysis. After this, 20 compounds were identified in negative and 15 in positive LC-MS mode (Figs 3. and 4.). A trend of increased levels of different types of glycerophospholipids and decreased levels of different types of carnitines and bile acids was found in PTSD subjects when compared to HC subjects (Fig 5).

Conclusions

This preliminary study reveald changes in several metabolites that are found to be mainly associated with processes of cell membrane dynamics, energy metabolism, (neuro-) inflammation and processes of biological aging. These findings could contribute to better understanding of PTSD etiology, but further research has to be carried out in order to validate current results

Materials and methods

Study included 100 blood plasma samples, 50 from male patients, aged 57.5 (52.3-66) years with combat related PTSD and 50 from matching healthy control (HC) subjects aged 57 (51.5-66) years. Samples were collected within the project „Genomic and glycomic biomarkers for PTSD" supported by Croatian Science Foundation with the approval of Ethics Committees of the Psychiatric hospital Vrapce. Patients were diagnosed according to the 5th edition of Diagnostic and Statistical Manual of Mental Disorders criteria. Both, HC subjects and PTSD patients met the same criteria and were evaluated using the same psychiatric scales.

Plasma samples	• Deproteinization by cold ethanol:methanol (1:1) in the proportion 1:3
LC (Agilent 1200)	• Reversed-phase column Discovery HS C18 15 cm x 2.1 mm, 3 µm; Supelco • Guard column Discovery HS C18 2 cm x 2.1 mm, 3 µm; Supelco • Solvent A: water with 0.1 % formic acid; B: acetonitrile with 0.1 % formic acid • Flow rate: 0.6 ml/min; gradient: 25 % B, 95 % B in 35 min, 25 % B in 1 min, 25 % B for further 9 min • Sample injection volume 10 µl
QTOF-MS (Agilent 6520)	• Positive and negative ionization mode in 2 separate runs • Full scan spectra (m/z range 50-1000) • Capillary voltage 3000 V; nebulizer gas flow rate 10.5 l/min; scan rate 1.02 scan/s

Figure 1. Blood plasma footprinting in patients with combat related PTSD and healthy control subjects conducted by LC-MS in positive and negative ionization mode.

Raw data processing	• Molecular Feature Extraction and Recursive Feature Extraction by Profinder B.06.00 (Agilent Technologies)
Data pretreatment	• filtering according to 80 % presence in at least one sample group and lower than 30 % RSD in respective QCs
Statistical analyses	• SIMCA 14.1 (Umetrics, Umeå, Sweden): Multivariate unsupervised (PCA) and supervised (PLS-DA and OPLS-DA) statistical analyses • MATLAB R2015a: Univariate statistical analyses (Student's t-test, Mann-Whitney U test)

Figure 2. Data treatment worklist

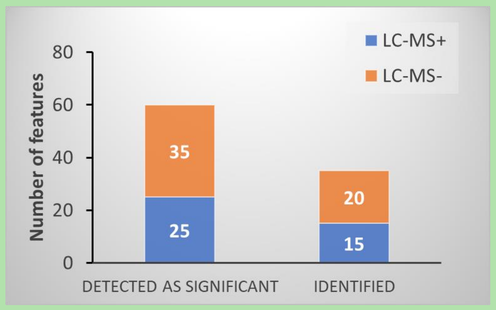
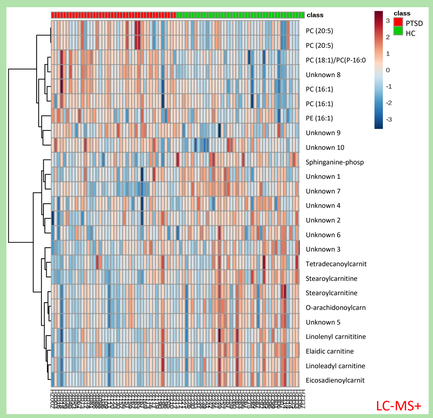


Figure 3. Number of significant compounds in positive and negative LC-MS mode



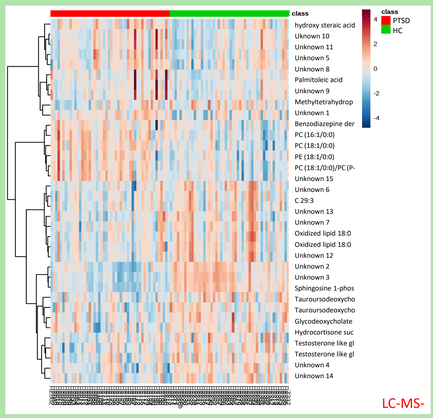
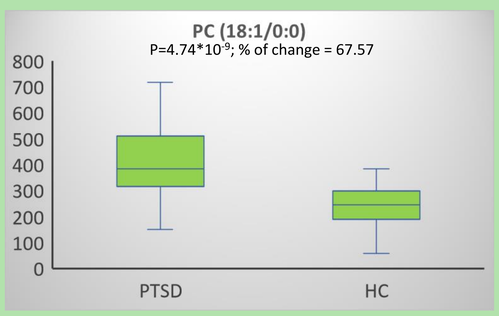
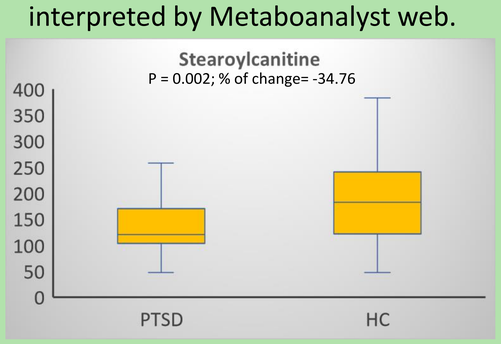


Figure 4. Heatmaps revealed medium contribution of significant metabolites to PTSD condition. Results are presented for LC-MS positive and negative modes and interpreted by Metaboanalyst web.





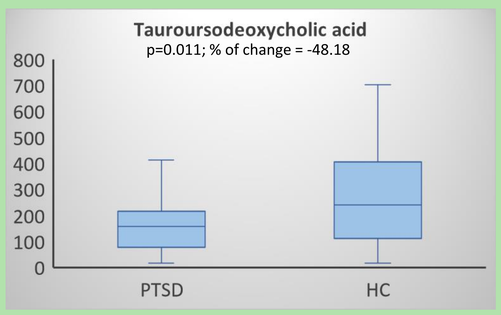


Figure 5. Incresed plasma levels of PC (18:1/0:0) and decreased levels of stearoylcarnitine and tauroursodeoxycholic acid detected in PTSD patients when compared to healthy control subjects