Biomarker Discovery in Cerebrospinal Fluid for Schizophrenia and Antipsychotic Drug Treatment-Induced Weight Gain.

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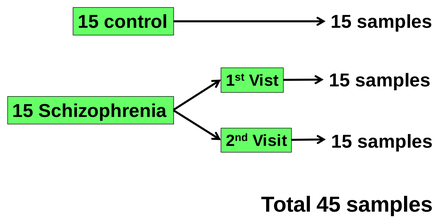
Introduction

Schizophrenia is a complex neuropsychiatric disorder with both behavioral and cognitive alterations and an onset in early adulthood. However, diagnosis still relies on interview-based processes and no useful biomarker has been found for clinical application. Cerebrospinal fluid (CSF) is a promising source for detecting neurological disease-related biomolecular alterations. Here, we performed a protein marker discovery study on CSF samples from schizophrenic and healthy subjects using a label-free proteomics approach previously used for finding Central Nervous System lymphoma biomarkers (1-3).

Methods

15 healthy individuals and 15 schizophrenic patients (age and gender matched) were recruited for this study. As part of an earlier technical study, a second CSF sample obtained one month later was analyzed concurrently and provided an extra measure or verification of observed changes. CSF samples from 15 healthy controls (n=15) and the two times visit of 15 schizophrenia patients each (n=15×2) were subjected to MARS-14 depletion of abundant proteins and digested with trypsin. Sample analysis was performed using 2D-LC-MS in combination with Strong Cation Exchange Chromatography and a Thermo QExactive LC-MS system. Statistical and pathway analyses were utilized to investigate biological signatures of the disease.



Results

Two time points for same patient are well correlated; Separate clustering of patients (at both time points) is observed vs. case controls

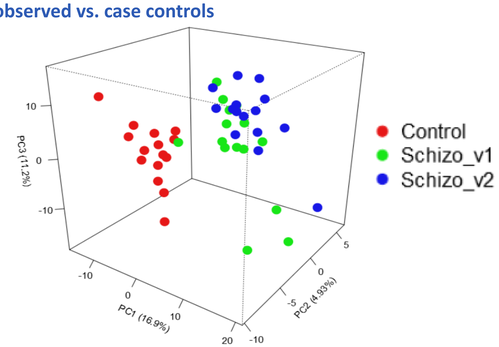


Figure 1. Principal Component Analysis on all detected proteins (1239)

Results continued

Total 9829 unique peptides are sequenced and 1239 proteins identified in CSF. 138 proteins are differentially expressed.

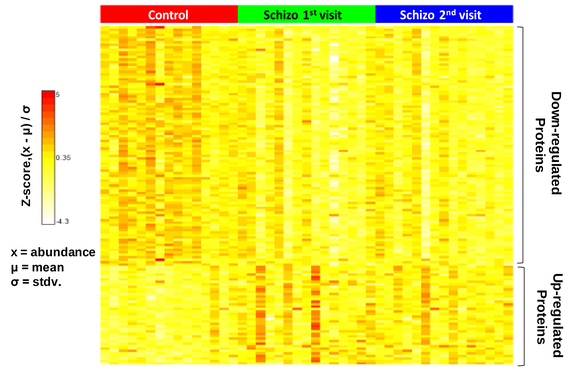


Figure 2. Heatmap of differentially expressed proteins in Schizophrenic patients vs. healthy individuals. Threshold: fold change 1.25, p & q value <0.01

Down-regulated Neuronal-Associated proteins

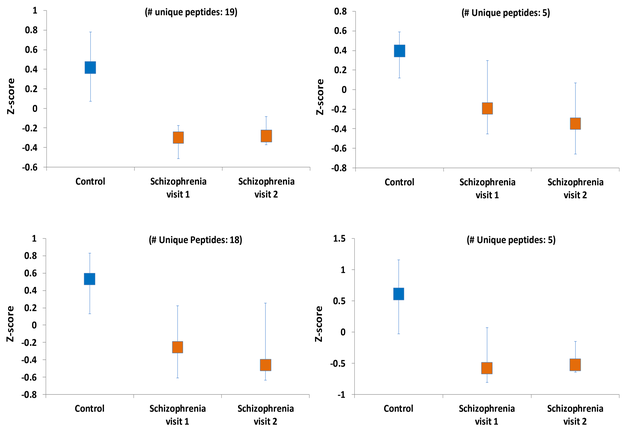


Figure 3. Averaged z-score (average of all peptide z-scores) per cohort for four different Neuronal associated example proteins (# peptides per protein is reported above)

Perturbed Biological Functions: Neurological disease

	Schizophrenia vs control		
Diseases or Functions Annotation	**p-Value**	**Activation z-score**	**# Proteins**
Neurological Disease			
seizures	2.02E-03	2.229	13
Astrocytosis	7.72E-04	1.753	5
Neurodegeneration	9.87E-05	-0.464	10

Results continued

Perturbed Biological Functions: Nervous System Development and Function

	Schizophrenia vs control		
Diseases or Functions Annotation	**p-Value**	**Activation z-score**	**# Proteins**
Nervous System Development and Function			
Development of central nervous system	2.35E-06	0.612	23
Synaptogenesis	9.12E-05	-1.491	9
Axonogenesis	4.38E-11	-1.06	17
Synaptic transmission	4.83E-06	-1.052	16
Neuritogenesis	1.95E-13	-1.218	30

Up-regulated proteins related to Inflammation

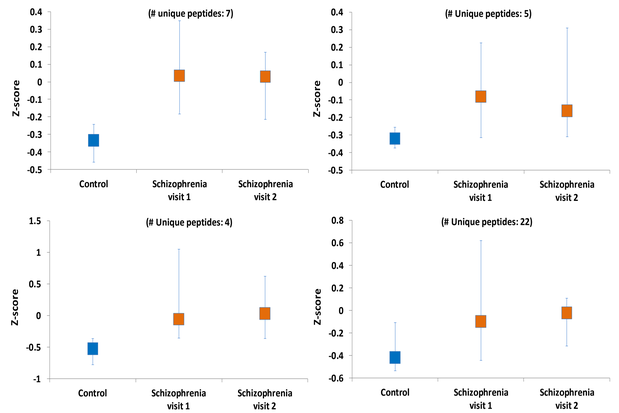


Figure 4. Averaged z-score (average of all peptide z-scores) per cohort for four example proteins related to inflammation (# peptides per protein is reported above)

Perturbed Biological Functions: Inflammatory Response

	Schizophrenia vs control		
Diseases or Functions Annotation	**p-Value**	**Activation z-score**	**# Proteins**
Inflammatory Response			
Activation of myeloid cells	1.19E-03	2.01	10
Adhesion of phagocytes	7.74E-04	1.664	7
Complement activation	2.68E-12	1.96	11
Activation of leukocytes	2.11E-03	1.709	18
Phagocytosis of cells	4.30E-05	0.362	12
Immune response of T lymphocytes	1.53E-03	-1.067	6

Results continued

Comparison of patients on anti-psychotic drug treatment with or without weight gain reveals 63 differentially expressed proteins

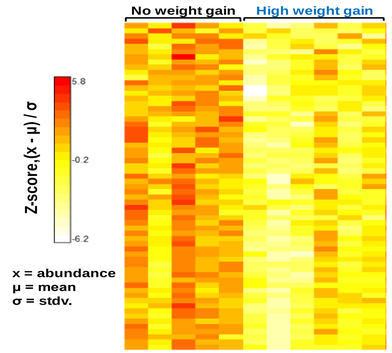


Figure 5. Heatmap of 63 differentially expressed proteins between anti-psychotic drug treat patients with and without weight gain

Main canonical pathways affected (IPA):

- Glycolysis I
- Gluconeogenesis
- Sucrose degradation
- Axonal guidance signaling

20/63 proteins specifically linked to body weight, weight gain or weight loss

Summary

- ❑ Relative intensities of detected proteins in the disease cohort were the same between the two visits a month apart.
- ❑ Down-regulated neuronal-associated proteins were observed in subjects with Schizophrenia.
- ❑ Inflammation-related proteins were up in Schizophrenia.
- ❑ Protein expression correlated with antipsychotic treatment leading to significant weight gain, a significant issue in therapy.
- ❑ An in-depth investigation of proteomic changes in CSF associated with Schizophrenia was obtained.
- ❑ The candidate biomarkers described here require verification and may facilitate effective diagnostics and utility in clinical trials for monitoring efficacy; biomarkers indicating adverse effects such as weight gain sue to antipsychotic agent therapy may be used for monitoring improved therapies.

References

1. Roy *et al.*, JCO 2008; 26(1):96-105
2. Wang *et al.*, Analytical chemistry 2003; 75(18):4818-26
3. Roy & Becker, Methods Mol. Biol. 2007;359:87–105

Acknowledgements

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