SHOTGUN PROTEOMICS APPLIED TO THE STUDY OF NEW BIOMARKERS IN SCHIZOPHRENIA

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INTRODUCTION

Shotgun proteomics allow the study of the complete proteome without the need to work with isolated proteins. In this techniques, from the enzymatic digestion of the proteins contained in a biological sample can be carried out a massive analysis of the peptides obtained by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Because this technique allows a proteomic analysis from a wide range of biological samples, shotgun proteomics turns out to be highly interesting in the identification of biomarkers of mental disorders like Schizophrenia (SZ), where the affected tissue cannot be analysed *in vivo* and in which case the study is made from biological fluids such as blood.

SZ is a highly disabling chronic neurodevelopmental disorder product of the combination of genetic and environmental factors that influence the correct development of brain functions. Due to the importance of establishing an effective treatment as soon as possible, find diagnostic biomarkers of this disease in hospitalized patients with a First-Episode Psychosis (FEP) is highly interesting.

In this search for biomarkers, the "glutamatergic hypothesis" that links this pathway of neurotransmission with the development of the disease, has acquired special relevance since the hypofunction of N-methyl-D-aspartate receptors (NMDAR) in patients diagnosed with SZ has been demonstrated. In addition, NMDARs have been found in the membrane of lymphocytes, which highlights the probable communication between nervous and immune systems. Based on this, it would be expected that the proteomic study of peripheral blood mononuclear cells (lymphocytes and monocytes, PBMCs) maybe can shows significant differences in the levels of proteins related with the glutamatergic via between healthy individuals and patients with a diagnostic of FEP.

OBJECTIVES

The main objective of this study is the search for peripheral protein biomarkers present in blood of patients diagnosed with a FEP through the LC-MS/MS technology, which through a routine blood test, provide information in the clinic about the firsts steps of a neuropsychiatric disorder at molecular level. In addition, it will seek to find associations between the obtained proteomic results and the glutamatergic neurotransmission, highly relevant in SZ according to recent evidences.

MATERIAL AND METHODS

Blood samples were taken from the 7 participants: 3 individuals with diagnosis of FEP hospitalized in the Acute Unit of the "Álvaro Cunqueiro Hospital" (two blood samples were taken from each one: the first was extracted during the hospitalization and the second at discharge) and 4 healthy volunteers taken as control (Table 1). PBMCs were isolated by Ficoll density gradient centrifugation. After purification, quantification and digestion of total protein present in PBMCs, 3.2 µg of the resulting peptides were analyzed via LC-MS/MS. Raw data was processed by Xcalibur™ software and protein identification and quantification were carried out with Proteome Discoverer 2.2 software through Sequest HT algorithm. Functional classification of the identified proteins by biological process (Table 2) and protein class (Table 3) was realised by PANTHER software. Finally, STRING 11.0 software was used for the study of physical and functional interactions among the modulated proteins (Figure 1).

Participant	Gender	Age (years)	Treatment	Via	Duration of the treatment (days)	Dose
Control 1	Male	30	-	-	-	-
Control 2	Female	38	-	-	-	-
Control 3	Female	32	-	-	-	-
Control 4	Male	38	-	-	-	-
FEP 1	Female	39	Risperidone	Oral	22	4 mg/day
FEP 2	Female	37	Aripiprazole	Oral	45	10 mg/day
			Clopixol	IM	32	600 mg/14 day
FEP 3	Male	23	Aripiprazol	Oral	128	15 mg/day
			Olanzapine	Oral	15	10 mg/day
			Risperidone	Oral	4	3 mg/day

Table 1. Clinical data of the patients included in the study.
Legend: IM – Intramuscular injection

RESULTS

Biological process of the proteins categorized by PANTHER				
Biological process	Number of genes (%)			
	FEP-BT vs Control	FEP-AT vs Control	FEP-AT vs FEP-BT	FEP-BT vs FEP-AT
Cellular process	35.3	35.1	37.9	34.8
Metabolic process	31.1	25.4	28.0	34.8
Biological regulation	24.4	21.9	15.9	13.9
Response to stimulus	13.4	11.4	9.1	4.9
Localization	9.2	12.3	15.9	9.4
Multicellular organismal process	7.6	9.6	3.8	2.5
Immune system process	5.0	6.1	3.8	3.3
Biological adhesion	2.5	1.8	2.3	0.8
Cellular component organization or biogenesis	2.5	0.9	3.0	1.2
Developmental process	2.5	2.6	1.5	1.2
Reproduction	1.7	0.9	1.5	1.2
Cell proliferation	0.8	0.9	-	-
Biological phase	-	-	0.8	-

Table 2. Biological process of the proteins upregulated in each comparison between 2 types of samples categorized by PANTHER

Protein classes categorized by PANTHER				
Protein classes	Number of genes (%)			
	FEP-BT vs Control	FEP-AT vs Control	FEP-AT vs FEP-BT	FEP-BT vs FEP-AT
Nucleic acid binding	18.5	10.5	16.7	18.0
Enzyme modulator	8.4	10.5	2.3	7.8
Hydrolase	10.1	17.5	6.1	9.8
Signaling molecule	4.2	4.4	4.5	2.5
Defense/immunity protein	1.7	3.5	1.5	0.8
Ligase	5.0	0.9	2.3	3.7
Transcription factor	4.2	6.1	5.3	4.5
Oxidoreductase	3.4	1.8	3.8	3.7
Transporter	3.4	4.4	3.0	2.5
Cell adhesion molecule	1.7	1.8	0.8	0.8
Cytoskeletal protein	2.5	6.1	4.5	2.9
Receptor	2.5	2.6	1.5	-
Transfer/carrier protein	1.7	-	1.5	2.9
Extracellular matrix protein	1.7	3.5	-	-
Calcium-binding protein	0.8	1.8	3.0	2.5
Cell junction protein	0.8	0.9	0.8	0.4
Chaperone	0.8	1.8	4.5	0.8
Lyase	0.8	-	0.8	0.4
Structural protein	0.8	-	1.5	0.4
Transferase	0.8	-	0.8	7.4
Membrane traffic protein	-	2.6	4.5	2.0
Isomerase	-	-	-	0.4
Storage protein	-	-	-	0.4

Table 3. Protein classes of the proteins upregulated in each comparison between 2 types of samples categorized by PANTHER
Legend: C – Control; FEP-AT – First Episode Psychosis After Treatment; FEP-BT – First Episode Psychosis Before Treatment

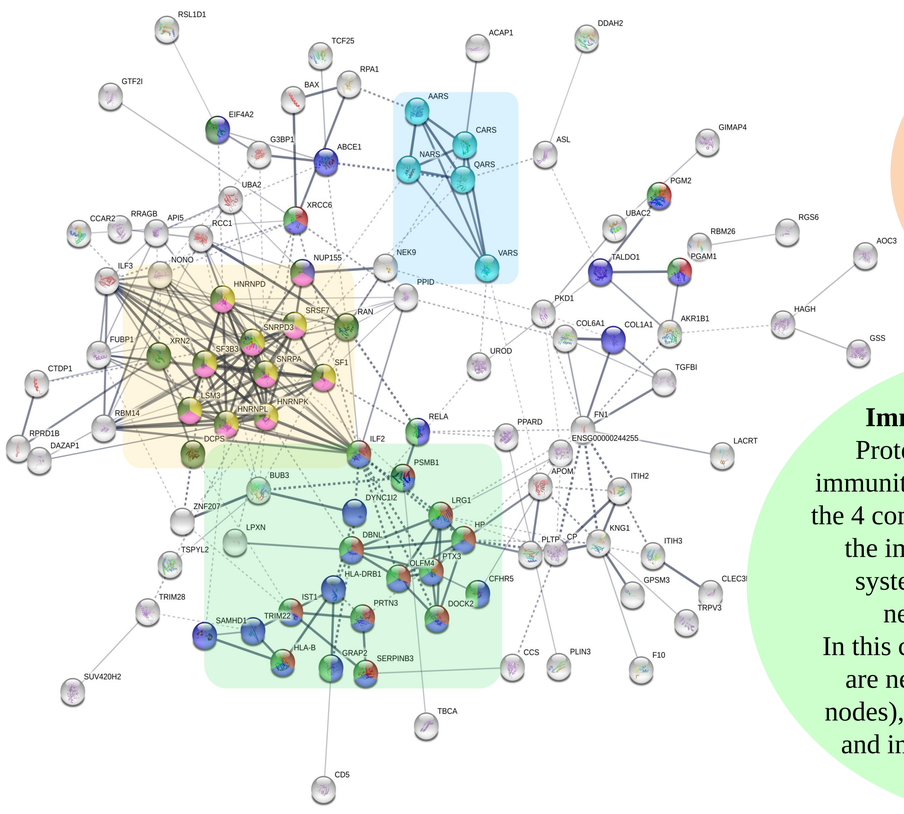
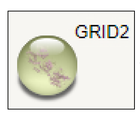


Figure 1. Protein interactome network of the proteins whose expression is upregulated in patients with FEP-BT in comparison with healthy controls using the STRING software.

The main proteins upregulated in the FEP-BT samples compared with controls, are proteins related with the cytosolic tRNA aminoacylation pathway (blue square) and with RNA processing (yellow square)

Immunity system pathways
Proteins implicated in different immunity pathways are upregulated in the 4 comparisons realized. This shows the important role of the immune system in the development of a neuropsychiatric disorder.
In this case, predominantly pathways are neutrophil degranulation (red nodes), immune system (blue nodes) and innate immune system (green nodes) pathways.

WITH THE FOCUS ON THE GLUTAMATERGIC PATHWAY…



Glutamate receptor ionotropic, delta-2 (GRID2) is part of a subfamily of ionotropic glutamate receptors known as "delta". GRID2 is selectively expressed in the dendritic spines of Purkinje cells, where it plays a crucial role in synaptogenesis, synaptic plasticity and motor coordination.

This study has shown that GRID2 is significantly upregulated in FEP-BT and FEP-AT *samples* compared with healthy controls, which may be pointing out a key role of this protein in the development of the first steps of a neuropsychiatric disorder.

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