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LETTER

Mass Spectrometry-Based Proteomics to Understand Schizophrenia

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Psychiatric disorders are the most disabling disorders of humankind. From an economic and social point of view, a person affected by these diseases may lose about 30 years of their lives in an unproductive manner [1]. Although psychiatric patients have been diagnosed and treated for more than a century, these diseases are incurable, and the current medications only partially alleviate symptoms. These hurdles are mostly because we do not understand the biology behind these diseases. Understanding the most elementary molecular processes involved in the development and establishment of these multifactorial diseases is mandatory for better treating them, which will in turn improve patients' lives.

Proteomics emerged in the post genomic era as an alternative toolbox that, by definition, is appropriate to study multifactorial diseases such as psychiatric disorders. In the case of schizophrenia, we can easily see that proteomics has opened roads that have been traveled by scientists in the last decade.

Around 20 years ago, the first proteomic study approaching schizophrenia was published, combining two-dimensional gel electrophoresis and mass spectrometry [2]. From then on, proteomic methods have evolved towards automated liquid chromatography coupled to high-resolution tandem mass spectrometry (LC-MS/MS) and its derivations in qualitative and quantitative terms. The whole proteomic toolbox for LC-MS/MS-based shotgun quantitative proteomics has been employed to decipher the pathobiology of schizophrenia from the molecular point of view: 1DLC or 2DLC in terms of liquid chromatography (offline and online); data-dependent analysis (DDA) and data-independent analysis (DIA) — even including ion mobility — in terms of mass spectrometry [3]. In quantitative terms, different stable isotope labeling techniques - e.g., Isotope-Coded Protein Labeling (ICPL), Isobaric tag for relative and absolute quantitation (iTRAQ) - and label-free approaches (spectral counting and MS^E) have also been used [4]. Finally, targeted proteomics (selected reaction monitoring, SRM) have also been employed [5,6]. These technologies were employed mostly to study postmortem brains and blood plasma or serum. However, other human organs such as the skin and liver were also explored as well as several *in vivo* and *in vitro* models.

While studying the schizophrenia brains, the most consistent differences observed in the proteomes were those associated with energy metabolism, myelination, cytoskeleton assembly, alternative splicing (mRNA processing), and synaptic transmission. Differences associated with synaptic deficits have largely been documented, not only by proteomic analysis but also by large genomic studies. Some of the most commonly found genes or proteins associated to schizophrenia with synaptic function, according to the UniProt, are depicted in Figure 1.

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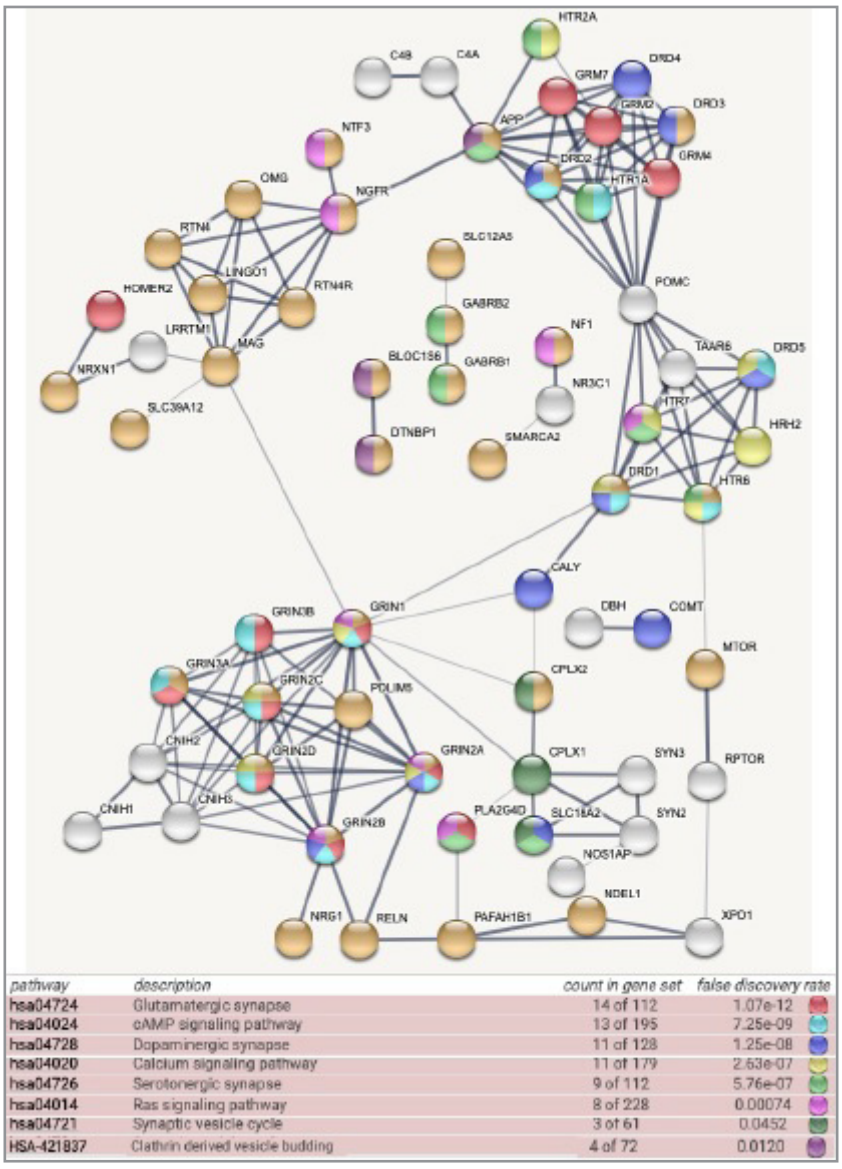

pathway	description	count in gene set	false discovery rate
hsa04724	Glutamatergic synapse	14 of 112	1.07e-12
hsa04024	cAMP signaling pathway	13 of 195	7.25e-09
hsa04728	Dopaminergic synapse	11 of 128	1.25e-08
hsa04020	Calcium signaling pathway	11 of 179	2.63e-07
hsa04726	Serotonergic synapse	9 of 112	5.76e-07
hsa04014	Ras signaling pathway	8 of 228	0.00074
hsa04721	Synaptic vesicle cycle	3 of 61	0.0452
HSA-421837	Clathrin derived vesicle budding	4 of 72	0.0120

Figure 1. Genes and proteins involved in the synaptic dysfunction associated with schizophrenia, (color coded to the major biochemical pathways they are involved in). Dopamine, serotonin, and glutamate receptors are represented majorly. In silico analysis performed in STRING [14].

Interestingly, proteomic alterations observed in postmortem schizophrenia brains have also been observed in induced pluripotent stem cell (iPSC)-derived cerebral organoids generated from the cells of schizophrenia patients (not published). Since proteomics have highlighted the most altered biological processes in schizophrenia, these need to be studied more deeply.

Using *in vivo* and *in vitro* pre-clinical models, we were able to prove that the energy-metabolism-associated differences observed in schizophrenia brains are likely to happen in oligodendrocytes, which are the cells that produce myelin [7]. In Figure 2, based on data from the literature, we can see how the main energy metabolism proteins are associated with classical myelination markers. More specifically, our results indicate that glycolysis seems to be essential in this regard, which is also supported by the analysis conducted in Figure 2. Oligodendrocytes became one of the main topics of our studies since we believe that schizophrenia is not only a neuronal disease, as it has been treated so far, but it can also be a disease

centered on glia cells [8]. It is known that current antipsychotic medication mostly affects the function of neurons. We have also been investigating whether antipsychotics target oligodendrocytes [9]. By realizing that this does happen, we have also been investigating alternative treatments, such as those associated with the endocannabinoid system, as a means of better treating schizophrenia [10].

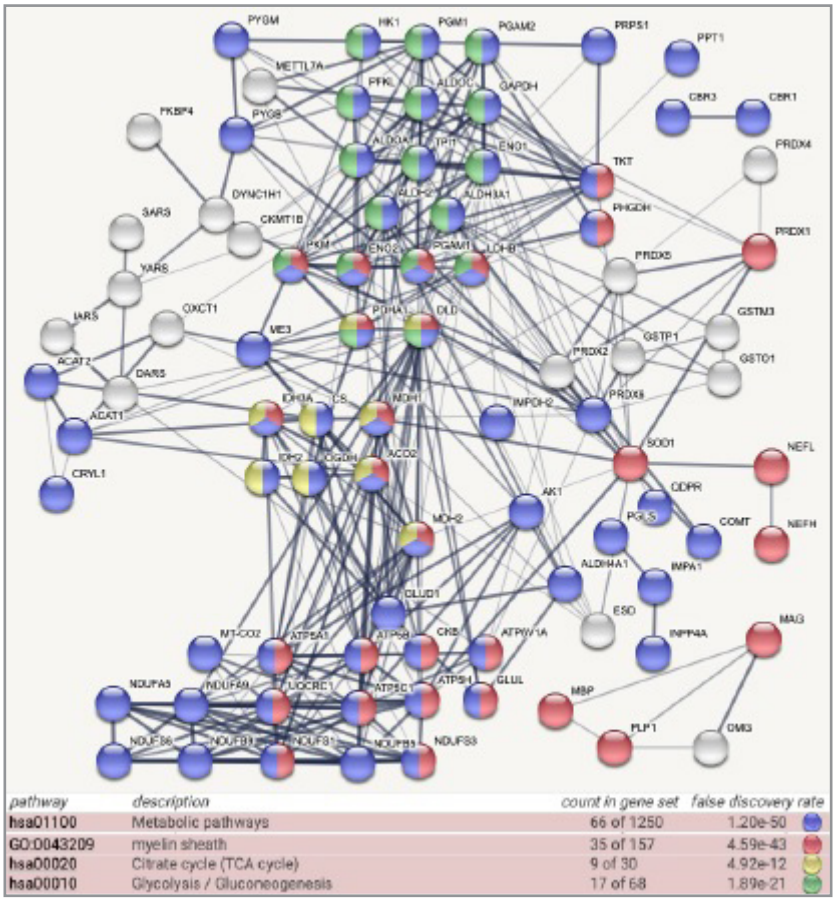

Figure 2. Differentially expressed proteins in schizophrenia samples associated with energy metabolism and myelination. Their strong connectivity shows their direct relation, as we demonstrated experimentally. The central role of glycolysis is also highlighted. In silico analysis perfomed in STRING [14].

In addition, more recently we have been searching for protein biomarkers that could predict an unsuccessful response to antipsychotics in the blood serum or plasma of schizophrenia patients. This is important because almost half of schizophrenia patients do not respond properly to the first round of medication. When medication does not function properly, the disease severity increases, and patients never recover their full brain performance. Moreover, given the side effects of antipsychotic medications, there has been a significant drop in medication usage by patients, which can only worsen the symptoms and, therefore, the mental health of the patients. Thus far, we were able to generate panels of lipid [11] and protein biomarkers [12], which may be implemented as biochemical tests for the prediction of a successful drug response. Proteomic signatures have also been used to build a diagnostic test for schizophrenia [13], which was even commercialized, but later discontinued.

In the last two decades, proteomics has added significant value to the understanding of schizophrenia, which is also true for the other psychiatric disorders. These investigations will end up improving the lives of patients since the next generation of medication may be based on the molecular underpinnings associated

with the disease. Eventually, we may also have biomarker tests in the future for better diagnosis and treatment outcomes from proteomic investigations.

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