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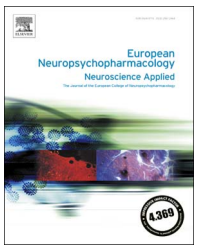

Pattern of gene expression in different stages of schizophrenia: Down-regulation of *NPTX2* gene revealed by a meta-analysis of microarray datasets

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Abstract
Schizophrenia (SCZ) is a severe psychiatric disorder with a genetic susceptibility. Alterations in neurochemical signaling, as well as changes in brain structure and function, manifest during the course of SCZ and are likely causative of the symptoms shown by affected individuals. However, little is known about the timing of these changes, particularly in the pre-morbid and prodromal phases of SCZ. Here, we performed a gene-based and pathway-based meta-analysis of 5 microarray datasets from human induced pluripotent stem cells (hiPSCs)-derived neurons and post-mortem brain tissue from SCZ and healthy controls (HC), with the underlying assumption they might represent the neurobiological make-up of SCZ in the pre-morbid and chronic stages of illness, respectively. Thus, we identified 1 microarray expression profiling dataset of hiPSCs-derived neurons (GSE25673) and performed a systematic search of microarray expression profiling datasets from SCZ post-mortem brain publicly available on the Gene Expression Omnibus (GEO) repository. We selected 4 different SCZ post-mortem brain microarray expression profiling datasets (GSE17612, GSE21935, GSE12649, and GSE21338)

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according to specific inclusion and exclusion criteria. We downloaded raw data and performed quality controls, differential expression analysis, and gene-based, as well as pathway-based meta-analysis. Neuronal pentraxin 2 (*NPTX2)* gene was consistently down-regulated across all datasets, with highly significant association in the meta-analysis (FDR < 1.0E-04). These results highlight the heuristic value of microarray meta-analysis and suggest a role of *NPTX2* as a disease biomarker, provided that it achieves biological validation in future studies examining whether this down-regulation has predictive value with respect to the developmental trajectory of SCZ.

1. Introduction

Schizophrenia (SCZ) is a severe and chronic psychiatric disorder affecting 1% of the population worldwide (Owen et al., 2016). It is well established that the liability to SCZ is modulated by the interplay of genetic and/or environmental determinants of risk (van Os et al., 2014). Indeed, twin studies have estimated the heritability of SCZ at 80% (Cardno and Gottesman, 2000), prompting molecular genetic studies. Recently, hypothesis-free genome-wide association studies (GWAS) have identified more than 128 significant association signals in 108 loci, increasing the knowledge of the genetic architecture of SCZ (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Importantly, GWAS-derived polygenic risk score profiling was able to explain roughly 7% of the variation on the liability scale to SCZ (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). It is plausible that the remaining proportion of phenotypic variation could be explained, at least in part, by environmental determinants, that, through the epigenetic machinery, might modify the liability threshold set by genetic factors.

Thus, it is conceivable that inherited DNA sequence variants and developmental (epigenetic) determinants might interact in modulating the neurobiological make-up of at-risk individuals to the point where illness becomes phenotypically evident. Indeed, alterations in neurochemical signaling (Rolls et al., 2008), as well as changes in brain structure and function (Chan et al., 2011) manifest during the course of SCZ and are likely underlying the symptoms shown by affected individuals. However, little is known about the timing of these changes, particularly in the premorbid and prodromal phases of SCZ. Such neurochemical and structural changes might be specific for each stage of illness (Millan et al., 2016). Consequently, specific gene expression alterations in the brain might correlate with these functional and structural changes.

In this context, a key aspect is to understand whether expression patterns of specific genes might show the same degree of change (i.e. up- or down-regulated) at diverse stages of illness. Such genes could represent illness biomarkers and be used for screening of at risk populations (for instance, individuals at high genetic risk for SCZ). However, one crucial limitation in the field of psychiatric genetics is that we do not have direct access to brain tissue during the active phases of illness, and even comprehensive datasets of human brain gene expression are based on post-mortem specimens (Hawrylycz et al., 2012). The development of human induced pluripotent stem cells (hiPSC)-derived neurons has helped to overcome these limitations (Brennand et al., 2012). Indeed, hiPSC-derived neurons reprogrammed from fibroblasts sampled in SCZ subjects showed specific phenotypic and gene-expression, characteristics, which were partly reversed by *in vitro* treatment with loxapine (Brennand et al., 2011). It is conceivable that these neurons may resemble the state of the central nervous system (CNS) in the premorbid phase of SCZ. Similarly, post-mortem brain tissue might provide a later stage view of the gene expression patterns of the brain when SCZ is entirely manifest clinically. In this context, it would be of importance to test for the presence of commonalities or differences in gene expression patterns at these two separate stages of SCZ by comparing datasets from hiPSC-derived neurons and post-mortem cerebral cortex gray matter.

To this end, we performed a meta-analysis of 5 gene expression microarray datasets, publicly available, from hiPSC-derived neurons and post-mortem brain tissue of SCZ patients, and healthy controls (HC), with the underlying assumption they might represent the neurobiological make-up of SCZ in the pre-morbid and chronic stages of illness, respectively. Specifically, we identified 1 microarray expression profiling dataset of hiPSC-derived neurons (GSE25673) and performed a systematic search of microarray expression profiling datasets from SCZ post-mortem brain publicly available on the Gene Expression Omnibus (GEO) repository. Further, we conducted a pathway-based meta-analysis with the aim of identifying biological pathways showing the same degree of perturbation at different stages of illness.

2. Experimental procedures

2.1. Systematic search of microarray expression profiling datasets from post-SCZ mortem brain

We conducted a systematic search of the GEO repository (https://www.ncbi.nlm.nih.gov/gds/), using the following terms: "*Schizophrenia*" and "*Expression profiling by array*". We obtained a total of 98 datasets. A dataset was incorporated in the analysis if the following inclusion and exclusion criteria were satisfied: 1) using a case-control study design; 2) obtained with a non-custom Affymetrix microarray platform; 3) derived by human post-mortem brain samples; 4) associated to a reference published in Medline; 5)