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Genome-wide expression in veterans with schizophrenia further validates the immune hypothesis for schizophrenia

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ABSTRACT

This study aimed to test whether a dysregulation of gene expression may be the underlying cause of previously reported elevated levels of inflammatory cytokines in veterans with schizophrenia. We performed a genome-wide expression analysis in peripheral blood mononuclear cells from veterans with schizophrenia and controls, and our results show that 167 genes and putative *loci* were differently expressed between groups. These genes were enriched primarily for pathways related to inflammatory mechanisms and formed networks related to cell death and survival, immune cell trafficking, among others, which is in line with previous reports and further validates the inflammatory hypothesis of schizophrenia.

1. Introduction

In addition to the most commonly studied mechanisms involving monoamines and acetylcholine in schizophrenia (which form the underlying basis of most of the currently available antipsychotic drugs), a growing body of evidence suggests a key role for inflammation in its pathophysiology (Khandaker and Dantzer, 2016). It is known that the interaction between the immune system and the brain can induce alterations in mood, cognition and behavior (Dantzer et al., 2008; Khandaker and Dantzer, 2016), and this immune-to-brain communication might play a key role in the pathophysiology and treatment of schizophrenia. Accordingly, patients with schizophrenia present a higher risk of developing auto-immune disorders (Benros et al., 2011) and show an imbalance between pro- and anti-inflammatory cytokines (Girgis et al., 2014). In addition, prenatal exposure to pathogenic microbes can increase the risk for the disorder (Brown and Derkits, 2010), suggesting a role for inflammatory cytokines in schizophrenia risk (Girgis et al., 2014).

In this context, we have previously shown that veterans with a diagnosis of schizophrenia exhibit an increased number of peripheral monocytes during episodes (Dimitrov, 2011), increased peripheral levels of GRO, MCP-1, MDC, and sCD40L, as well as decreased levels of IFN-γ, interleukin (IL)-2, IL-12p70, and IL-17, when compared to controls (Dimitrov et al., 2013). Moreover, more severe psychopathology in these patients, as assessed by the Positive and Negative Symptoms Scale (PANSS), was significantly correlated with molecules involved in the IL-17 pathway (Dimitrov et al., 2013) and with higher levels of high-sensitivity C-reactive protein (hsCRP) (Dimitrov et al., 2016). A follow-up sequential visit study confirmed the consistent increase in GRO, MCP-1, MDC, and sCD40L levels in patients across visits, as well as the decrease in IFN-γ levels (Dimitrov et al., 2015), while IL-17 and IL-4 were reduced only in specific visits (Dimitrov et al., 2015).

In another line of studies, genome-wide expression analyses, both in the periphery and the brain of patients with schizophrenia, have consistently shown alterations in immune system genes (Gardiner et al., 2013; Kumarasinghe et al., 2013; Narayan et al., 2008; Xu et al., 2012; Zheutlin et al., 2016), some of which are corrected by antipsychotic medications (Kumarasinghe et al., 2013). This dysregulation of gene expression may be the underlying cause of the elevated levels of inflammatory cytokines consistently observed in schizophrenia. To test this hypothesis, and in an effort to more clearly establish the relationship between peripheral inflammation and schizophrenia, we performed a genome-wide gene expression analysis in peripheral blood mononuclear

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cells from veterans with a diagnosis of schizophrenia and healthy controls.

2. Material and methods

2.1. Subjects

This study was carried out in accordance with the Declaration of Helsinki and with approval by the University of Texas Health Science Center at San Antonio and Veteran Affairs Research and Development Service. Fifty two veterans with a DSM-IV diagnosis of schizophrenia were enrolled at the Mental Health Intensive Care Management (MHICM) clinic of the South Texas Veterans Health Care System (STVHCS), as previously described (Dimitrov et al., 2015; Dimitrov et al., 2013), with some patients seen in sequential visits (Dimitrov et al., 2015). All patients had a long-standing chronic schizophrenia diagnosis and were treated with different antipsychotics (Table 1). Twenty age- and ethnicity-matched healthy veterans were screened with the SCID screening interview to rule out history of mental illness and family history of psychotic disorders (control group). Subjects were excluded if they had prior history of significant neurological disorder, head trauma, mental retardation, recent substance abuse, history of any psychiatric illness, personal or familial, or if they had ever been treated with any antipsychotic medication. All participants provided written informed consent.

2.2. Psychiatric assessments

Illness severity was evaluated in patients using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 2006). This scale is based on 30 items, rated according to a specific definition and detailed anchor criteria: 7 items are for positive symptoms, 7 for negative symptoms, and 16 for general psychopathology symptoms. Item scores range from 1 to 7, from symptom not present to extreme.

2.3. Genome-wide expression

Fasting blood samples were collected from each subject and processed for the isolation of peripheral blood mononuclear cells (PBMCs) using Ficoll-Paque PLUS (GE Healthcare), according to the manufacturer's instructions. RNA was isolated using the RNease Plus Mini kit (Qiagen). After quantification on NanoDrop™ (Thermo), the integrity of RNA samples was assessed by the Agilent RNA 6000 Nano Kit (Agilent Technologies) on a Bioanalyzer (Agilent Technologies), and samples were subsequently labelled with the TargetAmp™-Nano Labeling Kit (Epicentre). Genome-wide expression levels were measured using the Human HT-12 v4 Expression BeadChip Array (Illumina) in an iScan Microarray Scanner (Illumina). Processing and analysis of gene expression data was performed using the GenomeStudio™ V2011.1 software (Illumina) with the Gene Expression Module v1.0. Raw data were initially submitted to quantile normalization and background subtraction, followed by a differential expression analysis using the controls as the reference group and Illumina Custom as the error model. Probes with a detection p-value >0.01 were removed (22,599), resulting in 11,818 genes for further analysis.

Table 1
Demographic data.

	Schizophrenia patients ($N = 52$)	Controls ($N = 20$)	p-value
Age (years)	54.0 ± 10.1	49.8 ± 10.3	0.1181
Gender (male)	43 (82.7)	12 (60.0)	0.0274
Ethnicity:			0.9754
White	20 (38.4)	8 (40.0)	
Black	11 (21.1)	5 (25.0)	
Hispanic	17 (32.7)	6 (30.0)	
Asian	3 (5.76)	1 (5.0)	
Smoker	31 (59.6)	1 (5.0)	<0.0001
PANNS	78.4 ± 27.1	N/A	
Length of illness:			
5–10 years	3 (5.7)		
10–15 years	1 (1.92)		
≥ 15 years	48 (92.3)		
Medications:			
Paliperidone	15 (28.8)	0	
Clozapine	12 (23)	0	
Haloperidol	8 (15.4)	0	
Quetiapine	5 (9.6)	0	
Risperidone	5 (9.6)	0	
Fluphenazine	4 (7.7)	0	
Olanzapine	3 (5.7)	0	
Ziprasidone	3 (5.7)	0	
Aripiprazole	2 (3.8)	0	
Trifluoperazine	1 (1.92)	0	
Perphenazine	1 (1.92)	0	

Values are represented as Mean ± Standard Deviation or Frequency (Percentage). N/A – non applicable; PANNS – Positive and Negative Syndrome Scale.

2.4. Statistical analyses

Genome-wide expression levels were compared between groups using GenomeStudio™ V2011.1 (Illumina), and multiple testing corrections were applied using Benjamini and Hochberg procedure to control for false discovery rate. Differentially expressed genes were identified using the threshold of p-value <0.01 and absolute fold change >1.3. Pathway analysis was performed using Ingenuity® Pathway Analysis (IPA®, Qiagen).

3. Results and discussion

Demographic data from patients and controls are shown in Table 1. Veterans with schizophrenia and controls did not differ for age or ethnicity, but the patients' group presented a higher proportion of males and smokers compared to controls. All patients were symptomatic, with significant psychopathological scores obtained by the PANSS scale at the time of visit (Table 1). We found 167 differentially expressed genes or putative *loci* between schizophrenia veterans and controls, of which 137 were up-regulated (Table 2) and 30 were downregulated in patients (Table 3). We found no significant correlations between levels of expression of these genes and PANSS scores in the patients' sample ($p \geq 0.05$ for all analyses). Pathway analysis performed with the 167 differentially expressed genes resulted in an enrichment of 46 canonical pathways, of which at least 25 are linked to immune/inflammatory mechanisms (Table 4). The top-ranked pathways were: iNOS Signaling ($p = 0.00122$), Production of Nitric Oxide and Reactive Oxygen Species in Macrophages ($p = 0.00188$), PI3K/AKT Signaling ($p = 0.00288$), CD40 Signaling ($p = 0.00383$), and IL-17A Signaling in Airway Cells ($p = 0.00383$).

As hypothesized, pathway analysis confirmed the role of most of the differentially expressed genes in immune/inflammatory mechanisms. In addition, networks identified by IPA® suggest a role for these genes in processes related to cell death and survival, cellular compromise, function, and maintenance, cell-to-cell signaling and interaction, immune cell trafficking, immunological disease, among others. These results are in accordance with several genome-wide expression studies performed in blood from patients with schizophrenia, which also show immune pathways among the top-ranked pathways identified (Gardiner et al., 2013; Kumarasinghe et al., 2013; Xu et al., 2012; Zheutlin et al., 2016). Altogether, these results suggest that a dysregulation in peripheral gene expression might be contributing to the inflammatory alterations seen in patients, particularly here in veterans with schizophrenia. In addition, they support inflammation as a key target in schizophrenia and suggest the potential clinical relevance of anti-inflammatory agents in the treatment of patients. In this sense, the efficacy of anti-inflammatory medications to improve symptoms in patients with schizophrenia has been reported by independent studies and seems particularly promising (Sommer et al., 2012; Sommer et al.,

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