Psychiatry Research 225 (2015) 208–211

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Brief report

Metabolic and inflammatory genes in schizophrenia

Kayla A. Chase [a], Cherise Rosen [a], Hannah Gin [a], Olivia Bjorkquist [a], Benjamin Feiner [a], Robert Marvin [a], Sean Conrin [a], Rajiv P. Sharma [a,b,*]

[a] The Psychiatric Institute, University of Illinois at Chicago, 1601 West Taylor Street, Chicago, IL 60612, United States
[b] Jesse Brown Veterans Affairs Medical Center, 820 South Damen Avenue (M/C 151), Chicago, IL 60612, United States

ARTICLE INFO

Article history:
Received 24 July 2014
Received in revised form
15 October 2014
Accepted 3 November 2014
Available online 13 November 2014

Keywords:
Schizophrenia
Obesity
Inflammation

ABSTRACT

Energy metabolism and immunity are characterized as abnormal in schizophrenia. Because these two systems are highly coordinated, we measured expression of prototypic obesogenic and immunogenic genes in freshly harvested PBMC from controls and participants with schizophrenia. We report significant increases in PPARγ, SREBP1, IL-6 and TNFα, and decreases in PPARα and C/EPBα and mRNA levels from patients with schizophrenia, with additional BMI interactions, characterizing dysregulation of genes relating to metabolic-inflammation in schizophrenia.

Published by Elsevier Ireland Ltd.

1. Introduction

Energy metabolism and immunity are both reported to be abnormally regulated in schizophrenia. Abnormal energy metabolism, manifested as obesity and insulin resistance is an accepted major public health concern (McEvoy et al., 2005). More subtly, signatures of a chronic subclinical inflammatory state in schizophrenia are increasingly supported and published findings include increases in pro-inflammatory cytokines (Xiu et al., 2012); and a decrease in anti-inflammatory cytokines (Kim et al., 2009; Potvin et al., 2008).

Obesity can be categorized as a pro-inflammatory phenotype, with adipose tissue sitting at the crossroad of metabolism and immunity. Approximately 40% of the cell population in engorged adipose tissue consists of macrophages, which are activated by the abundance of necrotic adipocytes (Meijer et al., 2011; Shapiro et al., 2011; Weisberg et al., 2006). In parallel, adipocytes can release pro-inflammatory cytokines (Meijer et al., 2011) At the signaling level, both obesogenic and immunogenic tissues share common pathways and co-expressed molecules (Chase and Sharma, 2013), which may serve to coordinate their messages. Taken together, obesity and inflammation can induce a composite state titled metabolic-inflammation (Lumeng and Saltiel, 2011; Miller et al., 2011).

In this study, we examine the transcription of both obesogenic and immunogenic genes for the following reasons. Firstly, there is a dearth of studies examining *combined* obesogenic and immunogenic molecular gene expression in schizophrenia patients (Mansur et al., 2012; Miller et al., 2011; Na et al., 2014; Song et al., 2011). Secondly, due to the multiple sources of cytokine and adipose molecules found in the serum (originating from adipose, lymphoid, liver and muscle tissue) (Ferno et al., 2009; Koutnikova et al., 2003; Raschke and Eckel, 2013), we utilized mRNA from a single, identifiable source: the peripheral blood mononuclear cell (PBMC). Our selection of obesity-related genes is based on known developmental and regulatory properties of PPARγ2, PPARα, C/EBPα and SREBP1. The pro-inflammatory cytokines IL-6 and TNFα, both demonstrated in the literature to be were selected as they are up-regulated in obesity, inflammation and schizophrenia.

2. Methods

2.1. Patient information and clinical measures

Subjects ($n=62$) were recruited from the University of Illinois at Chicago Medical Center after receiving approval from the IRB, and provided written informed consent. General inclusion criteria for all subjects were: good physical health (with no reported infections), no history of neurological disease or head trauma, no lifetime history of substance/alcohol dependence or recent (2 months) substance abuse, and not pregnant. Healthy individuals ($n=31$) had no major Axis I disorder (as assessed by SCID interview). Patients with schizophrenia ($n=31$) were diagnosed by clinical consensus using DSM-IV-TR criteria. At the time of sampling, 71% ($n=22$) of the patients were inpatients, with the remainder outpatients. All antipsychotic use was converted to both Chlorpromazine (CPZ) units and the Defined Daily Dose (DDD), with two patients unmediated at time of sampling (Gardner et al., 2010; Nose et al., 2008; Rijcken et al., 2003; Wertheimer, 1986).

Abbreviations: PBMC, fresh peripheral blood mononuclear cells; DSM-IV-TR, Diagnostic and Statistical Manual of Mental Disorders, Edition IV-TR; CPZ, Chlorpromazine; DDD, defined daily dose

* Corresponding author at: The Psychiatric Institute, University of Illinois at Chicago, 1601 West Taylor St., Chicago, IL 60612, United States.
Tel.: +1 312 413 4508; fax: +1 312 413 4503.
E-mail address: rsharma@psych.uic.edu (R.P. Sharma).

http://dx.doi.org/10.1016/j.psychres.2014.11.007
0165-1781/Published by Elsevier Ireland Ltd.

Both BMI and waist circumference were collected on all participants. Tobacco consumption was collected and was categorically coded as yes/no. Patient demographics are presented in Supplemental Table 1.

2.2. PBMC collection

Blood was collected in the morning prior to breakfast. Fresh PBMC were isolated using the Ficoll gradient method (Chase et al., 2013; Gavin et al., 2009; Jayaraman et al., 1999).

2.3. mRNA extraction

Total RNA was isolated using TRIzol reagent (Life Technologies). Samples were treated with DNase (Amersham 27-0514-03) after extraction (Mannhalter et al., 2000).

2.4. Real time RT-PCR quantification

Total RNA was used to prepare cDNA using the Applied Biosystems High Capacity Archive Kit (#4368813). For detection and measurement of expression, Fermentas Maxima SYBR Green/ROX qPCR Master Mix (#K0222) was used. PCR mixtures were run in triplicate on a Thermo Scientific PikoReal System. Cycle threshold value was used for relative quantification, and all values were normalized to a geometric mean of three housekeeping genes: GAPDH, TFRC and β-Actin (Chase and Sharma, 2012; Vandesompele et al., 2002) Primer sequences are listed in the Supplemental Table 2.

2.5. Data Analysis

We performed a linear regression with each gene of interest as the dependent variable and diagnosis, medication use (in both CPZ and DDD units), age, BMI, sex, diabetes, age of onset of psychotic symptoms and tobacco consumption as the explanatory variables. To measure any interactions with weight "risk", BMI was separated out into two groups: "normal" weight (BMI = 18.5–24.9) and "risk" (BMI ≥ 25) (World Health Organization, 1995; Rajkovic et al., 2014; Salazar et al., 2014). This categorization is the most widely accepted predictor of morbidity in the metabolic literature. All mRNA values were tested for normality in SPSS, and variables not exhibiting a normal distribution were natural log transformed.

3. Results

3.1. Diagnosis, demographics and morphometrics

Four obesogenic genes were examined, PPARγ, PPARα, SREBP1 and C/EBPα. Both a diagnosis of schizophrenia ($\beta = 0.30$; $p = 0.013$; Fig. 1A) and a "risk" BMI of 25 or above ($\beta = -0.31$; $p = 0.007$; Fig. 1G) were significant predictors for increased levels of PPARγ mRNA ($F_{2,59} = 8.42$, $p = 0.001$). Further analysis revealed a significant interaction ($F_{1,60} = 6.47$, $p = 0.014$), with overweight patients with schizophrenia exhibiting the highest amounts of PPARγ mRNA.

A diagnosis of schizophrenia was the only predictor of decreases of both PPARα ($F_{1,60} = 7.97$, $p = 0.006$; Fig. 1B) and C/EBPα mRNA levels ($F_{1,60} = 4.9$, $p = 0.032$; Fig. 1C), while a diagnosis of schizophrenia was a significant predictor of increases of SREBP1 mRNA ($F_{1,6} = 4.8$, $p = 0.033$; Fig. 1D). We note here that SREBP1 mRNA was significantly increased in participants currently taking atypical antipsychotic medication. However, this effect cannot be separated from the previously noted diagnostic effect, given that only patients with schizophrenia were receiving antipsychotics. Additionally, this effect was lost if antipsychotic use was coded in CPZ equivalents.

Additionally, two immunogenic gene expression differences were examined: IL-6 and TNFα. Both a diagnosis of schizophrenia ($\beta = 0.38$; $p = 0.002$; Fig. 1E) and a "risk" BMI ($\beta = -0.24$; $p = 0.041$; Fig. 1H) were significant predictors for increased levels of IL-6 mRNA ($F_{2,59} = 8.63$, $p = 0.001$). Further analysis revealed no significant interactions between BMI and diagnosis. TNFα mRNA was significantly higher in men ($t_{61} = 3.11$; $p = 0.003$). A diagnosis of schizophrenia ($\beta = 0.32$; $p = 0.01$; Fig. 1F) was also a significant predictor of differences in TNFα mRNA levels. A sex × diagnosis interaction was also significant in the multiple regression, but this interaction would require replication in a diagnostic sample more evenly distributed for the sex demographic.

4. Discussion

The primary findings of this study were the significant variations of *both* obesity and immune gene expression namely increases in PPARγ, SREBP1, IL-6 and TNFα mRNA and decreases in PPARα and C/EPBα levels, in freshly extracted peripheral blood cells from patients with schizophrenia.

PPARγ is expressed in a variety of tissues, but plays a well-defined role in adipose tissue, and is necessary for the initiation of adipogenesis. Coordinately, in tissues of hematopoietic origin, PPARγ can also decrease expression and activity of pro-inflammatory pathways promoted by the transcription factor NF-κB, and may thereby serve as a mechanism of immune tolerance (Wen and Li, 2010). Martinez-Gras *et al.*, found reduced levels of PPARγ protein in PBMC *nuclear* extracts as well as *plasma levels* of its endogenous ligand 15d-PGJ2, in patients with schizophrenia (Martinez-Gras et al., 2011).

Generally, C/EPBα is significantly anti-mitotic, and is inhibited by inflammatory stimuli (Ramji and Foka, 2002). In fact, C/EBPα can be down-regulated at the transcriptional level by recombinant cytokines such as IL-6, IL-1, and TNFα (Akira et al., 1990; Alam et al., 1992; Poli, 1998). Taken together, increased levels of IL-6 and TNFα and decreases in C/EBPα mRNA are suggestive of interactions between obesogenic and immunogenic function in patients with schizophrenia. Our results with IL-6 and TNFα mRNA levels as a proxy for a pro-inflammatory state are consistent with the substantial literature indicating that pro-inflammatory cytokines are elevated in both schizophrenia and obesity (Eder et al., 2009; Potvin et al., 2008; Song et al., 2009). However, we interpret the sex differences seen in TNFα mRNA levels with caution, as our cohort had significantly more males with schizophrenia; thus any statistical difference seen here could be a result of an unbalanced sex ratio.

Waist circumference and BMI, which were found to be related to increases in both PPARγ and IL-6 mRNA levels, have been shown to be the best indicators of the presence and intensity of an inflammatory response and a metabolic syndrome (Rogowski et al., 2010). IL-6 has a PPRE sequence within its promoter, indicating PPAR binding, dimerization and manipulation of IL-6 gene transcription (Denner et al., 2012; Ramji and Foka, 2002). The confluence of these two systems is also predicted by a common epigenetic mechanism involved in their interaction with the environment (Chase and Sharma, 2013).

The findings of this study have several limitations. Firstly, our cohort consisted of significantly more male patients with schizophrenia. Additionally, in our naturalistic clinical study we did not control for dose, type or duration of antipsychotic. Data indicates a significant effect of atypical antipsychotics on SREBP1 mRNA that would be useful to confirm using a more controlled paradigm to separate a diagnostic from pharmacological effect in PBMCs, as the literature has indicated the ability of atypical antipsychotics to induce both inflammatory and obesogenic gene expression (Klemettila et al., 2014; Sarvari et al., 2014). Finally, many diseases and medical conditions can directly affect metabolism and inflammation, including, but not limited to autoimmune diseases, lipid disorders, the use of statins or even the duration of a diabetes diagnosis. This data was not collected systematically, and could not be controlled for in our data analysis.

Contributors

Kayla A. Chase (KAC), Cherise Rosen (CR), Hannah Gin (HG), Olivia Bjorkquist (OB), Benjamin Feiner (BF), Sean Conrin (SC), Robert Marvin (RM) and Rajiv P. Sharma (RPS).