Schizophrenia Research xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Schizophrenia Research

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

Changes in gene expression and methylation in the blood of patients with first-episode psychosis

Vanessa Kiyomi Ota [a,b,1], Cristiano Noto [b,c,d,1], Ary Gadelha [b,c], Marcos Leite Santoro [a,b], Leticia Maria Spindola [a,b], Eduardo Sauerbronn Gouvea [c,d], Roberta Sessa Stilhano [e], Bruno Bertolucci Ortiz [c], Patricia Natalia Silva [a,b,c], João Ricardo Sato [b,f], Sang Won Han [e], Quirino Cordeiro [c,d], Rodrigo Affonseca Bressan [b,c], Sintia Iole Belangero [a,b,c,*]

[a] Genetics Division of Department of Morphology and Genetics of Universidade Federal de Sao Paulo (UNIFESP), Brazil
[b] LiNC-Interdisciplinary Laboratory of Clinical Neurosciences of UNIFESP, Brazil
[c] Department of Psychiatry of UNIFESP, Brazil
[d] Department of Psychiatry of Irmandade da Santa Casa de Misericórdia de São Paulo (ISCMSP), Brazil
[e] Department of Biophysics of UNIFESP, Brazil
[f] Center of Mathematics, Computation and Cognition, Universidade Federal do ABC, Brazil

ARTICLE INFO

Article history:
Received 30 July 2014
Received in revised form 2 September 2014
Accepted 5 September 2014
Available online xxxx

Keywords:
gene expression
BH4
biopterin
GTP cyclohydrolase I
schizophrenia

ABSTRACT

Schizophrenia is a severe mental health disorder with high heritability. The investigation of individuals during their first-episode psychosis (FEP), before the progression of psychotic disorders and especially before treatment with antipsychotic medications, is particularly helpful for understanding this complex disease and for the identification of potential biomarkers. In this study, we compared the expression of genes that are involved in neurotransmission and neurodevelopment of antipsychotic-naive FEP in the peripheral blood of patients ($n = 51$) and healthy controls ($n = 51$). In addition, we investigated the differentially expressed genes with respect to a) DNA methylation, b) the correlation between gene expression and clinical variables (PANSS), and c) gene expression changes after risperidone treatment. Expression levels of 11 genes were quantified with SYBR Green. For methylation analysis, bisulfite sequencing was performed. A significant decrease in *GCH1* mRNA levels was observed in FEP patients relative to controls. Also, when we compare the FEP patients after risperidone treatment with controls, this difference remains significant, and no significant differences were observed in *GCH1* mRNA levels when comparing patients before and after risperidone treatment. Additionally, although the differences were non-significant after Bonferroni correction, the expression of *GCH1* seemed to be correlated with PANSS scores, and the *GCH1* promoter region was more methylated in FEP than in controls, thus corroborating the results obtained at the mRNA level. Few studies have been conducted on *GCH1*, and future studies are needed to clarify its potential role in the progression of schizophrenia.

Abbreviations: FEP, first-episode psychosis; PCR, polymerase chain reaction; PANSS, Positive and Negative Syndrome Scale; *GABRR2*, gamma-aminobutyric acid (GABA) receptor, rho 2; GABA, gamma-aminobutyric acid; mRNA, messenger ribonucleic acid; RNA, ribonucleic acid; qPCR, quantitative polymerase chain reaction; SCID, Structured Clinical Interview for DSM-IV; HKG, housekeeping genes; *B2M*, beta-2-microglobulin; *HPRT1*, hypoxanthine phosphoribosyltransferase 1; *RPL13A*, ribosomal protein L13a; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *ACTB*, actin, beta; Ct, threshold cycle; SAGE, serial analysis of gene expression; *GAD67*, glutamic acid decarboxylase 67; *RELN*, reelin; *ABAT*, 4-aminobutyrate aminotransferase; *TSPO*, translocator protein (18 kDa); *CHRNB1*, cholinergic receptor, nicotinic, beta 1 (muscle); *CHRNE*, cholinergic receptor, nicotinic, epsilon (muscle); *COMT*, catechol-O-methyltransferase; *GABRR2*, gamma-aminobutyric acid (GABA)receptor, rho 2; *GCH1*, GTP cyclohydrolase 1; *GCHFR*, GTP cyclohydrolase I feedback regulator; *TACR2*, tachykinin receptor 2; *NRG1*, neuregulin 1.

* Corresponding author at: Disciplina de Genética/UNIFESP, Rua Botucatu, 740, Ed. Leitao da Cunha, 1° andar, CEP 04023-900, Sao Paulo, Brazil. Tel.: +55 11 5576 4260, +55 11 5576 4264.

E-mail addresses: vanessakaota@gmail.com (V.K. Ota), csnoto@gmail.com (C. Noto), aryararipe@yahoo.com.br (A. Gadelha), santorogen@gmail.com (M.L. Santoro), leticia.n.spindola@gmail.com (L.M. Spindola), esgouvea@yahoo.com (E.S. Gouvea), robertasessa@gmail.com (R.S. Stilhano), ortiz_bru@hotmail.com (B.B. Ortiz), patriciaoliveira.unifesp@gmail.com (P.N. Silva), joao.sato@ufabc.edu.br (J.R. Sato), universo.han@gmail.com (S.W. Han), qcordeiro@yahoo.com (Q. Cordeiro), rodrigoabressan@gmail.com (R.A. Bressan), sinbelangero@gmail.com (S.I. Belangero).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.schres.2014.09.008
0920-9964/

1. Introduction

Schizophrenia is a severe mental disorder that is characterized by a heterogeneous clinical presentation with a wide variation in the specific symptom dimensions, including positive symptoms (i.e., delusions and hallucinations), negative symptoms (i.e., flattened affect), and disorganized symptoms (i.e., impaired cognitive function, disorganized speech and behavior). The onset of schizophrenia typically occurs in late adolescence and early adulthood, leading to the severe impairment of schizophrenia patients' occupational and personal lives (Onwumere et al., 2011). The first 5 years of the disorder is believed to be particularly critical for prognosis because symptomatic and psychosocial deterioration progresses rapidly during this period (Birchwood et al., 1998). The investigation of individuals during their first-episode psychosis (FEP), before the progression of psychotic disorders and especially before treatment with antipsychotic medications, is particularly helpful for understanding this complex disease.

Although schizophrenia has a high heritability (approximately 80%), the underlying genes related to the disease remain largely unknown (Sullivan et al., 2003). Until now, several studies have been conducted on large samples; these studies have identified rare and common variants that are associated with the disorder (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011; Shi et al., 2009; Stefansson et al., 2009, 2008), but additional approaches are still necessary to fully understand the molecular underpinnings of schizophrenia.

Numerous studies have investigated gene expression changes in post-mortem samples from individuals with schizophrenia and reported changes in the levels of genes associated with myelin, gamma-aminobutyric acid (GABA), glutamate, signaling and synaptic function (for a review, see Sequeira et al., 2012). Although analyzing gene expression in post-mortem samples is helpful to understand the biological pathways underlying psychotic disorders, the investigation of potential biomarkers is still needed to identify progression and even treatment response signatures. In this way, several studies have suggested that gene expression in the blood can serve as a diagnostic tool for brain-related diseases (Middleton et al., 2005; Tsuang et al., 2005; de Jong et al., 2012; Lee et al., 2012; Maschietto et al., 2012), subtype classification (Bowden et al., 2006), or symptomatology, such as delusions and hallucinations (Kurian et al., 2011).

DNA hypermethylation within a gene's promoter usually silences or downregulates the expression of the gene (Baylin, 2005) and has been studied in the blood of patients with schizophrenia in order to identify potential biomarkers for the disease (Melas et al., 2012; Liu et al., 2014).

Few studies have investigated these variables in antipsychotic-naive FEP patients (Numata et al., 2008; Suzuki et al., 2008; Zhang et al., 2008; Kuzman et al., 2009; Gutierrez-Fernandez et al., 2010; Takahashi et al., 2010; de Jong et al., 2012; Kordi-Tamandani et al., 2012, 2013b; Melas et al., 2012; Kinoshita et al., 2013; Kordi-Tamandani et al., 2013a; Kumarasinghe et al., 2013; Nishioka et al., 2013). Given that schizophrenia is a chronic condition that requires lifelong treatment, the progression of the disease and the use of antipsychotic medication can confound results on gene expression and DNA methylation.

Assuming that lymphocytes express several neurotransmitter receptors, including dopaminergic, cholinergic and serotonergic receptors (Gladkevich et al., 2004), we selected 11 neurotransmitter and neurodevelopment-associated genes, based on their expression in blood according to our previous study (Ota et al., 2013) and GeneCards database (http://www.genecards.org/) to investigate gene expression differences in blood of antipsychotic-naive FEP patients and controls. In addition, we evaluated the differentially expressed genes with respect to a) DNA methylation, b) the correlation with clinical variables (Positive and Negative Syndrome Scale – PANSS), and c) gene expression changes after risperidone treatment. The present study is one of the largest to evaluate gene expression and DNA methylation in the blood of an antipsychotic-naive sample.

2. Method

2.1. Study population

Antipsychotic-naive patients ($n = 51$) were recruited from a psychiatric emergency unit in São Paulo, Brazil. FEP was defined by a distinct period, characterized by the emergence of the first psychotic symptoms in the individual. To determine the beginning of the psychotic episode, we determined the last time point at which the individual clearly did not show psychotic symptoms. The diagnosis of a psychotic disorder was established according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), using the Structured Clinical Interview of the DSM-IV (SCID-I). Only FEP patients that were less than 40 years of age with no prior history of antipsychotic medication exposure were selected. Prior treatment with other psychotropic drugs, but not antipsychotics, was allowed. Patients with psychotic episodes due to a general medical condition, substance-induced psychotic disorder, or intellectual disability or psychotic episodes that were associated with bipolar or major depressive disorders were excluded. All patients fulfilled the criteria for one of the following psychotic diagnoses according to the DSM-IV Text Revision: schizophrenia (59.6%), schizophreniform disorders (21.3%), brief psychotic disorders (12.7%) and psychotic disorders not otherwise specified (6.4%). Alcohol and drug misuse was assessed by the SCID and familiar interview, but was not an exclusion criterion. Patients who fulfilled the diagnosis of substance-induced psychotic disorder and patients who suffered from acute intoxications were excluded to participate. Acute and chronic general medical conditions such as infections, HIV, allergies, pregnancy or the postpartum period, rheumatologic or immunological conditions were exclusion criteria for both cases and controls.

Once patient consent was obtained, peripheral whole blood samples were collected and risperidone was prescribed. Risperidone was standardized at doses between 1 and 6 mg. A subgroup of forty-four patients was followed-up for 10.23 (SD = 4.02) weeks under pharmacological treatment and reassessed after this period.

The patients were evaluated using the a) PANSS (Positive and Negative Syndrome Scale) (Vessoni, 1993), b) CGI (Clinical Global Impression Scale) (Lima et al., 2007), c) GAF (Global Assessment of Functioning Scale), and d) CDSS (Calgary Depression Scale for Schizophrenia).

In the group that received follow-up after treatment, whole blood was recollected, and the patients underwent analysis using the same clinical scales under the supervision of the same experienced psychiatrist. The response to treatment was defined as a reduction of 50% in the PANSS total scores after treatment (Leucht et al., 2009).

The healthy control subjects ($n = 51$) were age- and gender-matched volunteers who had no abnormal psychiatric diagnoses or family history of severe psychiatric illness. The Research Ethics Committee of UNIFESP approved the research protocol, and all participants gave informed consent (CEP No. 0603/10).

2.2. Genetic analyses

We assessed the expression of 11 neurodevelopment or neurotransmitter-related genes and five housekeeping genes (HKGs: *B2M*, *HPRT1*, *RPL13A*, *GAPDH* and *ACTB*) using custom RT^2 Profiler PCR Arrays (Qiagen). The threshold cycle (Ct) of each gene and the geometric mean (GM) of all the HKG Ct values were calculated for each sample, and these values were used to estimate ΔCt ($\Delta Ct = Ct_{target\ gene} - Ct_{GM\ of\ HKG}$). ΔCt values were included in the Statistical Package for the Social Sciences (SPSS version 15.0) dataset. For the DNA methylation analysis, we performed sodium bisulfite modification (Epitect® Bisulfite Kit, Qiagen), cloning, and sequencing of individual clones. Details of the methods are provided in Supplementary Information S1.

Please cite this article as: Ota, V.K., et al., Changes in gene expression and methylation in the blood of patients with first-episode psychosis, Schizophr. Res. (2014), http://dx.doi.org/10.1016/j.schres.2014.09.008