Psychiatry Research 217 (2014) 158–162

Contents lists available at ScienceDirect

Psychiatry Research

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

Chronic schizophrenia is associated with over-expression of the interleukin-2 receptor gamma gene

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ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form
8 December 2013
Accepted 15 March 2014
Available online 25 March 2014

Keywords:
Schizophrenia
Interleukin-2 receptor gamma
mRNA
Cytokine
Over-expression

ABSTRACT

Altered immune response, including low-grade inflammatory processes, is involved in the pathogenesis of schizophrenia, a chronic psychiatric disorder with complex etiology. Distinct gene variants of a number of pro-inflammatory and chemotactic cytokines together with their receptors associate with this disorder. Interleukin-2 receptor gamma (IL-2RG) represents an important signaling component of many interleukin receptors and so far, no data on the functional state of this receptor in schizophrenia have been reported. The aim of this study was to investigate mRNA expression of the IL2RG gene (*IL2RG*) in schizophrenia patients in comparison with healthy subjects (controls). Total RNA was isolated from peripheral blood of 66 schizophrenia patients and 99 healthy subjects of Armenian population. The mRNA expression was determined by quantitative real-time polymerase chain reaction (RT-PCR) using *PSMB2* as housekeeping gene. *IL2RG* mRNA expression was upregulated in peripheral blood of patients in comparison with controls (patients vs. controls, median [interquartile range]: 2.080 [3.428–1.046] vs. 0.324 [0.856–0.000], $p < 0.0001$). In conclusion, our findings suggest that over-expression of the IL2RG gene may be implicated in altered immune response in schizophrenia and contribute to the pathomechanisms of this disorder.

1. Introduction

Schizophrenia is a complex severe chronic psychiatric disorder with a heterogeneous clinical phenotype (Faludi et al., 2011). Rate for schizophrenia is approximately 1.1% of the population over the age of 18, and currently 25 million people worldwide are affected by this disorder (Perälä et al., 2007). Recently it was shown that countries characterized by a large rich–poor gap may be at increased risk of schizophrenia (Burns et al., 2013). In Armenia it affects about 0.18% of population (Soghoyan et al., 2003)

Gene–environmental interactions have been found to play a crucial role in the development of schizophrenia (van Os et al., 2008). Among various factors, altered immune response and the inflammatory processes have been reported to contribute to pathophysiology of schizophrenia (Drexhage et al., 2011; Fan et al., 2007). Previous studies, including our own findings, suggested alterations in inflammatory cytokine gene expression and plasma levels in schizophrenia-affected subjects. Moreover, polymorphic variations in the genes for many cytokines have been associated with schizophrenia (Monji et al., 2010; Watanabe et al., 2010; Boyajyan et al., 2012; Fineberg and Ellman, 2013). Alterations in the gene expression profiles of serum cytokines have been also found in first-episode psychosis. (Di Nicola et al., 2013).

Being important mediators of inflammation (Meyer, 2013), pro-inflammatory cytokines also regulate synaptic plasticity (Ben Achour and Pascual, 2010), neurotransmission (Dunn et al., 1999), and neurogenesis (Monje et al., 2003). Particularly, both IL-6 and TNF-alpha were found to inhibit hippocampal neurogenesis in adults (Monje et al., 2003). This may disrupt neurodevelopment in the striatum, which is involved in the pathology of schizophrenia (Novak et al., 2013).

Interleukin (IL)-2 receptor gamma (*IL2RG*) (referred to as the common gamma chain – γ_c) is an important signaling component of receptors for many cytokines, including IL-2, -4, -7, -9, -15, and -21, which display functional redundancy in the regulation of the immune response (Sugamura et al., 1995; Sugamura et al., 1996; Recher et al., 2011; Meazza et al., 2011). Each of these cytokines binds to a specific high affinity receptor complex formed by a cytokine-specific α chain and the γ_c (Potvin et al., 2008).

IL2RG associates with the Janus family tyrosine kinase 3 (JAK-3), which is required for signal transduction. Both the γ_c and JAK3 are essential for the function of all cytokine receptors of this family. Genetic defects of γ_c or JAK-3 results in a severe combined

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http://dx.doi.org/10.1016/j.psychres.2014.03.020
0165-1781/

immune deficiency characterized by the lack of T, B, and NK cells in both mice and humans JAK-3 phosphorylates different downstream signal transducer and activator of transcription (STAT) molecules, in relationship to the type of the receptor complex involved. Thus, IL-4 predominantly signals through STAT-6, whereas IL-2, IL-7, IL-9, and IL-15 mainly activate STAT-5, and IL-21 acts through STAT-3 and STAT1 (Noguchi et al., 1993; Leonard, 1996; Heim, 1999; Kisseleva et al., 2002; Kawai et al., 2012). Among those cytokines, IL-2 and IL-4 have been implicated in schizophrenia: e.g., decreased *in vitro* secretion of IL-2 and increased *in vitro* secretion of IL-4 by peripheral blood leukocytes/mononuclear cells from schizophrenia patients were reported (Avgustin et al., 2005; McAllister et al., 1995). Also, changes in CSF and plasma levels of IL-2 and IL-4 in patients with schizophrenia and increased plasma levels in exacerbations of schizophrenia were reported (Kim et al., 2004; O'Brien et al., 2008; Schwarz et al., 2006). Moreover, functional polymorphisms of the IL-2 and IL-4 encoding genes were shown to be associated with schizophrenia in Germans (Schwarz et al., 2006). However, there is no data in the literature concerning the functional state of IL2RG in schizophrenia.

The aim of the present study was, therefore, to evaluate mRNA expression of the IL2RG gene (*IL2RG*) localized on chromosome X (Xq13.1) in peripheral blood mononuclear cells from schizophrenia patients in comparison with healthy subjects.

2. Methods

2.1. Study population

In this study 66 chronic patients with paranoid schizophrenia (males/females: 33/33, mean age ± S.D.: 51 ± 11.2 years; mean age at the first onset of disease ± S.D.: 41.4 ± 14.2; mean duration of illness ± S.D.: 9.6 ± 11.4) diagnosed by two independent psychiatrists according to DSM-IV-TR (code: 295.30) and 99 age- and sex-matched healthy control subjects (males/females: 45/44, mean age ± S.D.: 50 ± 13.9 years) with no family, past or present history of any mental disorder as determined by the non-patient version of the Structured Clinical Interview for DSM-IV-TR Axis I Disorders have been enrolled (First et al., 2001). All patients were receiving typical antipsychotic haloperidol during their treatment (1 mg 3 times daily *per os*) and were recruited from the clinics of Psychiatric Medical Center of the Ministry of Health of the Republic of Armenia (MH RA). Healthy control subjects were recruited among the blood donors of the Erebouni Medical Center MH RA, and were interviewed by psychiatrists. Exclusion criteria for all study participants included any serious neurological, endocrine, oncological, inflammatory, autoimmune, cerebrovascular, heart, or metabolic disorder. The informed consents were obtained from all study participants. All study subjects were Armenians born and lived in Armenia. The study was approved by the Ethical Committee of the Institute of Molecular Biology NAS RA (IRB #00004079).

2.2. Blood sampling, isolation of total RNA from peripheral blood mononuclear cells, assessment of the quantity and quality of RNA samples

From each study subject 5 ml of peripheral blood was collected in EDTA-containing tubes. Peripheral blood mononuclear cells were isolated from whole blood by the following protocol: to 5 ml of fresh blood was added 10 ml of Red Cell Lysis Buffer (RCLB) (0.144M ammonium chloride, 1 mM sodium bicarbonate). After 5 min the mixture was centrifuged at 1000*g* for 10 min. Supernatant was discarded, and pellet gently rinsed by RCLB, resuspended in 5 ml of the same buffer and centrifuged at 1000*g* for 10 min. The final purified pellet was stored in RNAlater (Ambion, Austin, TX, USA) at -20 °C. Total RNA was extracted using High Pure miRNA Isolation Kit (Roche Applied Science, Penzberg, Germany) according to manufacturer's instruction. The quantity and quality of RNA samples were assessed by 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) using RNA 6000 Nano kit (Agilent Technologies) according to manufacturer's protocol and stored at −80 °C until further use. Average RNA yield was 25 μg per 5 ml of blood sample.

2.3. Reverse transcription

Reverse transcription (0.5 μg total RNA, total volume of 20 μl) was performed with Transcryptor First Strand cDNA Synthesis Kit (Roche Applied Science) using anchored dT primers (0.4 μg; ABgene, Waltham, MA, USA) at 47 °C for 45 min. cDNA samples were stored at −20 °C until further use.

2.4. Measurement of IL2RG expression by quantitative real-time polymerase chain reaction (qRT-PCR)

Fluorescently labeled Locked Nucleic Acid (LNA) probe was selected from the Universal Probe Library (http://www.roche-applied-science.com). The primers for *IL2RG* (Gene ID 3561, accession number AY692262; GenBank database of the National Center of Biotechnology Information, www.ncbi.nlm.nih.gov/genbank) were designed using Probe Finder web-based software in the Universal Probe Library (http://probefinder.com/roche3.html) as follows:

GACAGGCCACACAGATGCTA (left primer), GCTGGGATTCACTCAGTTTGT (right primer); amplicon size 90 basepairs.

PCR reaction mix was prepared as follows: cDNA (5 μl, corresponding to 20 ng calculated on input total RNA) for *IL2RG* was added to 20 μl PCR-mix. The final reaction mix contained 900 nM each sense and antisense primers (Roche Applied Science), 100 nM LNA probe (Roche Applied Science; Universal Probe Library number #50), 3.5 mM $MgCl_2$, 200 μM each dNTPs (ABgene), 1 U Thermo-Start TAQ polymerase and 1 × Thermo-Start Buffer (ABgene). After initial denaturation (one cycle at 94 °C for 15 min), 40 cycles of amplification (94 °C for 45 s, 60 °C for 30 s) were performed on RotorGene 3000 real time DNA detection system (Corbett Research, Sydney, Australia) followed by calculation of the *IL2RG* relative expression by RotorGene Software 6.1.71 (Corbett Research) using a second derivative of the raw amplification data. cDNA from human universal reference RNA (Stratagene, La Jolla, CA, USA) was used as calibrator at concentration of 1.25 ng/reaction calculated on input RNA. As a housekeeping gene proteasome subunit beta type-2 gene (*PSMB2*) was used. Data were expressed as arbitrary units (*IL2RG*/*PSMB2* ratio). The absolute expression of *PSMB2* in all study participants was approximately the same. It has to be mentioned that *PSMB2* is currently one of generally accepted housekeeping genes for normalizing gene expression by qRT PCR. Thus, it was showed that *PSMB2* is a good housekeeping gene for bronchoalveolar cells (Kriegova et al., 2008), and according to our unpublished data it is stably expressed also in PBMC, contrary to *b-actin*, *GAPDH* etc., which expression in these cells varies. In addition, there are no reports on *PSMB2* alterations in any specific disease including schizophrenia.

2.5. Statistical analysis

The Shapiro–Wilk test for normality revealed non-parametric distribution of the obtained data. Therefore, the significance of difference in gene expression levels between patients and controls as well as between males and females within each study group was analyzed by the Mann–Whitney *U* test. Spearman's correlation analysis including calculation of Spearman rank correlation coefficient (Rs) and ordinal descriptive statistics were used. *P* values less than 0.05 were considered as significant. Statistical analysis was performed using GraphPad Prism 5 software.

3. Results

The median mRNA expression levels of *IL2RG* in our schizophrenia patients were on average 6.5 times significantly higher than in healthy control subjects (patients vs. controls, median [interquartile range]: 2.080 [3.428–1.046] vs. 0.324 [0.856–0.000], $p < 0.0001$), see Fig. 1. Significant difference in the *IL2RG* expression was observed between males and females in the control group (males vs. females, median [interquartile range]: 0.2 [0.47–0.00]

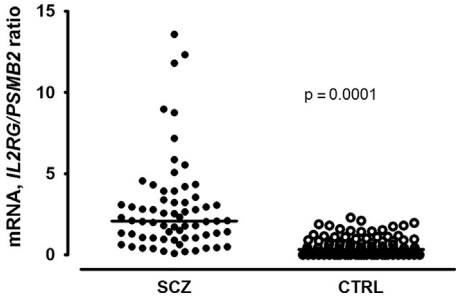

Fig. 1. Interleukin-2 receptor gamma (IL2RG) mRNA expression levels in peripheral blood mononuclear cells from schizophrenia patients (1) and healthy control subjects (2). Data is presented as a dot-plot graph. Median is indicated by horizontal line.