Expression of micro-RNAs miR-31, miR-146a, miR-181c and miR-155 and their target gene IL-2 are altered in schizophrenia: a case-control study [version 1; peer review: awaiting peer review]

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Abstract

Background: Schizophrenia is a severe psychiatric disorder with a heterogeneous clinical phenotype. The association of interleukins and other cytokines and their receptors with schizophrenia has been previously reported. Additionally, a number of studies have reported altered micro-RNA (miRNA) expression in schizophrenia and other psychiatric disorders. The aim of our study was to explore the possible association of miR-31, miR-146a, miR-181c and miR-155 with schizophrenia pathogenesis, as well as their link to IL2 gene expression in disease.

Methods: For this case-control study, 225 patients with paranoid schizophrenia and 225 sex- and age-matched controls with no family history of schizophrenia were recruited. The expression of studied miRNAs and the IL2 gene was measured using qPCR. DNA samples of all patients and controls were genotyped for IL2 rs2069778 single nucleotide polymorphism (SNP) using PCR with sequence specific primers (PCR-SSP). Statistical analyses include the Mann-Whitney U-test and Fischer’s exact test.

Results: All studied miRNAs were over-expressed in schizophrenic patients IL2 gene expression was down-regulated in schizophrenic patients. The IL2 rs2069778 SNP is not associated with schizophrenia but regulates expression of the IL2 gene.

Conclusions: Over-expression of studied miRNAs and down-regulation of IL2 gene expression may be considered as genetic risk factors for chronic schizophrenia. Abnormalities in studied miRNA expressions result in the deregulation of the T-cell receptor signaling pathway in schizophrenia.

Keywords

schizophrenia, miRNA, interleukin-2, cytokines, SNP, expression, pathway
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Introduction

Schizophrenia (OMIM code: 181500) is a severe psychiatric disorder with a heterogeneous clinical phenotype. While the etiology of this disorder remains largely unknown, it has become evident that immune-inflammatory processes play an important multilevel role in disease development and progression. Both cellular and humoral components of the innate and adaptive immune system were shown to be altered at different stages of disease development, both locally within the central nervous system as well as at a systemic level. Cytokines and chemokines are essential signal mediators of the immune system that modulate and guide immune/inflammatory responses as well as perform a wide range of other functions related to cell survival, proliferation, differentiation and migration. Considering their biological importance, cytokines, especially interleukins (IL), have received considerable attention in the context of schizophrenia. The association of interleukins and other cytokines and their receptors with schizophrenia has been previously reported both on the level of genetic variants as well as the levels of gene expression and protein abundance, indicating their essential role in disease predisposition, development, progression and treatment response.

IL-2 controls a wide range of biological activities, largely depending on the biological context. It is essential for T lymphocyte proliferation and differentiation, but it is also implicated in the generation and maintenance of regulatory T (Treg) cells. The role of the IL2 gene in schizophrenia is still unclear and conflicting. However, previous studies, including our own findings, suggest the involvement of IL-2 in the pathogenesis of schizophrenia. Decreased lymphocyte production of IL-2 and increased IL-2 receptors have been reported previously. Interleukin-2 receptor gamma (IL2RG) is an important signaling component of receptors for many cytokines, including IL-2, -4, -7, -9, -15 and -21. Moreover, the IL2RG gene is over-expressed in the blood of schizophrenia patients. On the other hand, other groups reported increased IL-2 serum levels in schizophrenia. Some IL2 genetic polymorphisms were also reported to be associated with schizophrenia. Expression of the IL2 gene is controlled at multiple layers. The IL2 gene contains at least two cis elements for transcript stability regulation, located in both the 3' and 5' untranslated regions (UTRs). Single nucleotide polymorphisms (SNPs) in the promoter region of IL2 influence the expression levels of this cytokine. Finally, expression of IL2 is also regulated by micro-RNAs.

Micro-RNAs (miRNAs) are class of small, non-coding RNAs (comprised of about 22 nucleotides). MiRNAs are found in animals, plants and some viruses. They function in the regulation of gene expression at posttranscriptional level and RNA silencing. As miRNAs are involved in the normal functioning of eukaryotic cells, deregulation of miRNAs has become associated with disease. There is a manually curated “miR2Disease” database, which aims to provide a comprehensive resource of microRNA deregulation in various human diseases. Also, a number of studies have reported altered miRNA expression in schizophrenia, bipolar disorder and major depression and anxiety disorders.

The aim of our study was to explore the possible association of miR-31, miR-146a, miR-181c and miR-155 with schizophrenia pathogenesis, as well as their link to IL2 gene expression in disease. This study is the first to report genetic association between schizophrenia and mentioned above miRNAs; however, several studies have reported a role for the IL2 gene in schizophrenia. All of these four micro-RNAs play a major role in regulating expression of the cytokine network. Particularly, miR-31, miR-146a and miR-181c are regulators of IL2 gene expression, while miR-155 expression is greatly enhanced following stimulation of macrophages and dendritic cells by Toll-like receptors. We also studied the possible association of the IL2 rs2069778 SNP genotype with IL2 and miRNA levels. This SNP was chosen due to its high minor allele frequency, clinical significance in autoimmune diseases, as well as its location near the regulatory elements of the IL2 gene.

Methods

Ethical statement

Informed written consent was obtained from all study participants. The study has been approved by the Ethical Committee of the Institute of Molecular Biology of the National Academy of Sciences RA (IRB00004079, IORG0003427).

Study population

This case-control study was conducted from January 2016 to February 2017. A total of 225 patients with paranoid schizophrenia (SCZ) and 225 sex- and age-matched controls (CTRL) with no family history of schizophrenia were involved in this study (Table 1). This was the maximum available number of schizophrenia patients in Armenia who agreed to participate in this study. From these subjects, 61 patients and 60 controls were tested for micro-RNA expression and 66 patients and 99 controls for IL2 gene expression. There was no specific criteria for dividing subsets in this study; subjects were divided into groups according to availability of biological material (DNA and RNA). All subjects were genotyped for this study.

Paranoid schizophrenia (OMIM code: 181500, ICD-10-CM code: F20.0, DSM-5 code: 295.90) was diagnosed by two independent psychiatrists. Schizophrenia patients were recruited from the clinics of the Psychiatric Medical Center of the Ministry of Health of the Republic of Armenia (MH RA). Healthy subjects with any psychiatric illness during their lifetime, any serious endocrine or neurological disorder, any treatment or medical condition known to affect the brain or meeting the DSM-5 criteria for intellectual disability were excluded from this study. Exclusion criteria for all study subjects included any treatment with immune-modulating drugs and serious medical disorder.

<table>
<thead>
<tr>
<th></th>
<th>SCZ</th>
<th>CTRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>46.34±13.78</td>
<td>46.16±15.01</td>
</tr>
<tr>
<td>Sex male/female</td>
<td>132/93</td>
<td>137/88</td>
</tr>
<tr>
<td>Age of first onset of schizophrenia</td>
<td>30.83±12.92</td>
<td>NA</td>
</tr>
</tbody>
</table>
Healthy control subjects were recruited among the blood donors of the Erebouni Medical Center (MH RA) and were interviewed by psychiatrists.

**Blood sampling and isolation of genomic DNA and total RNA from peripheral blood mononuclear cells**

A total of 10 ml of peripheral blood was collected in EDTA containing tubes (5ml for RNA and 5ml for DNA isolation) from each study participant.

Peripheral blood mononuclear cells were isolated from whole blood using the following protocol, as described in 13: 10 ml of Red Cell Lysis Buffer (RCLB) (containing 0.144M ammonium chloride, 1 mM sodium bicarbonate) was added to 5 ml of fresh blood. After 5 minutes, the mixture was centrifuged at 1000g for 10 minutes, the supernatant was discarded and the pellet was gently rinsed with RCLB. The pellet was re-suspended in 5 ml of the RCLB buffer and centrifuged at 1000g for 10 minutes. The final purified pellet was stored in RNAlater (Ambion, Austin, TX, USA) at -20°C for later use. Total RNA was extracted using High Pure miRNA Isolation Kit (Cat. No. 05080576001 Roche Applied Science, Penzberg, Germany) according to the manufacturer’s instructions. The quantity and quality of RNA and DNA samples were assessed using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer’s protocol and stored at -80°C until further use. The average RNA yield was 25 mg per 5 ml of blood sample.

The method for genomic DNA isolation was a modification of Miller’s salting-out procedure where a chloroform extraction phase is added (2ml of chloroform added to each tube supernatant and centrifuged at 3000g for 10 minutes)40.

**cDNA synthesis**

cDNA synthesis was performed by reverse transcription (0.5 μg total RNA, total volume of cDNA 20 μl) using Transcriptor First Strand cDNA Synthesis Kit (Cat. No. 04897030001, Roche Applied Science) with 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.

**Quantification of IL2 gene expression**

mRNA levels of *IL2* (target) and *PSMB2* (housekeeping) genes and were measured by quantitative real-time polymerase chain reaction with the Rotor Gene 3000 instrument (Corbett Research, USA). cDNA (5 μl, corresponding to 20 ng calculated on input total RNA) was added to 20 μl PCR-mix. The final reaction mix contained 900 nM of each sense and antisense primer (Roche Applied Science), 100 nM LNA probe (Roche Applied Science), 3.5 mM MgCl2, 200 μM of each dNTP (ABgene), 0.2μl Thermo-Start TAQ polymerase with concentration 5 U/μl and 1x ThermoStart Buffer (ABgene).

qPCR was performed using following thermal cycling conditions: initial denaturation for 15 minutes at 95°C; 40 cycles of denaturation for 20 seconds at 95°C, annealing for 30 seconds at 60°C and extension for 60 seconds at 72°C; and a final extension for five minutes at 72°C.

The primers and fluorescnetly-labeled Locked Nucleic Acid (LNA) probe from the Universal Probe Library for the *IL2* gene were selected using the Probe Finder web-based software as follows:

**IL2 gene:**

Left primer: 5’-AAG TTT TAC ATG CCC AAG AAG G-3’
Right primer: 5’AAG TGA AAG TTT TTG CTT TGA GCT A-3’
Probe: #65

**PSMB2 gene:**

Left primer 5’-GTG AGA GGG CAG TGG AAC TC-3’
Right primer 5’-GAA GGT TGG CAG ATT CAG GA-3’
Probe #50

**Measurement of miRNA expression levels**

Reverse transcription for selected micro-RNAs and measurement of micro-RNA expression by quantitative real-time polymerase chain reaction (RT-PCR) were performed using TaqMan Micro-RNA Assays (see Table 2) and TaqMan Universal PCR Master MIX II (no UNG) (Cat. No. 4440040, Thermo Fisher Scientific, Waltham, USA), according to the manufacturer’s instructions. qPCR was performed using Realist-DX IAB real-time PCR system (GeneTiCA, Czech Republic).

qPCR was performed using following thermal cycling conditions: polymerase activation for 10 minutes at 95°C; 40 cycles of denaturation for 20 seconds at 95°C, annealing for 30 seconds at 60°C and extension for 60 seconds at 72°C; and a final extension for five minutes at 72°C.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>miRBase Accession Number</th>
<th>Mature miRNA Sequence</th>
<th>TaqMan Assay cat number</th>
<th>Assay ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-16</td>
<td>M100000070</td>
<td>UAGCAGCAGUAAUAUUGGCG</td>
<td>4427975</td>
<td>000391</td>
</tr>
<tr>
<td>miR-31</td>
<td>M10002673</td>
<td>GGCAAGAUGUGGCAGAUAGCG</td>
<td>4427975</td>
<td>001100</td>
</tr>
<tr>
<td>miR-146a</td>
<td>M10000477</td>
<td>UAGAAACUGAUUCCAGGUGG</td>
<td>4427975</td>
<td>000468</td>
</tr>
<tr>
<td>miR-155</td>
<td>M10000681</td>
<td>UUAUUGCAUAUCUGAUAAGGG</td>
<td>4427975</td>
<td>002623</td>
</tr>
<tr>
<td>miR-181c</td>
<td>M10000271</td>
<td>AACAUUCAACCUGUCGGUGAG</td>
<td>4427975</td>
<td>000482</td>
</tr>
</tbody>
</table>

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**Table 2. List of miRNA assays used in the study.**
of denaturation for 15 seconds at 95°C and annealing/extension for 60 seconds at 60°C.

We used miR-16 as a housekeeping micro-RNA. Data were expressed as arbitrary units (miR-X/miR-16 ratio). miR-16 is currently one of generally accepted housekeeping micro-RNAs for normalizing micro-RNA expression in blood cells by qRT PCR. In addition, there are no reports on miR-16 alterations in any psychiatric disease including schizophrenia.

**PCR-SSP analyses**

DNA samples of all patients and controls were genotyped for *IL2* rs2069778 SNP using a polymerase chain reaction with sequence-specific primers (PCR-SSP). The sequences of specific primers were designed based on relevant DNA sequences available in the NCBI GenBank database (RefSeq Accession: NG_016779.1). Primer sequences for the selected SNP were as follows:

Reverse standard: 5’-CAC CAC TAC AAA TTC TAC AAA TTC G-3’
Reverse mutant: 5’-CAC CAC TAC AAA TTC TAC AAA TTC A-3’
Forward constant: 5’-CTG GTG CCA GAA AGA GCT TG-3’

The presence/absence of allele-specific amplicons were visualized by electrophoresis using 2% agarose gel in 0.5x Tris-Borate-EDTA (TBE) buffer stained with ethidium bromide fluorescent dye. To check the reproducibility of results, randomly selected DNA samples of study subjects (10% of total) were genotyped twice.

Genotyping was carried out at Laboratory of Human Genomics and Immunomics, Institute of Molecular Biology NAS RA (Yerevan, Armenia).

**Statistical analyses**

The Shapiro–Wilk test for normality revealed non-parametric distribution of the obtained data. Therefore, the significance of difference in gene expression levels between each study group was analyzed by the Mann–Whitney U test. *p*-values less than 0.05 were considered as significant. Statistical analysis was performed using GraphPad Prism (version 5) software. Allele and genotype frequencies were checked for Hardy-Weinberg equilibrium and were in equilibrium. No investigation of potential sources of bias was undertaken.

**Pathway analysis**

For characterization of enriched functions and biological pathways of the studied miRNAs targets, we used miRsystem, which performs enrichment analyses, accounting both for target genes as well as the levels of individual miRNA expression.

**Results**

**Expression of IL2 in schizophrenia**

In total, 66 SCZ patients (male/female: 33/33, mean age±S.D.: 51±11.2 years) and 99 healthy controls (male/female: 45/44, mean age±S.D.: 50±13.9 years) participated in *IL2* gene expression step.

We studied mRNA expression levels of the *IL2* gene in schizophrenia and its possible association with the *IL2* rs2069778 C/T SNP. The median mRNA expression levels of *IL2* in the patient group were significantly lower than in healthy control subjects (patients vs. controls, median [interquartile range]: 0.06889 [0.6499–0.007519] vs. 1.469 [3.858–0.000], *p*=0.0095) (Figure 1).

Furthermore, analysis revealed a significant difference in *IL2* expression between those with and without the *IL2* rs2069778 C/T SNP in both the control and schizophrenia groups. Particularly, in schizophrenic patients, *IL2* mRNA expression levels were significantly higher in rs2069778*T* minor allele carriers (CT+TT) than in CC homozygotes (CC vs. CT+TT, median [interquartile range]: 0.033711 [0.5433–0.00453] vs. 0.2178 [2.618–0.03726], *p*=0.0003) (Figure 2). The same difference was found in control groups (CC vs. CT+TT, median [interquartile range]: 1.083 [2.840–0.000] vs. 2.625 [4.966–0.000], *p*=0.0495) (Figure 2). It is worth noting that expression of *IL2* in schizophrenic patients carrying the CC genotype was lower than in corresponding controls (*p*=0.0216), while the difference in expression between carriers of the *T* minor allele was not significant (*p*=0.22).

**Levels of miRNAs in schizophrenia**

A total of 61 SCZ patients (male/female: 37/24, mean age±S.D.: 45.4±13.9 years) and 60 healthy controls (male/female: 37/23, mean age±S.D.: 44.5±13.6 years) were tested for micro-RNA expression.

Median expression levels of all studied miRNAs were significantly higher in schizophrenic patients as compared to healthy controls (Table 3 and Figure 3).

Further analysis indicated that in *T* allele carriers, miR-181c had significantly lower expression compared to CC homozygous variants, both in schizophrenia patients (TT+CT vs. CC, median [interquartile range]: 1.99 [2.92–1.39] vs. 3.46 [4.84–2.14], *p*=0.0045) and controls (TT+CT vs. CC, median [interquartile range]: 0.41 [2.41–0.10] vs. 1.90 [5.31–0.59], *p*=0.011) (Figure 4). Interestingly, in *T* allele carriers, miR-31 also had significantly lower expression compared to CC homozygous variants in controls (TT+CT vs. CC, median [interquartile range]: 0.99 [1.61–0.03] vs. 1.84 [3.35–0.48], *p*=0.0015) but in schizophrenia patients there was no significant difference (TT+CT vs. CC, median [interquartile range]: 3.93 [7.74–2.91] vs. 5.03 [8.12–3.16], *p*=0.36) (Figure 4). We have not found any association between *IL2* rs2069778 variants and expressions of miR-155 and miR-146a.

**Functional annotation of biological pathways containing targets for studied miRNAs**

Our analysis demonstrated significant up-regulation of *IL2* expression-modulating miRNAs in schizophrenia. However,
it is known that miRNAs can affect multiple targets. For characterization of enriched functions and biological pathways of the studied miRNAs targets, we used a freely available online integrated system called miRsystem\(^5\). The analysis resulted in 30 pathways from 4 databases (KEGG\(^6\), Biocarta, Reactome, Pathway Interaction Database) significantly enriched with miRNA targets. The majority of these were related to immune/inflammatory system pathways where IL2 is either an effector or a target gene (Table 4). Figure 5 is an example of miR target enriched pathway\(^5\).

**Discussion**

In this study, we observed decreased levels of IL2 expression in peripheral blood mononuclear cells of schizophrenic patients, paralleled with increased expression of IL2-regulating miRNAs (miR-31, miR-146a, miR-155 and miR-181c). In addition, we demonstrated that carriage of the minor allele for IL2 rs2069778 is associated with increased IL2 expression levels which might suggest either a regulatory role for this SNP or a linkage with other SNPs that can modulate gene expression.

**Table 3. Levels of miR-31, miR-146a, miR-155 and miR-181c expression in schizophrenia patients and controls.**

<table>
<thead>
<tr>
<th>miRNA</th>
<th>SCZ patients (median [interquartile range])</th>
<th>Controls (median [interquartile range])</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-31</td>
<td>4.57 [8.12–3.38]</td>
<td>1.39 [2.998–1.1288]</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>miR-146a</td>
<td>0.802 [1.26–0.5205]</td>
<td>0.487 [1.318–0.1223]</td>
<td>(p=0.0254)</td>
</tr>
<tr>
<td>miR-155</td>
<td>1.155 [2.1–0.8223]</td>
<td>0.04065 [0.1037–0.02585]</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>miR-181c</td>
<td>3.086 [4.637–1.97]</td>
<td>1.442 [3.454–0.3059]</td>
<td>(p=0.0008)</td>
</tr>
</tbody>
</table>
Figure 3. Levels (miR-X/miR-16 comparative expression) of miR-31, miR-146a, miR-155 and miR-181c expression in schizophrenic patients (SCZ) and controls (CTRL).

Figure 4. IL2 rs2069778 minor allele carriage-dependent expression (miR-X/miR-16 comparative expression) of miRNAs in patients (SCZ) and controls (CTRL). CC – homozygous for rs2069778 SNP, TT+CT – carrier for rs2069778 SNP.
Table 4. Top pathways enriched with target genes for miR-31, miR-146a, miR-155, miR181c.

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>TERM</th>
<th>Targets In The Term</th>
<th>% Of Targets In Genes</th>
<th>Raw p-value</th>
<th>Empirical p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>KEGG</td>
<td>TOLL-LIKE RECEPTOR SIGNALING PATHWAY</td>
<td>10</td>
<td>9.8</td>
<td>3.08E-07</td>
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<td>KEGG</td>
<td>T CELL RECEPTOR SIGNALING PATHWAY</td>
<td>8</td>
<td>7.41</td>
<td>3.58E-05</td>
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<tr>
<td>KEGG</td>
<td>CHEMOKINE SIGNALING PATHWAY</td>
<td>9</td>
<td>4.76</td>
<td>0.000325</td>
<td>0.003505</td>
</tr>
<tr>
<td>KEGG</td>
<td>B CELL RECEPTOR SIGNALING PATHWAY</td>
<td>5</td>
<td>6.67</td>
<td>0.001634</td>
<td>0.003546</td>
</tr>
<tr>
<td>KEGG</td>
<td>FCGAMMAR-MEDIATED PHAGOCYTOSIS</td>
<td>5</td>
<td>5.32</td>
<td>0.004168</td>
<td>0.025695</td>
</tr>
<tr>
<td>KEGG</td>
<td>NEUROTROPHIN SIGNALING PATHWAY</td>
<td>6</td>
<td>4.72</td>
<td>0.003129</td>
<td>0.047234</td>
</tr>
<tr>
<td>BIOCARTA</td>
<td>BIOCARTA BCR PATHWAY</td>
<td>3</td>
<td>8.57</td>
<td>0.007175</td>
<td>0.027159</td>
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<tr>
<td>BIOCARTA</td>
<td>BIOCARTA MAPK PATHWAY</td>
<td>5</td>
<td>5.75</td>
<td>0.003043</td>
<td>0.030354</td>
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<tr>
<td>PATHWAY INTERACTION DATABASE</td>
<td>IL2 SIGNALING EVENTS MEDIATED BY STATS</td>
<td>4</td>
<td>13.33</td>
<td>0.000374</td>
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<td>PATHWAY INTERACTION DATABASE</td>
<td>CALCINEURIN-REGULATED NFAT-DEPENDENT TRANSCRIPTION IN LYMPHOCYTES</td>
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<td>10.0</td>
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<tr>
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<td>8.82</td>
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<tr>
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<td>IGF1 PATHWAY</td>
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<td>13.79</td>
<td>0.000328</td>
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<tr>
<td>PATHWAY INTERACTION DATABASE</td>
<td>PDGFR-BETA SIGNALING PATHWAY</td>
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<td>5.56</td>
<td>0.000602</td>
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<td>REACTOME</td>
<td>INTERFERON GAMMA SIGNALING</td>
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<td>6.85</td>
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<td>0.008003</td>
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<tr>
<td>REACTOME</td>
<td>CYTOKINE SIGNALING IN IMMUNE SYSTEM</td>
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<td>4.09</td>
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<tr>
<td>REACTOME</td>
<td>INTERFERON SIGNALING</td>
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<td>CD28 CO-STIMULATION</td>
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<td>0.009527</td>
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<td>REACTOME</td>
<td>INTERFERON ALPHA BETA SIGNALING</td>
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<td>REACTOME</td>
<td>ADAPTIVE IMMUNE SYSTEM</td>
<td>13</td>
<td>2.70</td>
<td>0.002974</td>
<td>0.011809</td>
</tr>
</tbody>
</table>

Conflicting results on the levels of IL2 have been reported for this disease both in medicated and undedicated patients. The data published by Singh et al.,55 and Theodoropoulou et al.,56 are in line with our findings. However, Ebrinc et al.,57 and Zhang et al.,58,59 have found elevated levels of IL2 in their subjects. Furthermore, there are studies reporting no difference in the IL2 levels of schizophrenic patients as compared to controls33. Because expression was studied in different populations, we can speculate that the observed discrepancies could be partially explained by different genetic backgrounds. Moreover, the validity of our results is supported by the detected increase of IL2 expression-modulating miRNAs measured using independent assay techniques. Finally, consistent with IL2’s role in maintenance of Treg cells, their low levels were also reported in schizophrenia59.

Though the studies of miRNA involvement in schizophrenia are a relatively new direction, there are already results implicating miRNA deregulation in the pathogenesis of schizophrenia. miR-137 is the micro-RNA best known for its role in schizophrenia pathogenesis44-46. This micro-RNA is also well known due to a genetic polymorphism (SNP variant) in its gene, which was described as a genetic risk factor for the development of schizophrenia in a European population7-9. There are other recent studies which confirmed the role of distinct micro-RNAs such as miR-195, miR-181b, miR-301a, miR-19, miR-206, miR-30a and miR-219 in the pathogenesis of schizophrenia40-44. In this study, we reported four miRNAs (miR-31, miR-146a, miR-155, miR-181c) that were up-regulated in schizophrenic patients. Besides targeting IL2 expression, these molecules have many other targets that are involved in immune/inflammatory pathways, confirming the essential role of immune system disturbances in disease development and progression.

The limitation of the present study is the inability to recruit medication-free patients for assessment of the effect of treatment on IL2 and miRNA expression. However, in many studies cited in this paper,53,54 regardless the direction of difference in IL2 levels, no differences were observed between treated and untreated patient groups. It should also be noted that we measured IL2 gene and miRNA expression in two different patient groups with little overlap, which prevented us from performing direct correlation analysis between the levels of IL2 and miRNAs.

Overall, our findings further strengthen the role of immune system deregulation in the development and progression of schizophrenia and necessitate further research towards understanding the changes of the Th1/Th2/T-reg response in this disease and in response to antipsychotic treatment.

Conclusions

All studied miRNAs (miR-31, miR-146a, miR-155, miR-181c) were over-expressed in schizophrenic patients, suggesting...
a role for them in disease pathogenesis. IL2 gene expression was down-regulated in schizophrenic patients. The IL2 rs2069778 C/T SNP is not associated with schizophrenia but regulates expression of the IL2 gene. Abnormalities in studied miRNA expressions result in the deregulation of the T-Cell receptor signaling pathway in schizophrenia.

Data availability
Underlying data

Figure 5. Regulation of T cell signaling pathway by miR-31, miR-146a, miR-155 and miR-181c. Red boxes indicate target genes for studied miRNAs. This figure has been reproduced with permission from the KEGG PATHWAY database46.

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