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Expression pattern of long non-coding RNAs in treatment-naïve and medicated schizophrenia patients

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Schizophrenia is a disabling mental disorder that affects 1% of people over their lifetime. The etiology and mechanism of schizophrenia are very complex, and many genes are involved in many different signaling pathways in the etiology of this disease. According to recent studies, one of the important mechanisms altered in this disorder is the regulation of immune system and the inflammation mechanism. In the present study, we evaluated the peripheral blood expression pattern of four lncRNAs and three protein-coding genes in the treatment- naïve patients, and medicated patients compared with sex and age-matched controls. In the medicated-patients, expression levels of *IFNG*, *IL18RAP*, *AC007278.2* were significantly up-regulated ($P < 0.05$); and the expression level of *IFNG-AS1-001* was significantly down-regulated compared to healthy controls ($P < 0.05$). However, levels of *IL18R1*, *AC007278.3* and *IFNG-AS1-003* were not different between these groups. In the treatment-naïve patients, *IFNG*, *IL18R1*, *IL18RAP*, *IFNG-AS1-001*, *AC007278.2*, and *AC007278.3* were significantly up-regulated compared to controls. On the other hand, *IFNG-AS1-003* was significantly down-regulated in the treatment-naïve patients compared to controls. Based on the Spearman correlation matrix, there was a significant correlation between genes in the treatment-naïve patients. We also showed the high sensitivity and specificity of *IFNG-AS1-003*, *IFNG*, *IL18R1*, and *AC007278.3* in the identification of treatment-naïve patients from controls. The current study contributes further evidence to the understanding of the role of lncRNAs in the pathogenesis of schizophrenia. Future research is necessary to establish the validity of lncRNAs as peripheral markers for this condition.

Keywords Schizophrenia, IFNG, IL18R, IL18RAP, IFNG-AS1-001

Schizophrenia is one of the most disabling mental disorders that affects about 1% of people over their lifetime[1]. The prevalence of this disease is approximately 0.3 to 0.7% worldwide. The disease usually occurs sporadically. Its symptoms usually appear in adulthood and early adolescence, leading to severe disability and high level of stress[1]. This disorder is determined by the existence of various symptoms, including positive symptoms (hallucinations, delusions, unusual behaviors, unusual speech, abnormal thought, and movement disorder), negative symptoms (insensibility, lack of delight, attention impairment, and sociality withdrawal), and cognitive symptoms (imperfection in executive function and precision, and agnosia)[2]. The etiology of schizophrenia has not been completely identified[3]. However, it is clear that mechanisms of schizophrenia are very complex, and many environmental and genetic factors play a pivotal role in causing this disease. There is massive evidence indicating that changes in gene expression in the immune system contribute to the pathogenesis of this disorder[4,5]. Dysregulation of the immune system and its intricate interplay with the nervous system might play a part in the etiology and physiological mechanisms of schizophrenia[6]. The reciprocal relationship between the immune system and the brain has sparked a rising curiosity regarding the involvement of the immune system

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in neuropsychiatric disorders. Notably, the atypical blood lymphocyte parameters, such as the levels of total T lymphocytes and T helper cells, have specifically attracted attention[7].

Numerous studies have shown that inflammation and immunity play significant roles in the development of symptoms associated with schizophrenia[8,9]. These studies have provided compelling evidence that systemic inflammation can have a profound impact on the brain, resulting in alterations in mood, cognition, and behavior[10].

Long non-coding RNAs are a subset of non-coding transcripts and have a length of more than 200 nucleotides[11]. Studies have demonstrated that some lncRNAs are effective in controlling the behavior of immune cells and immune responses[12], including the differentiation and activity of T and B cells, macrophages, and NK cells. Several lncRNAs play pivotal roles in cell function and participate in the pathogenesis and development of various diseases such as cancer[13]and especially neuropsychiatric disorders and neurodegenerative diseases such as Alzheimer's disease[14], Parkinson's disease[15], major depressive disorder[16], autism spectrum disorders[17]and multiple sclerosis[18].

Based on the functional roles in the regulation of immune responses, we selected four lncRNAs, namely *IFNG-AS1-001*, *IFNG-AS1-003*, *AC007278.2*, and *AC007278.3*; and three protein-coding genes, namely *IFNG*, *IL18R1*, and *IL18RAP* to assess their expression in peripheral blood of treatment-naive and medicated schizophrenia patients compared with matched healthy controls. *IFNG*has been suggested to partake in the pathogenesis of schizophrenia[19,20]. Methylation of the *IFNG* locus is regulated by *IFNG-AS1-001*[21]. *IFNG-AS1-003* gene is also located on the same chromosome as the *IFNG*[22], possibly contributing to regulation of this gene. Similarly, *IL-18*is involved in the pathologic events seen in this disorder[23]. This cytokine exerts its effect through its receptor being encoded by *IL18R1* and *IL18RAP*genes[24]. Two functionally related lncRNAs, namely *AC007278.2* and *AC007278.3* are located on chromosome 2 inside the introns of *IL18R1* and *IL18RAP*, respectively.

This study provides new insights into the altered expression of protein-coding and non-coding genes related to the immune system and proposes them as contributors in the pathogenesis of schizophrenia and as novel biomarkers for the diagnosis of schizophrenia.

Materials and methods

Study participants

The present study was performed on 50 medicated and 25 treatment-naive schizophrenia patients, and 50 sex and age-matched healthy controls. Cases were recruited from Razi hospital, Tabriz, Iran. The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V)[25] was applied in the diagnostic process. The participants with alcohol drinking and substance abuse or cigarette smoking were excluded from the study. Healthy controls were selected from those were referred to the health centers of Tabriz University of Medical Sciences. The Mini-International Neuropsychiatric Interview was used for the assessment of healthy controls. Exclusion criteria were the existence of any systemic disorder, psychiatric condition, pregnancy, or a history of psychopathology in a first-degree biological relative. The study protocol was approved by the Shahroud University of Medical Sciences Ethical Committee (IR.SHMU.REC.1398.113). Informed consent forms were signed by all participants/or their guardians.

RNA extraction and cDNA synthesis

In the first step, 5 ml of the peripheral blood was gathered in K2-EDTA-containing tubes. The RNA of all samples was extracted by using the Hybrid-R blood RNA extraction Kit (Gene All, Seoul, Korea). The Quality of the extracted RNAs was verified by 1% agarose gel electrophoresis, and to eliminate any genomic DNA contamination, samples were treated with DNase I (Thermo scientific, Deutschland, Germany). The quantity of RNA was appraised by Nanodrop equipment (Thermo Scientific, MA, USA). The extracted RNA was subsequently converted to cDNA by using the High-Capacity cDNA Reverse Transcription FIRE Script RT cDNA Synthesis Kit (Solis Bio Dyne, Estonia).

Primer design

Primer design was accomplished using the NCBI Primer designing tool and verified through blasting in the nucleotide BLAST database to confirm specific binding to target sequences. The primer sequences are detailed in Table 1.

qRT-PCR

Expression levels of genes were assessed in all participants using the RealQ Plus 2 × PCR Master Mix Green with high ROX (Amplicon, Odense, Denmark). Cycling reactions were carried out in Step One Plus Real-Time PCR equipment (Applied Biosystems, Foster City, CA, USA). After evaluation of Ct values of *UBC* and *YWHAZ*, we used *UBC* gene as the reference gene based on its constant expression in the peripheral blood of schizophrenia patients. The stable expression of *UBC* was also confirmed by NormFinder software (https://www.moma.dk/software/normfinder).

Statistical analysis

Relative expression levels of genes were measured in all samples. The Ln [Efficiency^ΔΔCT] method considers the transcript levels of *UBC* as normalizer. The Shapiro-Wilk test was performed to evaluate the normality of the data. One-way ANOVA and Bonferroni's multiple comparisons test were used for comparison of expression data between study groups. Correlations between expressions of genes were valued by calculation of Spearman correlation coefficients. Data was analyzed using the GraphPad Prism 8.0.0 software. The diagnostic power of the transcript levels of genes was measured by depicting receiver operating characteristic (ROC) curves.

Primers		Sequence 5' 3'	Tm (C°)
IFNG-AS1-001	F	ACATACTTCCACCAGAGA	62
	R	TTCCACAACACTATCAACT	
IFNG-AS1-003	F	CCAGCACCATAATTCCAG	60
	R	GAAGACATGAGCACTGAC	
AC007278.2	F	ATCATCTGTATGCTGTCTAAC	60
	R	AACCATATAATGAGGCTGTC	
AC007278.3	F	TTGAAGAGGAGATTAAGTATTAGG	64
	R	TCGATCATCTTCACATTCACATC	
IFNG	F	GTTCTCTTGGCTGTTACTG	58
	R	CATTATCCGCTACATCTGAAT	
IL18R1	F	GAGAAACATTTTGGGTATAAGTTATG	62
	R	CTCTATCAGTGAGTGGATTTC	
IL18RAP	F	AACACTCTACTCTGGCAAA	62
	R	ATCCTTTAATTCGCTCTCCT	
UBC	F	GGATTTGGGTCGCAGTTCTTG	60
	R	TGCCTTGACATTCTCGATGG	
YWHAZ	F	ACTTTTGGTACATTGTGGCTTC	60
	R	CCGCCAGGACAAACCAGTA	

Table 1. Primers sequences.

Results

General data of patients and controls

A total of 50 medicated patients, 25 treatment-naïve patients, and 50 healthy controls were recruited for the current case-control study. No significant difference was found between the age and sex ratios of cases and controls. Demographic data of the participants in the study are summarized in Table 2.

Expression assays

Relative expression of genes was compared between medicated patients, treatment-naïve patients, and healthy controls. Expression level of *IFNG-AS1-001* was significantly downregulated in medicated-patient ($P < 0.05$); while expression levels of *IFNG*, *IL18RAP*, and *AC007278.2* were significantly upregulated in medicated patients compared to controls ($P < 0.05$). Expression levels of *IFNG-AS1-003*, *AC007278.3* and *IL18R1* were not significantly different between this group of patients and healthy controls (Fig. 1).

Expression levels of *IFNG*, *IFNG-AS1-001*, *IL18R1*, *IL18RAP*, *AC007278.2*, and *AC007278.3* were significantly upregulated in treatment-naïve patients compared to controls ($P < 0.0001$). On the other hand, expression level of *IFNG-AS1-003* was significantly downregulated in treatment-naïve patients compared to controls ($P < 0.0001$) (Fig. 1).

ROC curve analysis

In the present study, we evaluated the diagnostic power of transcript quantities of *IFNG*, *IL18R1*, *IL18RAP*, *IFNG-AS1-003*, *AC007278.2*, and *AC007278*.3 in identifying between the treatment-naïve patients and controls by depicting ROC curve (Table 3).

Based on the area under cover (AUC) value, *INFG-AS1-003*, *IFNG*, *IL18R1*, and *AC007278.3* had powerful diagnostic power (AUC = 0.90, 0.9, 0.87, and 0.80, $P < 0.0001$) (Fig. 2).

Additionally, we assessed the diagnostic power of all differentially expressed genes in *3* in identifying between treatment-naïve patients and controls (Fig. 3). Transcript levels of these genes could separate these groups with AUC = 0.93, sensitivity = 0.96 and specifiicity = 0.73.

Correlation matrix analysis

The analysis of the Spearman correlation matrix among genes in all subjects allows us to examine the connections between genes, as evidenced by their correlation coefficients and corresponding p-values. Significant positive and negative correlations between genes were observed in all three groups of medicated patients, treatment-naïve patients, and healthy controls, which indicated their expression relationship in the disease (Figs. 4, 5 and 6).

Finally, we assessed correlation between expression of genes and clinicopathological scores (Tables 4 and 5). In the medicated patients, we found positive correlations between expression of *IL18RAP* and BPRS, expression of *IFNG-AS1-001* and both PANNS and negative symptoms score, and expression of *AC007278.2* and PANNS. Moreover, inverse correlations were detected between CRP levels and expression levels of both *IFNG-AS1-001* and *AC007278.3*.

Among treatment-naïve patients, expression of *IFNG-AS1-001* was inversely correlated with BPRS and positive symptoms.

Study groups	Parameters		Values
Treatment-naive patients	Gender (number, %)	Male	20 (80%)
		Female	5 (20%)
	Age (Years, mean ± SD)	Male	27 ± 7.33
		Female	24 ± 3.71
	Family History (number, %)	Yes	15 (60%)
		No	10 (40%)
	Education (%)	Illiterate	40%
		School	32%
		High School	24%
		University	4%
	PANNS (mean ± SD)	72.52 ± 8.47	
	BPRS (mean ± SD)	51.16 ± 4.69	
	Positive symptoms score (mean ± SD)	17.12 ± 3.52	
	Negative symptoms score (mean ± SD)	16.84 ± 4.32	
	CRP (mg/L) (mean ± SD)	11.32 ± 3.23	
Medicated Patients	Gender (number, %)	Male	42 (84%)
		Female	8 (16%)
	Age (Years, mean ± SD)	Male	42.26 ± 8.42
		Female	37.33 ± 11.5
	Age at onset (Years, mean ± SD)	Male	28.36 ± 6
		Female	29 ± 3.6
	Duration (Years, mean ± SD)	Male	14.45 ± 6.8
		Female	8 ± 8.18
	Family History (number, %)	Yes	27 (54%)
		No	23 (46%)
	Education (%)	Illiterate	20%
		School	30%
		High School	44%
		University	6%
	PANNS (mean ± SD)	58.12 ± 7.87	
	BPRS (mean ± SD)	33.62 ± 6.33	
	Positive symptoms score (mean ± SD)	12.86 ± 3.38	
	Negative symptoms score (mean ± SD)	10.68 ± 2.85	
	CRP (mg/L) (mean ± SD)	5.88 ± 2.22	
	Clozapine dose (mg/day) (mean ± SD)	172 ± 82.16	
Controls	Gender (number, %)	Male	42 (84%)
		Female	8 (16%)
	Age (Years, mean ± SD (range))	Male	39.5 ± 8.81
		Female	41 ± 9.51
	Education (%)	Illiterate	0
		School	10%
		High School	48%
		University	42%

Table 2. Demographic data of patients and controls.

Discussion

After intensive research, it is now clear that a gene or protein cannot explain such a complex disease as schizophrenia. Schizophrenia is a multifactorial disease in which a large number of genes and different cellular signaling pathways are involved in its initiation and development. One of the important pathways involved in the development of schizophrenia is the inflammatory pathway[26]. Cytokines have a critical role in initiating and maintaining immune responses. They can easily cross the blood-brain barrier and act as a major mediator between the brain and the immune system[27]. Many studies showed that there is a significant alteration in the levels of inflammatory cytokines in the blood of schizophrenia patients compared to healthy controls[28].

The aim of this study was to evaluate the expression level of *IFNG*, *IL18R1*, *IL18RAP*, and *IFNG-AS1-001*, *IFNG-AS1-003*, *AC007278.2*, and *AC007278.3* in treatment-naïve schizophrenic patients who were in the acute

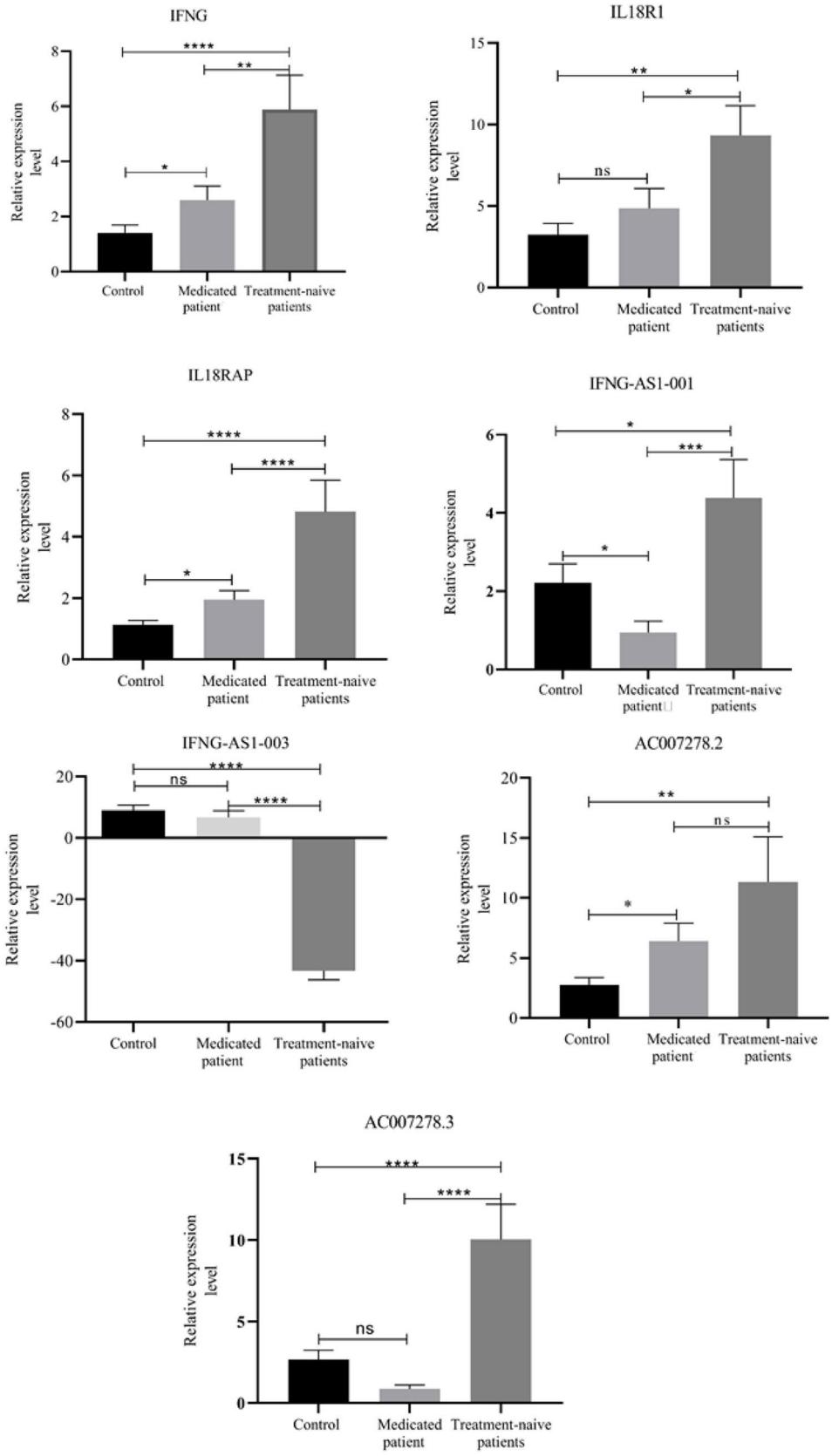

Figure 1. Expressions levels of genes in treatment-naïve and medicated patients compared to healthy controls. Expression levels of genes were calculated using the (Efficiency^-ΔΔCT) method. ns: not significant, $P < 0.05$(*), $P < 0.01$(**), $P < 0.001$(***), $P \leq 0.0001$(****).

Gene	Specificity	Sensitivity	AUC	Cutoff	*p*-value
IFNG-AS1-003	0.94	0.7	0.9	11.7	< 0.001
IL18R1	0.9	0.84	0.879	8.4	< 0.001
IFNG	0.9	0.8	0.94	7.4	< 0.001
AC007278.3	0.82	0.84	0.8	4.66	< 0.001

Table 3. Result of ROC curve analysis between treatment-naïve patients and controls.

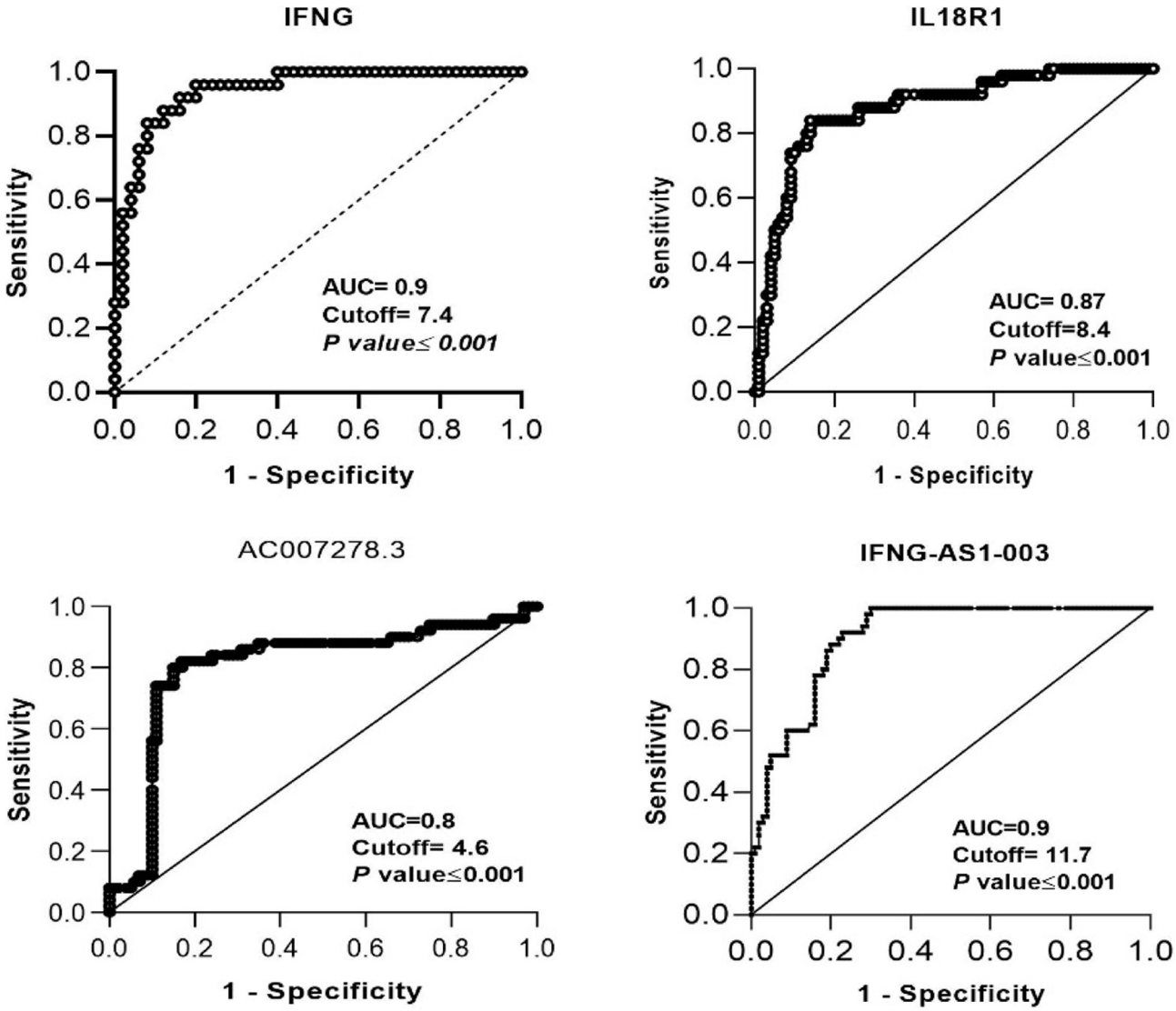

Figure 2. The diagnostic power of transcript quantities of *IFNG*, *IL18R1*, *IFNG-AS1-003*, and *AC007278.3* in identifying between treatment-naïve patients and controls.

phase of the disease as well as patients who were in the remission phase of the disease (medicated) compared to matched controls.

Interleukin 18 (IL-18) is one of the multifunctional cytokines that is structurally similar to the IL-1 family and is one of the main factors inducing the secretion of IFNG from T-helper cells[29]. IL-18 has a role in many psychiatric disorders. IL-18 signaling has been found to be interrupted in the central amygdala in an animal model of post-traumatic stress and alcohol use disorder[30]. This cytokine has a possible role in the modulation of the hypothalamic–pituitary–adrenal axis and might mediate the CNS dependent impacts on the susceptibility to related disorders[31]. Moreover, in many neurodegenerative and inflammatory diseases, the expression of IL-18 receptor (IL18R) and also the expression of *IL18*were increased[32]. The IL18R has two subunits, including IL18R1 (IL18α) and IL18RAP or (IL18ß)[24]. Two functionally related lncRNAs, namely *AC007278.2* and

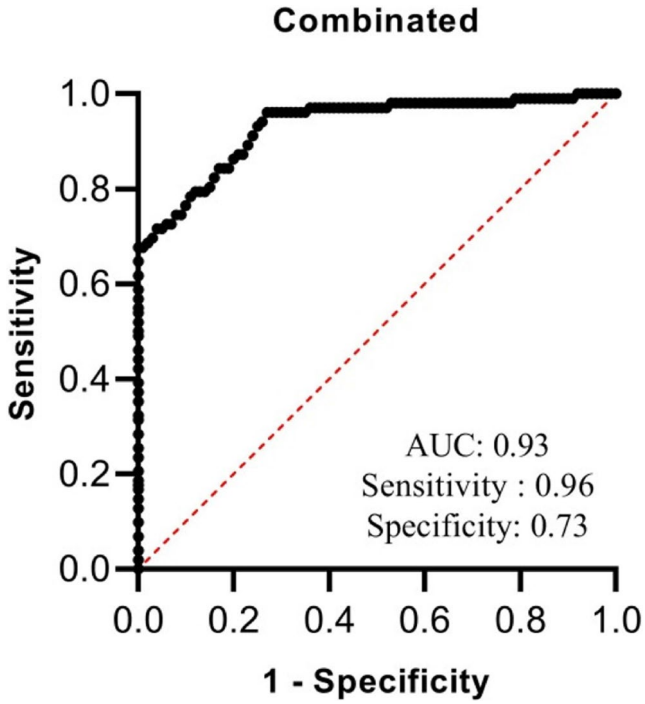

Figure 3. The diagnostic power of combination of transcript quantities of *IFNG*, *IL18R1*, *IFNG-AS1-003*, and *AC007278.3* in identifying between treatment-naïve patients and controls.

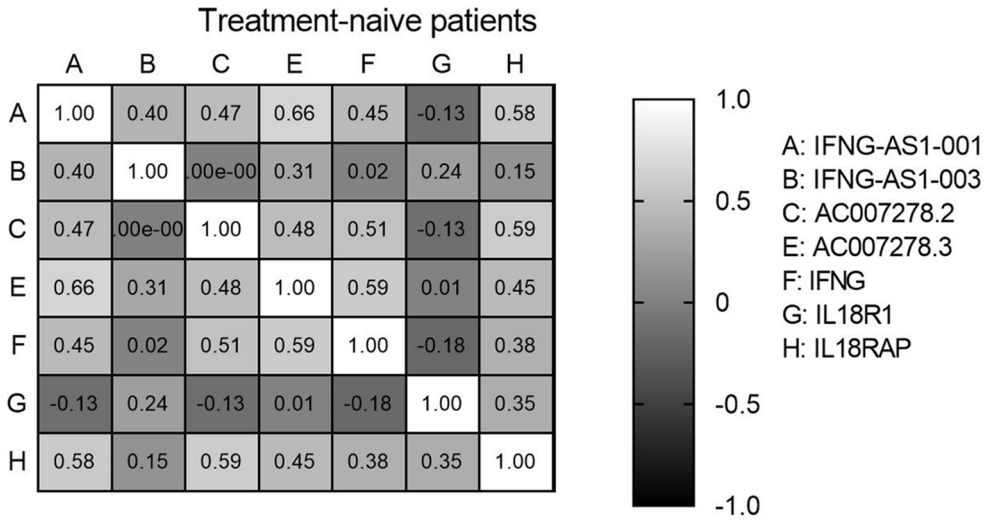

Figure 4. Spearman correlation between genes in treatment-naive patients.

AC007278.3 are located on chromosome 2 inside the introns of *IL18R1* and *IL18RAP*, respectively. IL-18 is secreted by macrophage-like cells and plays a pivotal role in the response of helper T cells[33]. IL18R is widely present in neurons. Thus, the IL-18 that is synthesized in the CNS, can affect neuronal growth, differentiation, and apoptosis[34]. This interleukin exerts its inflammatory role by increasing IFNG production from T cells and NK cells. Expression level of *IL18*in the blood of patients with schizophrenia has been shown to be higher than controls[23]. This finding has also been confirmed by Szabo et al., the serum level of IL18 significantly increased in schizophrenia patients compared to HCs[35].

Luo et al. have shown that the concentration of IL-18 in the serum of patients is not significantly altered between treatment-naïve and medicated patients, and the concentration level of IL-18 is increased in both groups compared to healthy controls[36]. Thus, one can infer that antipsychotic treatment does not change the concentration of IL-18. This finding was also verified in our study.

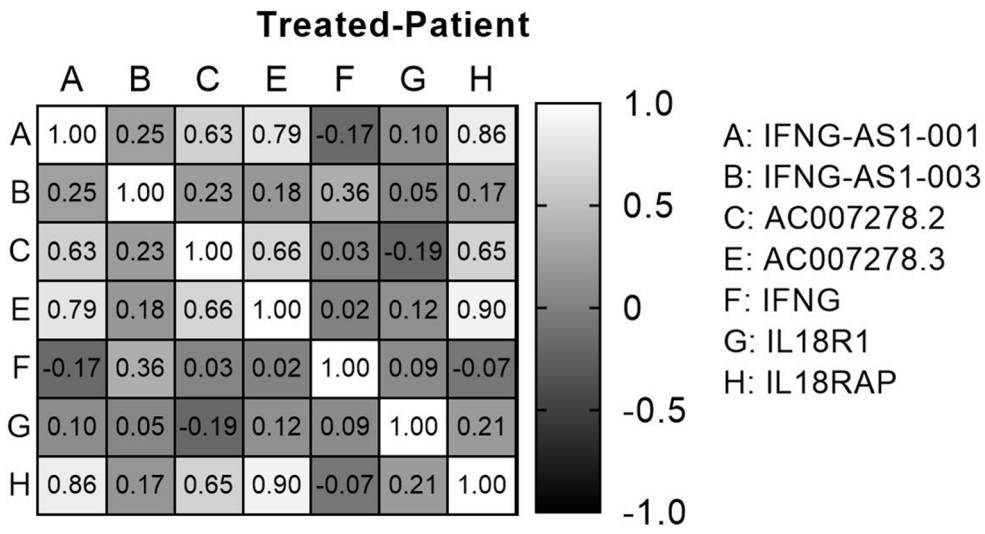

Figure 5. Spearman correlation between genes in medicated patients.

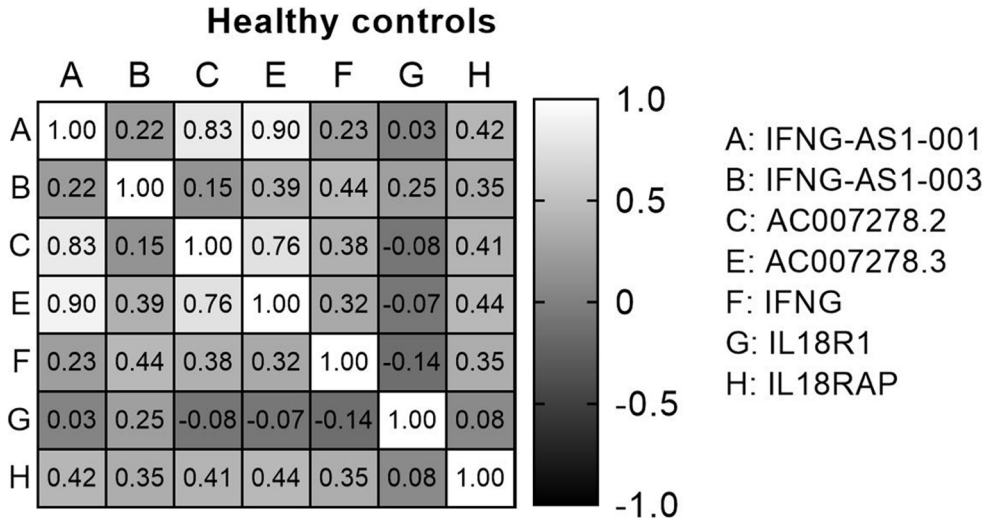

Figure 6. Spearman correlation between genes in healthy controls.

In the present study, we also evaluated the expression level of IL-18 receptor subunits, namely *IL18R1* and *IL18RAP*. The findings from the earlier research indicate that the binding of IL-18 to IL18R1 induces a sequence of processes that activates multiple signaling pathways, such as NF-kB and MAPK pathways, resulting in the generation of inflammatory cytokines and chemokines[37]. Therefore, based on previous studies, there is a direct relationship between the level of IL-18 and IL18R, IL18RAP. In the present study, we showed that the expression levels of *IL18R1* and *IL18RAP* were higher in both treatment-naive and medicated groups compared to healthy controls. We did not observe any significant expression changes between medicated and treatment-naive patients, which indicates that the use of antipsychotic drugs does not have a significant effect on the expression of IL-18 receptor subunits. In the ROC curve analysis of IL18R1, the sensitivity of 84% and the specificity of 90% indicated that it can be used as a suitable diagnostic biomarker to identify treatment-naïve patients from healthy with a expression cutoff of 8.4, which is statistically significant.

Another subunit of the IL18 receptor is IL18RAP. The expression of *IL18RAP* was significantly increased in treatment-naïve and medicated patients.

In line with our results, Xu et al. have analyzed GWAS and replicated the results in an independent cohort of schizophrenia patients. They showed association signals within *IL18R1* and *IL18RAP* genes, with the most significant marker being *IL18R1*rs1035130. They have also reported altered levels of IL-18 and IL18R1 in schizophrenia patients compared with controls[38].

We suggest that by increasing the expression of IL-18 receptor subunits, the expression of IL-18 also increases, which causes an increase in the amount of IFNG. IL-18 and IFNG are closely related cytokines that play important roles in the immune response. IL-18 is known to enhance the production of IFNG, and both

Genes	Parameters	PANNS	BPRS	Positive symptoms	Negative symptoms	CRP	Clozapine dose
IFNG	r	0.017	0.048	0.042	0.151	-0.118	−0.166
	P value	0.905	0.739	0.771	0.296	0.421	0.248
IL18R1	r	-0.182	-0.259	-0.174	0.201	-0.142	−0.212
	P value	0.206	0.069	0.227	0.161	0.331	0.139
IL18RAP	r	0.249	0.341*	0.175	0.029	0.051	0.144
	P value	0.081	0.015	0.225	0.843	0.729	0.318
IFNG-AS1-001	r	0.280*	0.080	0.130	0.309*	-0.287*	0.073
	P value	0.049	0.579	0.370	0.029	0.045	0.613
IFNG-AS1-003	r	0.162	0.073	0.090	0.081	-0.135	0.031
	P value	0.261	0.616	0.533	0.574	0.354	0.083
AC007278.2	r	0.3*	0.165	0.241	0.209	-0.161	0.69
	P value	0.034	0.252	0.092	0.145	0.270	0.632
AC007278.3	r	0.220	0.099	0.138	0.275	-0.319*	0.19
	P value	0.125	0.495	0.339	0.053	0.026	0.89

Table 4. Spearman correlation matrix analysis between genes and schizophrenia assessments criteria in the medicated patients.

Genes	Parameters	PANNS	BPRS	Positive symptoms	Negative symptoms	CRP
IFNG	r	-0.157	-0.108	0.219	-0.265	0.319
	P value	0.454	0.606	0.293	0.201	0.128
IL18R1	r	0.209	0.01	-0.265	0.092	-0.069
	P value	0.317	0.962	0.201	0.662	0.748
IL18RAP	r	-0.053	-0.355	-0.162	0.267	-0.066
	P value	0.801	0.082	0.438	0.197	0.758
IFNG-AS1-001	r	-0.209	-0.557	-0.719	0.221	0.162
	P value	0.316	0.004	0.000	0.289	0.449
IFNG-AS1-003	r	0.248	0.197	-0.205	0.387	0.18
	P value	0.232	0.346	0.327	0.056	0.4
AC007278.2	r	0.021	0.14	0.147	-0.105	-0.175
	P value	0.92	0.505	0.484	0.618	0.415
AC007278.3	r	-0.072	-0.057	-0.231	0.367	-0.132
	P value	0.731	0.786	0.268	0.071	0.54

Table 5. Spearman correlation matrix analysis between genes and schizophrenia assessments criteria in treatment-naive patients.

cytokines can synergistically amplify immune responses[39]. In Spearman's correlation matrix analysis, there was no significant relationship between the expression of *IL18R1* and *IL18RAP* in any of study subgroups.

We found positive correlation between expression of *IL18RAP* and BPRS in the medicated patients. In a previous study, elevation of serum concentration of IL-18 and a certain polymorphisms within *IL-18*gene have been reported to be positively associated with the PANSS general psychopathology subscore and the PANSS total score[40]. However, there was no data about correlation between expression of IL-18 receptor subunits and mentioned scores.

IFNG is a soluble cytokine of the type II class of IFNs[41]. It is a key activator of macrophages and inducer of MHC II molecule expression[42]. Abnormal expression of *IFNG*has been associated with numerous autoinflammatory and autoimmune diseases[43]. This cytokine is synthesized by T helper cells (particularly, Th1 cells), cytotoxic T cells, macrophages, mucosal epithelial cells, and NK cells. Moreover, it acts as a crucial autocrine signal for professional antigen-presenting cells (APCs) in early innate immune responses and a key paracrine signal in the adaptive immune response. Notably, expression of this molecule is induced by a number of cytokines, including IL-18 and type I IFN[44]. It is a cytokine of T helper 1[45]and regulates the presentation of antigens and the division and differentiation of lymphocytes. Meta-analyses of cytokine changes in schizophrenia have shown that IFNG can be a marker of disease diagnosis. The IFNG secretion is controlled by APCs, IL12 and IL18[46]. When the IFNG ligand binds to the receptor, the receptor is dimerized and causes JAK1 and JAK2 to come close to each other, leading to the phosphorylation and activation of STAT1. Activated STAT1 causes activation of STAT4. Activated STAT4 goes to the nucleus and increases the expression of the *IFNG*gene[47]. Figure 7 shows the relationships between IL-18, IFNG, JAK1, JAK2, STAT1, and STAT4.

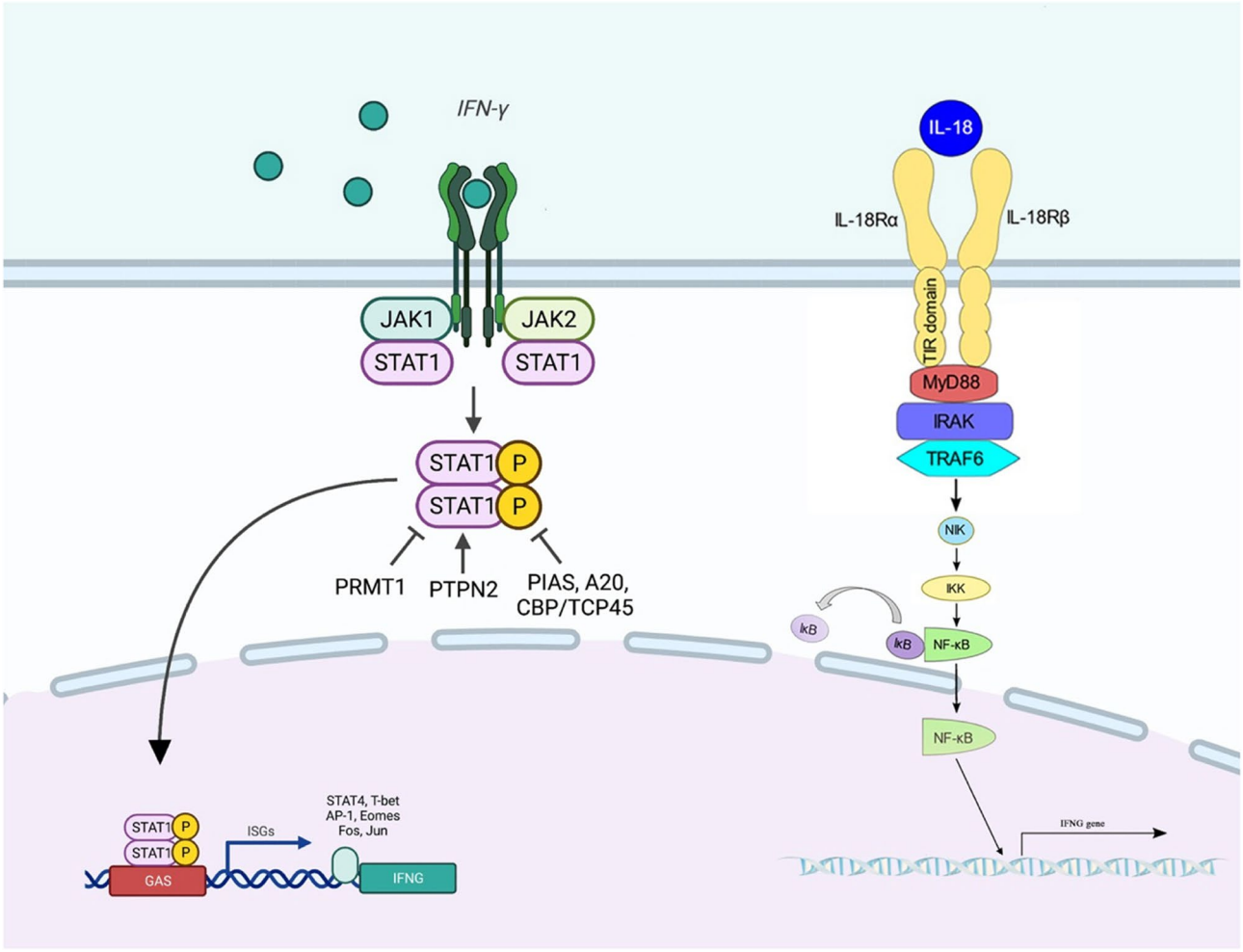

Figure 7. The relationships between IL18, IFNG, JAK1, JAK2, STAT1, and STAT4. When the IFNG ligand binds to the receptor, the receptor is dimerized. Then, JAK1 and JAK2 become close to each other. This leads to the phosphorylation and activation of STAT1. Activated STAT1 causes activation of STAT4. This cascade of events leads to up-regulation of IFNG.

*IFNG*has been shown to be significantly decreased in the medicated schizophrenia patients compared to healthy controls, while its expression has been up-regulated in the treatment-naïve patients[48]. Another study has shown that the expression level of this gene is higher in schizophrenia patients compared with controls[49]. In the medicated patients, we found positive correlations between expression of *IFNG-AS1-001* and both PANNS and negative symptoms score, and expression of *AC007278.2* and PANNS. Moreover, inverse correlations were detected between CRP levels and expression levels of both *IFNG-AS1-001* and *AC007278.3*. However, among treatment-naïve patients, expression of *IFNG-AS1-001* was inversely correlated with BPRS and positive symptoms. Thus, it can be inferred that medication affects correlation between mentioned genes and clinicopathological scores.

IL-18 and IFNG, two closely related cytokines, have a significant impact on the immune response as they often interact with each other. Both IL-18 and IFNG are classified as pro-inflammatory cytokines and play crucial roles in regulating immune responses, particularly in the context of defending the host against infections and managing the inflammatory response. One of the key aspects of their interaction is that IL-18 can stimulate the production of IFNG from various immune cells, such as T cells and NK cells. In response, IFNG can further enhance the expression of IL-18 receptors on immune cells. This creates a positive feedback loop within the immune response, where IL-18 and IFNG mutually reinforce each other's effects. This interaction between IL-18 and IFNG serves to amplify the inflammatory response, leading to a more robust immune defense against pathogens. By working together, IL-18 and IFNG contribute to the coordination and regulation of immune responses, ultimately aiding in the protection of the host organism[50]. Based on previous studies, the elevated *IL18R1* and *IL18RAP* expression can positively up-regulate the expression level of *IFNG*. In the present study, we showed that the expression level of *IFNG* in treatment-naïve patients significantly increased, and on the other hand, the level of *IFNG* expression in medicated patients compared decreased compared to controls. Based on the ROC curve analysis, *IFNG* had the highest AUC among the genes, at 0.94, and sensitivity of 0.8, specificity of 0.9 which significantly indicate its diagnostic ability ($P < 0.0001$).

IFNG-AS1, which is an intergenic lncRNAs, is located on the same chromosome as the *IFNG* gene, and is known as *Tmevpg1*; *NEST*[51]. *IFNG-AS1*has a number of variants[52], and in the present study, we investigated the expression level of two variants, including *IFNG-AS-001* and *IFNG-AS-003*. The expression level of the IFNG-AS1-001 variant decreased twofold in the medicated patients compared with controls, while its expression level was higher in the treatment-naïve patients. Most notably, while the expression level of the *IFNG-AS-003* gene was similar between the medicated patients and controls, its expression was significantly lower in the treatment-naïve patients compared to controls.

Expression level of the *IFNG-AS1-001* gene was decreased in the medicated patients compared with controls. *IFNG-AS1-001* contributes to the methylation of the *IFNG*locus and decreases its expression by binding to WDR5, a scaffolding protein in the H3K4 methyltransferase complex[21]. Thus, down-regulation of *IFNG-AS1-001* is expected to result in the up-regulation of IFNG. *IFNG-AS1-003* gene is also located on the same chromosome as the *IFNG*[22]. The observed downregulation of the *IFNG-AS1-003*variant in the current study is consistent with the previous studies that were conducted on other diseases such as Hashimoto's disease[53]. In fact, *AC007278.2* and *IFNG-AS1-001* have been shown to be up-regulated in the relapsing phase in multiple sclerosis patients, while *IFNG-AS1-003*has been up-regulated in the remitting phase compared with relapsing phase[52].

Expression levels of *AC007278.2* and *AC007278.3* were higher in the treatment-naïve patient compared to the controls. These lncRNAs are directly related with the increase of IL18R1 and IL18RAP genes. These genes are located on the positive strand of chromosome 2q12, and inside the *IL18R1*gene intron and play an important role in the expression and differentiation of T helper1 cells[54]. It has been disclosed that the expression level of *AC007278.2* and *AC007278.3* lncRNAs is aligned with the *IL18R1* and *IL18RAP* gene expression. Up-regulation of the mentioned lncRNAs has been correlated with over-expression of *IL18R1* and *IL18RAP* genes[52]. The increase in the expression of *IL18R1* and *IL18RA*genes causes the activation of STAT4 and IL-2, as well as activation of histone acetyltransferase and DNA methylase complex. Finally, IL18R1 and IL18RAP cause the differentiation of T helper1 cells, and this differentiation causes the production of pro-inflammatory cytokines from T helper1 cells, which is one of the main causes of autoimmune diseases[55]. Therefore, it is likely that the expression level of *AC007278.2* and *AC007278.3* is directly related to the level of expression of genes coding for the IL-18 receptor protein, and the use of antipsychotic drugs may reduce their levels. Based on the ROC curve analysis, *AC007278.3* had the sensitivity of 84% and the specificity of 82% with AUC = 80%, thus it can be used as a suitable diagnostic biomarker to identify treatment-naïve patients from controls with an expression cutoff of 4.66.

Finally, assessment of pairwise correlation in the patients group showed that there was a significant direct relationship between genes. In both medicated and treatment-naïve patients, *IFNG-AS1-001* showed significantly strong positive correlations with *AC007278.2*, *AC007278.3*, and *IL18RAP*. Moreover, *AC007278.2* had a significant correlation with *IFNG-AS1-001*, *AC007278.3* and *IL18RAP* in both groups. However, the correlations between *AC007278.2*/*IFNG* and *AC007278.3*/ *IFNG* were only significant in the treatment-naïve patients. On the other hand, the correlation between and *IFNG* and *IFNG-AS1-003* was only significant among medicated patients. Thus, it can be inferred from the correlation data that medication affects the correlation between *IFNG* and other genes.

Conclusion

In summary, this study showed changes in the expression of a number of lncRNA genes and related protein-coding genes in the blood of patients with schizophrenia. Notably, *IFNG*, *IFNG-AS1-003*, *IL18R1*, and *AC007278.3* genes were found to be suitable biomarkers in this disorder. In order to confirm the biomarker power of genes and gene expression changes in the acute phase and remission of the disease, more studies in a larger statistical population are suggested. Our study had some limitations including lack of assessment of expression of mRNA coding genes at protein level and lack of functional studies.

Data availability

The datasets generated and/or analysed during the current study are available in the NCBI repository: IFNG (NC_000012.12), IL18R (NC_058098.1), IL18RAP (NC_000002.12), IFNG-AS1(NC_000012.12).

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Research involving human participants and/or animals
The study was carried out according to the principles of the 1964 Declaration of Helsinki. Each enrolled subject provided a written informed consent, and all study data were obtained and elaborated in accordance with our institutional ethical committee regulations. The study protocol was approved by the Shahroud University of Medical Sciences Ethical Committee (IR.SHMU.REC.1398.113).

Informed consent
Informed consent was obtained from patients.

Consent to participate
Not applicable.

Author contributions
Kamran Javidi and Shima Rahmani performed the majority of experiments and data analysis. Faezeh Mehdizadeh, Fatemeh Manafzadeh, Seyed Gholamreza Noor Azar and Shahrokh Aghayan contributed to the experiments and interpreted the results. Faezeh Mehdizadeh wrote the manuscript and helped with the experiments. Behzad Baradaran and Soudeh Ghafouri-Fard revised the manuscript critically for important intellectual content. Asghar Shayannia designed and conducted the project.

Declarations

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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